

Extraction of Lipids and Cholesterol from Squid Oil with Supercritical Carbon Dioxide

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Abstract—The oil obtained from waste squid viscera consists of multi-compounds such as EPA, DHA and other valuable polyunsaturated fatty acids. The refining of this squid oil, using supercritical carbon dioxide plus ethanol, was performed in a semi-continuous flow extractor at 8 to 17 MPa and 25 to 50 °C. When 1.5% w/w ethanol was added to the solvent, the solubility of lipids was increased by up to 50% over the neat CO₂ value. The extraction curves indicated mass transfer to be solubility limited. Cholesterol was co-extracted with the lipids but, with its lower solubility, less than 54% appeared in the refined oil. The results of the carbon dioxide/multi-compound squid oil system at applied to the extraction conditions were correlated with the mole fraction of the cholesterol and the density of the pure solvent.

Key words: Fatty Acids, Squid Viscera Oil, Cholesterol, Supercritical Carbon Dioxide, Solubility

INTRODUCTION

Squid is a popular seafood that is used in various ways as part of the human diet. It is widely consumed in Southeast Asia and other parts of the world. In southern Europe squid is known as calamari. Not only is squid consumed fresh, but it is also processed into other forms of food in huge quantities [Bandarra et al., 1992]. Squid inhabit all the world's major oceans and seas. The viscera are usually discarded during processing, yet the by-product oil obtainable from this waste is high in polyunsaturated fatty acid oils (PUFA), particularly the ω -3 fatty acid oils.

The effect of increased dietary intake of ω -3 fatty acid oils on health has received much attention in recent years, in particular, eicosapentaenoic acid (EPA, 20 : 5 ω -3) and docosahexaenoic acid (DHA, 22:6 ω -3), are reputed to have prophylactic properties for the reduction of cardiovascular and inflammatory diseases [Lands, 1986; Ackman, 1988, 1989; Rambjar et al., 1996; Karmali et al., 1984]. Recovery of refined oil can provide a valuable dietary supplement as well as reduce the amount of high BOD waste to be disposed of [Kim et al., 1997].

The application of supercritical fluid extraction to biomaterials has been recognized for several years [Coenen and Kriegel, 1984; Dziezak, 1986; Eisenbach et al., 1983; Grimmer, 1982; Mansoori et al., 1988; Stahl et al., 1988; Hong et al., 1990]. Conventional methods for the extraction, fractionation and isolation of polyunsaturated fatty acids (PUFAs) include the use of highly flammable or even toxic solvents or energy-intensive vacuum distillation, as normal near atmospheric pressure high-temperature distillation can result in degradation of thermally labile compounds. Consideration of such factors has led investigators to apply supercritical fluid extraction (SFE) techniques to the separation [Mehugh and Krukonsis, 1986].

The technology is of special interest to the food and cosmetics industries because carbon dioxide, the most commonly applied supercritical solvent, is non-toxic and does not leave a residue. Super-

critical fluid extraction and fractionation of fish oils has been the subject of ongoing research, to the extent that workers have recently published data on solubilities and phase equilibria of polyunsaturated ω -3 fatty acid fish oils and cholesterol in supercritical fluids [Hardardottir and Kinsella, 1988; Nilsson et al., 1988; Chun and Wilkinson, 1999; Chrastil, 1982; Iwai et al., 1993; Foster et al., 1993]. Kosal et al. [1992] and Noh et al. [1995] measured the solubilities of cholesterol and fatty acids in supercritical CO₂ both with and without cosolvent, and the data were correlated by using the Peng-Robinson equation of state.

Recently Hartono et al. [2001] reported on six different cubic equations of state used to predict the solubility of cholesterol as a representative biomolecule in supercritical fluids. Valderrama and Silva [2003], and Baek et al. [2004] correlated the solubility data obtained from the supercritical fluid-solid and liquid mixtures with the equations of state.

Based on simple (pure components) systems, the solubility of PUFA oils in SCO₂ is sufficiently greater than the cholesterol to anticipate good rejection of cholesterol in the extraction process. However, no results have been reported on the relative extraction from mixtures. The presence of lipids in the SCO₂ may enhance the solubility of cholesterol. This work examines the solubility of cholesterol in SCO₂ in the presence of PUFAs and the degree of rejection of cholesterol from the extracts. Further, changes in polarity of solvent can affect relative solubility and so the effect of ethanol entrainer is examined. Ethanol (GRAS - generally regarded as safe for foodstuffs) was chosen as compatible with food requirements.

EXPERIMENTAL

1. Materials

A local (Korean) fish processor provided the crude oil used in this study. It was obtained by conventional expression of the internal organs of squid. The oil was stored under N₂ at 4 °C until required. The carbon dioxide was food grade, 99.9% pure and the ethanol entrainer was analytical grade. The components of the fatty acid oil were identified by comparison with standards (fatty acid methyl

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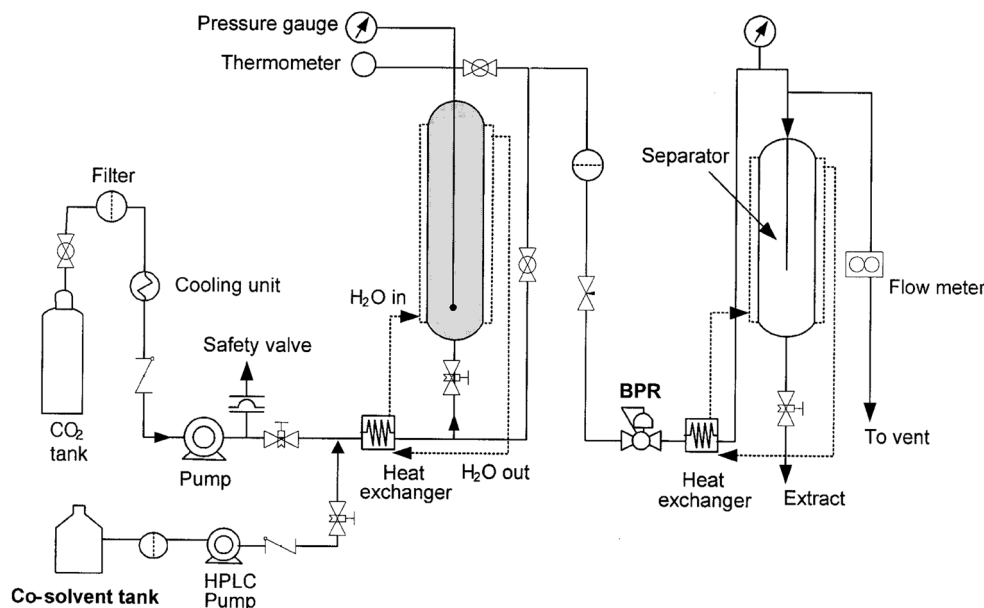


Fig. 1. Flow diagram of the supercritical fluid extraction system.

ester mixture-FAME: Sigma). The cholesterol was also identified by comparison with standards.

2. Apparatus and Extraction Procedures

The extractions were performed in a semi-continuous flow-type apparatus shown in Fig. 1 and described previously [Foster et al., 1993]. The 75 ml extractor, tubing and fittings were 316 stainless steel. Carbon dioxide was pumped to the extractor by a positive-displacement controlled-volume metering-pump. It was pre-cooled to -20°C prior to the pump inlet to avoid NPSH and compressibility problems (A net positive suction head - NPSH - is needed at the pump inlet to avoid fluid vaporization occurring in the pump during the suction stroke.). The same flow rate of 40 ml/min was used for all runs equivalent to a solvent mass-flow to charge-quantity ratio of 2.68 min^{-1} . An HPLC pump was used to supply ethanol entrainer, again using the same flow rate of 0.82 g/min for each run with entrainer.

Pressure in the extractor was controlled by a back-pressure regulator and CO_2 flow was measured, after expansion to ambient conditions, by a wet gas meter, then converted to mass flow rate. The extraction vessel was loosely packed with glass wool, and for each extraction a 20 g aliquot of crude oil was added to and distributed throughout the packing. A small plug of glass wool was then placed in the outlet end before closure to reduce entrainment. Extract was collected in a separator and chilled with ice, by expansion of the loaded solvent to ambient pressure. At 10-minute intervals, extracts were collected and analyzed for fatty acids.

3. Analysis

Samples were prepared for fatty acid analysis according to the AOAC (FAME method) standard [Grimmett, 1982] and analyzed by capillary GC-FID (HP 5890 series II, HP-Innowax cross-linked PEG column). GC conditions were as follows: injector 250°C ; detector 300°C ; oven 210°C ; carrier - nitrogen 1 ml/min. Cholesterol and individual fatty acid peaks in the chromatograms were identified by comparison with authentic standards (cholesterol: 99%, Sigma Chem. Co., C8667; fatty acid methyl ester mixture: Sigma Chem. Co., 189-19).

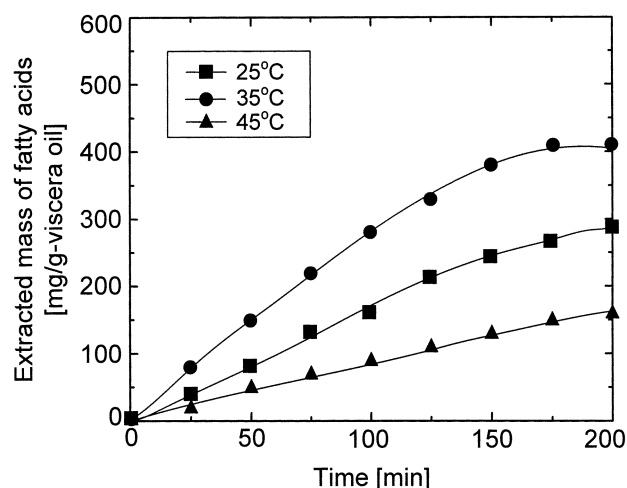


Fig. 2. Effect of temperatures on fatty acids extraction from squid viscera oil (Pressure= 8 MPa, Flow rate=40 ml/min).

RESULTS AND DISCUSSION

1. Extraction of Squid Viscera Oil

Fig. 2 demonstrates the effect of temperature on the degree of extraction. These isobars were obtained at a flowrate of 40 ml/min and pressure 8 MPa. These results are in general agreement with Nilsson et al. [1988]. Their work did not report sub-critical temperatures where we found that 25°C isotherm gave lower solubility than 35°C . It would appear this isotherm maximum solubility of lipids occurs at a temperature close to the critical point of the pure solvent. More work is needed to better define the optimum. This solubility behavior follows changes in the density of the solvent [Baek et al., 2004; Kramer and Thodos, 1989].

The effect of pressure on lipid extraction by pure CO_2 is shown in Fig. 3. It can be seen that solubility improves with an increase in pressure, as is expected from the density increase. However the 8

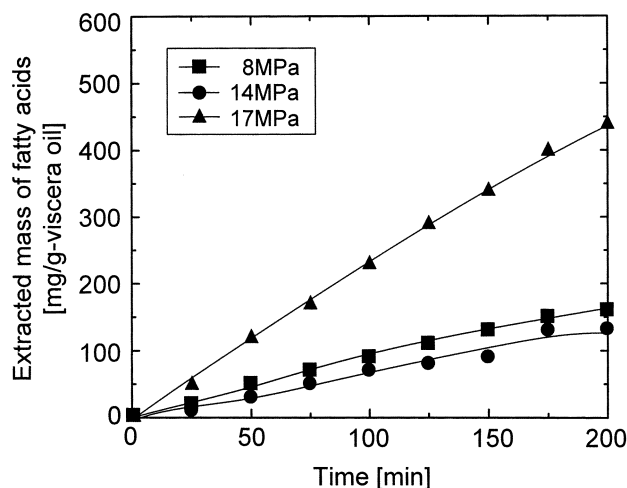


Fig. 3. Effect of pressures on fatty acids extraction from squid viscera oil (Temperature=35 °C, Flow rate=40 ml/min).

MPa shows a slightly higher solubility than the 14 MPa. This reversal is thought to be due to the unique properties such as density, viscosity and diffusivity related to transferring a solute into the supercritical fluids with temperature and pressure changes in the system [McHugh and Krukons, 1986]. The effect of solvent density is clearly evident where the higher pressure of 17 MPa resulted in higher oil solubility than at 14 MPa and the same temperature. The 25% greater pressure gave a 3¼-fold increase in solubility.

Fig. 4 shows the effect of ethanol entrainer. Generally the amount of ethanol used as entrainer is less than 3% w/w owing to the low solubility of supercritical carbon dioxide. The use of 1.5% w/w ethanol entrainer increased oil solubility markedly. In the case of liquid carbon dioxide extractions, the increased solubility was almost twice that obtained with no entrainer. This result is similar to that of Bulley et al. [1992]. The linearity of the curves indicates that mass transfer was limited by solubility; although at the highest extraction rate

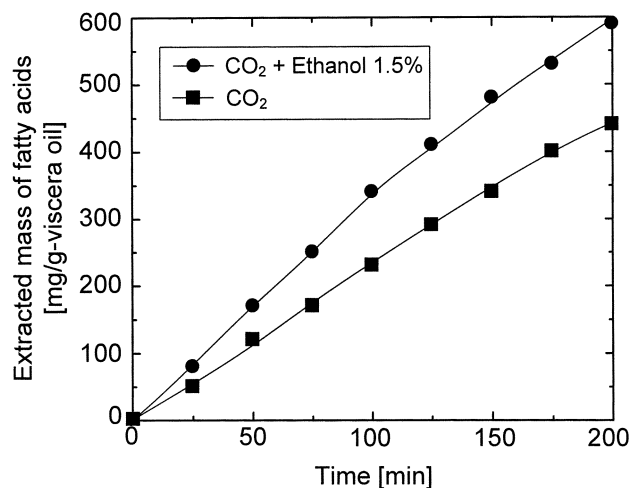


Fig. 4. Effect of entrainer on the extracted mass of fatty acids from squid viscera oil (Temperature=35 °C, Pressure=17 MPa, CO₂ flow rate=40 ml/min).

achieved (17 MPa, 35 °C) some curvature became apparent after about 2-hour's extraction. In the design of a commercial extraction process, this suggests that the mass-transfer characteristics of the extractor are of less importance than establishing the best (equilibrium) extraction conditions. One advantage in using an entrainer lies in the lower pressures that will achieve recoveries similar to the neat solvent. In any commercial application, this advantage must be weighed against any increased complexity of the extraction process and potential problems. With lipid extracts, ethanol can be removed to acceptable levels simply by evaporation, with or without vacuum.

2. Fatty Acid Composition

Fatty acid compositions of the extracts are shown in Table 1, where the wt% of specified fatty oils (as acids) are shown for extractions at 8 and 14 MPa and temperatures from 25 to 50 °C. Palmitic (C16:0),

Table 1. Fatty acid compositions of squid viscera oil extracted by SC-CO₂ at different temperature

Pres. [MPa]	Temp. [°C]	Fatty acid [%] ¹								
		Myristic C14 : 0	Palmitic C16 : 0	Palmitoleic C16 : 1	Oleic C18 : 1	Octadecatetraenoic C18 : 4	Arachidic C20 : 0	Eicosatetraenoic C20 : 3	EPA ² C20 : 5	DHA ³ C22 : 6
8	25	5.83	23.43	6.18	16.78	2.92	3.10	1.72	15.14	24.91
	30	6.86	23.55	6.20	16.17	2.81	4.66	1.62	14.52	23.62
	35	6.11	23.67	6.07	16.57	2.63	4.77	1.60	14.13	24.46
10	30	7.45	22.06	6.62	15.23	3.32	4.01	1.68	15.82	23.81
	35	8.06	21.99	6.64	14.60	3.42	3.60	1.63	15.73	24.34
	40	6.12	22.41	5.96	16.21	2.87	4.82	1.74	15.10	24.78
	45	7.21	24.12	6.27	16.18	2.71	4.21	1.60	14.02	23.26
12	35	6.79	22.46	6.54	15.41	3.25	4.10	1.68	15.64	24.12
	40	7.61	22.47	6.58	15.23	3.28	3.97	1.66	15.56	23.63
	45	8.64	24.97	6.85	15.52	2.97	4.06	1.58	14.01	21.40
	50	7.97	22.63	6.76	14.77	3.36	3.68	1.63	15.58	23.62
14	40	6.69	22.12	6.21	15.39	2.93	4.23	1.62	14.62	26.20
	45	6.13	23.70	6.12	16.42	2.69	4.75	1.63	14.25	24.30
	50	6.99	22.91	6.19	15.50	2.98	4.20	1.64	14.88	24.69

¹Percentage of gas chromatography area. ²EPA : eicosapentaenoic acid. ³DHA : docosahexaenoic acid.

oleic (C18 : 1), EPA (C20 : 5) and DHA (C22 : 6) were the major fatty acids followed. The greatest recoveries of DHA and EPA were obtained at 14 MPa and 40 °C and 10 MPa and 30 °C, respectively. To investigate the composition of the fatty acids in the oil extracts, the extraction time and conditions were changed, but the trends for other components and conditions were similar and are detailed in

Table 2.

The data show that the relative proportions of individual fatty acids remained relatively constant throughout an extraction and this is confirmed by linear regressions of the data as shown in Fig. 5. The CO₂ loadings of individual components were thus roughly the same under the conditions used. We would have expected the pro-

Table 2. Fatty acid oil compositions of extracts at various extraction times at 25 °C (8 MPa) and 45 °C (17 MPa)

25 °C (8 MPa)										
Area % of gas chromatography										
Extraction time [min]	10	20	30	40	50	60	70	80	90	100
Fatty acid										
C14 : 0	7.16	7.29	6.20	7.29	6.78	7.19	6.73	6.77	6.82	6.17
C16 : 0	23.28	23.41	21.89	23.10	22.47	22.82	22.81	22.31	22.25	22.04
C16 : 1	6.61	6.70	6.28	6.77	6.47	6.67	6.61	6.40	6.37	6.35
C18 : 1	16.21	16.03	16.23	16.32	16.08	15.69	16.52	15.84	15.87	15.65
C20 : 0	7.15	6.96	7.44	6.86	7.17	6.85	4.73	7.17	7.19	7.29
C20 : 5	15.96	16.06	17.06	16.04	16.44	16.45	16.94	16.34	16.30	16.54
C22 : 6	23.73	23.55	26.12	23.60	24.59	24.31	25.63	25.44	25.20	25.40
Extraction time [min]	110	120	130	140	150	160	170	180	190	200
Fatty acid										
C14 : 0	6.59	7.29	7.08	6.66	6.43	6.44	6.38	6.72	6.57	6.52
C16 : 0	22.10	23.58	22.43	22.31	22.31	22.22	21.65	22.86	22.22	22.20
C16 : 1	6.27	6.74	6.61	6.33	6.19	6.22	6.10	6.43	6.26	6.22
C18 : 1	15.94	16.47	15.58	15.94	16.00	15.89	15.59	16.05	16.07	15.53
C20 : 0	7.34	7.44	4.61	7.41	7.61	7.39	7.52	7.34	7.47	7.31
C20 : 5	16.37	17.05	17.28	16.16	16.02	16.24	16.33	16.01	16.03	16.26
C20 : 6	25.40	26.14	26.40	25.19	25.44	25.61	26.43	24.59	25.38	25.96
45 °C (17 MPa)										
Area % of gas chromatography										
Extraction time [min]	10	20	30	40	50	60	70	80	90	100
Fatty acid										
C14 : 0	7.72	6.94	7.15	6.43	6.43	5.95	5.84	5.66	5.52	5.91
C16 : 0	25.25	22.87	23.21	22.39	22.49	21.60	21.47	21.58	21.34	22.09
C16 : 1	7.31	6.57	6.63	6.20	6.21	5.99	5.90	5.76	5.85	5.97
C18 : 1	18.02	16.42	16.4	16.31	16.27	16.18	15.99	16.17	16.50	16.23
C20 : 0	5.03	4.88	4.87	7.61	7.58	7.72	7.73	5.11	5.29	7.88
C20 : 5	18.12	16.69	16.46	15.93	15.89	16.41	16.41	17.39	17.15	16.02
C22 : 6	27.15	25.63	25.28	25.14	25.13	26.14	26.64	28.32	28.32	25.90
Extraction time [min]	110	120	130	140	150	160	170	180	190	200
Fatty acid										
C14 : 0	6.35	5.59	5.89	6.01	5.94	5.51	5.23	6.09	5.35	5.24
C16 : 0	22.62	21.65	22.10	22.72	22.52	21.68	21.11	22.87	21.81	21.47
C16 : 1	6.15	5.77	5.88	6.05	6.03	5.74	5.61	6.07	5.73	5.62
C18 : 1	16.36	16.21	16.16	16.49	16.35	5.74	5.61	6.07	5.73	5.62
C20 : 0	5.20	8.14	8.01	5.28	5.33	7.97	8.34	7.89	8.18	8.37
C20 : 5	16.38	15.99	15.71	16.30	06.43	15.85	16.14	15.60	15.93	16.03
C22 : 6	26.94	26.64	26.25	27.14	27.41	27.24	27.35	24.99	26.75	26.95

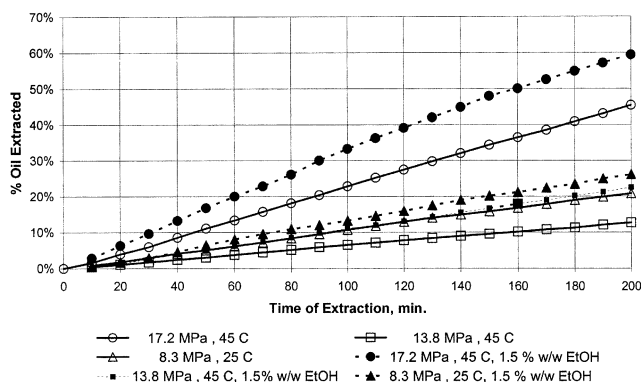


Fig. 5. Extraction curves for crude squid oil. Open symbols: neat CO₂; filled symbols: CO₂ with 1.5% w/w ethanol entrainer.

portions to change noticeably as one or other component became depleted. A comparison of extract analyses showed no noticeable preferences for particular fatty acids.

3. Extraction of Cholesterol

The extracts were also analyzed for cholesterol content in order to address the effect of extraction conditions on its recovery. At different conditions the solubilities of cholesterol in carbon dioxide are summarized in Table 3. The data show how the solubility of cholesterol in supercritical carbon dioxide from squid oil depends

Table 3. Cholesterol solubility in squid oil extracts recovered by dense CO₂ at different extraction conditions. Extraction time: 60 min, CO₂ flow rate: 40 ml/min

P (MPa)	T (°C)	$\rho_{CO_2}^*$ (kg/m ³)	$m_{ext} \times 10^6$ (kg)	$y_{chole} \times 10^{6***}$ (mole fraction)	R_{chole}^{***} (%)
8	30	717	8.061	0.710	38.5
	40	319	8.563	1.271	34.6
	45	264	9.327	1.116	28.8
	50	236	9.987	0.802	23.8
10	30	724	7.474	0.889	42.9
	40	651	7.521	0.938	41.6
	45	541	7.583	0.797	40.1
	50	421	8.235	0.793	37.1
12	30	816	7.461	0.866	43.1
	40	730	7.487	1.166	40.9
	45	676	8.121	1.260	38.0
	50	611	9.590	0.820	36.8
14	30	834	6.417	0.874	51.0
	40	761	6.489	1.096	46.5
	45	718	7.913	1.423	39.6
	50	669	8.141	1.153	37.9
17	30	841	6.030	0.885	54.0
	40	812	7.104	1.130	48.8
	45	780	7.251	1.258	44.6
	50	746	7.432	1.132	43.3

*Density of pure CO₂ obtained from published data.

**Cholesterol solubility (mole fraction) measured by Eq. (2)

***Cholesterol reduction (%) calculated by Eq. (1), initial cholesterol amount: 13.1 mg/g source

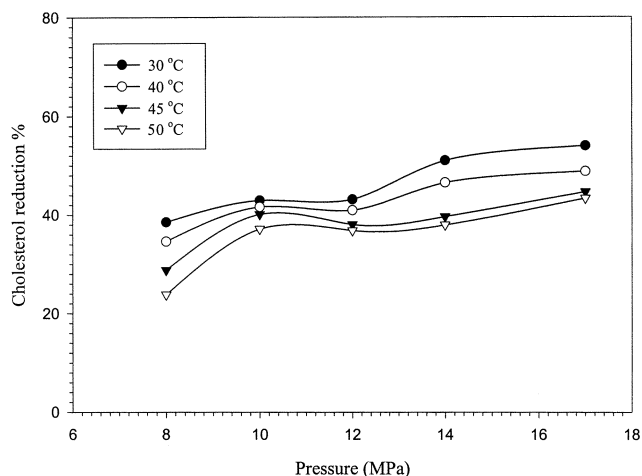


Fig. 6. Cholesterol reduction in the oil extracted with dense CO₂ at various conditions.

on temperature and pressure. The amount of cholesterol in the oil extracted from the crude squid oil increased with high density of carbon dioxide which provides a strong solvating power at liquid-liquid or solid extraction system.

Fig. 6 shows the relative reduction in cholesterol in the extracts at different temperatures and pressures. The lower temperatures favor cholesterol reduction as does increased pressure. The slight dip in the curves at 12 MPa is unexplained. Initially, 1 g of the crude oil contained 13.1 mg of cholesterol. The reduction in cholesterol achieved, and shown in Fig. 6, was between 38.5% (8.06 mg, in 8 MPa, 30 °C) and 43.3% (7.43 mg, in 17 MPa, 50 °C), respectively. At 17 MPa and 30 °C condition the cholesterol reduction was highly achieved with 54%. The reduction was calculated from the following equation:

$$R_{chole}(\%) = \left[1 - \frac{m_{ext}}{m_{ini}} \right] \times 100 \quad (1)$$

where m_{ext} and m_{ini} represent the amounts of cholesterol in 1 g of the extracted and initial oil, respectively, R_{chole} is the percentage of cholesterol reduction in the extracted oil.

Mole fractions of cholesterol in dense carbon dioxide were obtained by using the following equation:

$$y_{chole} = \frac{n_{chole}}{n_{chole} + n_{CO_2}} \quad (2)$$

Where y_{chole} represents the mole fraction of cholesterol and n_{chole} , n_{CO_2} are moles of cholesterol and carbon dioxide, respectively.

To model the supercritical carbon dioxide extraction, a simple approach was adopted. The enhancement factor, η , was correlated with the corresponding density of the pure CO₂ according the following equation:

$$\ln \eta = a + b \rho_{CO_2} \quad (3)$$

$$\eta = \frac{p y_{chole}^o}{p^o} \quad (4)$$

The saturated vapor pressure of the solute is represented as p^o , assuming 1 Pa.

The correlation is shown in Fig. 7 demonstrating a good linear relationship between $\ln \eta$ and the density of CO₂ across all condi-

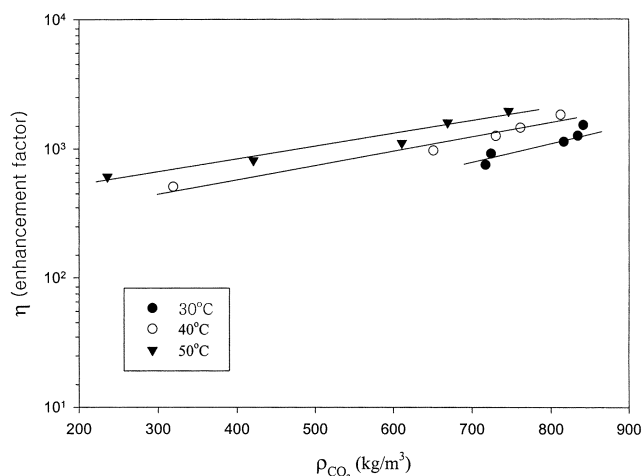


Fig. 7. Relationship between the enhancement factor and density of CO₂ at various temperatures.

Table 4. Coefficients of Eq. (4) obtained from Fig. 6

Conditions	a (-)	b × 10 ³ (m ³ /kg)
● 30 °C	1.5876	1.8395
○ 40 °C	2.3485	1.0603
▼ 50 °C	2.5265	0.9677

tions. The density of CO₂ was obtained from the published data. The coefficients in Eq. (3) are summarized in Table 4. The values of a and b are independent of temperature and are similar to those reported by Iwai et al. [1993].

The values of solubility obtained here on mixtures were less than those reported by Foster et al. [1993] which were obtained by using pure lipids and a gas circulation system. The data are, however, in close agreement with those of Mohamed et al. [1998] using a butter oil mixture.

Hartono et al. [2001] correlated the solubility of cholesterol in supercritical fluids using a two-parameter equation of state and compared the predictions with those using other cubic equations, including van der Waals, Redlich-Kwong, Peng-Robinson and Patel-Teja. The solubilities of cholesterol calculated by using the Redlich-Kwong and van der Waals equations were 2 to 3 and 4 to 7 orders of magnitude lower than the experimental data, respectively. It was difficult to make meaningful comparisons between theirs and this work since we worked with multi-component mixtures rather than pure oils. The data of solubilities, cholesterol and fatty acids of supercritical carbon dioxide, obtained in this work were similar to the values reported by previous works [Iwai et al., 1993; Noh et al., 1995].

CONCLUSION

The rate of extraction of fatty acid oil from crude squid viscera oil was shown to be solubility limited. The rates of extraction of individual fatty acid components were roughly similar, and so the composition profiles were relatively uniform throughout the whole extraction. The use of ethanol entrainer at 1.5% w/w significantly improved recoveries of oil by up to 90%, the greatest increase oc-

curing under conditions when the carbon dioxide was marginally sub-critical liquid.

Cholesterol was preferentially rejected in the extractions. The level of the cholesterol was reduced in the extracts by greater than 54% of the crude oil value, with high pressure and low temperature conditions favoring rejection. Supercritical fluid extraction of squid viscera oil results in high quality lipids lower in cholesterol content.

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NOMENCLATURE

- a, b : parameters, Eq. (3)
m : amount of cholesterol in oil extracted [kg]
n : mole [g/molecular weight]
P : pressure [MPa]
P^o : saturated vapor pressure of solute [MPa]
R : percentage of cholesterol reduction
T : temperature [°C]
y : mole fraction of solute in vapor phase

Greek Letters

- ρ : density [kg/m³]
η : enhancement factor

Subscripts

- chole : cholesterol
ext : extracts
ini : initial

REFERENCES

- Ackman, R. G., "The Year of Fish Oils," *Chem. and Ind.*, **Mar.**, 139 (1988).
Ackman, R. G., "Problems in Fish Oils and Concentrates, in Fats for the Future," R. C. Cambie (Ed.), *Ch.*, **13**, 189 (1989).
Ackman, R. G., Ratnayake, W. M. N. and Macpherson, E. J., "EPA and DHA Contents of Encapsulated Fish Oil Products," *J. Am. Oil Chem. Soc.*, **66**, 1162 (1989).
Baek, J. K., Kim, S. W., Lee, G. S. and Shim, J. J., "Density Correlation of Solubility of C. I. Disperse Orange 30 Dye in Supercritical CO₂," *Korean J. Chem. Eng.*, **21**, 230 (2004).
Bandarra, M. M., Nunes, M. L., Campos, R. M. and Batista, I., *Variacão Sazonal dos Lípidos de Sardinha *sardina pilchardus**, Relatório Técnico e Científico (1992).
Bulley, N. R., Labay, L. and Amfield S. D., "Extraction/Fractionation of Egg Yolk Using Supercritical CO₂ and Alcohol Entrainers," *J. Supercritical Fluids*, **5**, 13 (1992).
Chrastil, J., "Solubility of Solids and Liquids in Supercritical Gases," *J. Physical Chemistry*, **86**, 3016 (1982).
Chun, B. S. and Wilkinson, G. T., "Ternary Phase Equilibria of the iso-

- Propanol+Water+Carbon Dioxide System at High Pressure," *Korean J. Chem. Eng.*, **16**, 187 (1999).
- Coenen, H. and Kriegel, E., "Applications of Supercritical Gas Extraction Processes in the Food Industry," *Ger. Chem. Eng.*, **7**, 335 (1984).
- Dziedzic, J. D., "Innovative Separation Process Finding Its Way into the Food Industry," *Food Technol.*, **40**, 66 (1986).
- Eisenbach, W., Gottsch, P. J., Niemann, K. and Zosel, K., "Extraction with Supercritical Gases: the First Twenty Years," *Fluid Phase Equilibria*, **10**, 315 (1983).
- Foster, N. R., Singh, H., Jimmy, Yun, S. L., Tomasko, D. L. and Macnaughton, S. J. M., "Polar and Nonpolar Cosolvent Effects on the Solubility of Cholesterol in Supercritical Fluids," *Ind. Eng. Chem. Res.*, **32**, 2849 (1993).
- Grimmett, C., "The Use of Liquid Carbon Dioxide for Extracting Natural Products," *Chem. Ind.*, **15**, 359 (1982).
- Hardardottir, I. and Kinsella, J. E., "Extraction of Lipid and Cholesterol from Fish Muscle with Supercritical Fluids," *J. Food Sci.*, **53**, 1656 (1988).
- Hartono, R., Mansoori, G. Ali and Suwono, A., "Prediction of Biomolecules in Supercritical Solvents," *Chemical Engineering Science*, **56**, 6949 (2001).
- Hong, I. K., Rho, S. W., Lee, K. S., Lee, W. H. and Yoo, K. P., "Modeling of Soybean Oil Bed Extraction with Supercritical Carbon Dioxide," *Korean J. Chem. Eng.*, **7**, 40 (1990).
- Iwai, Y., Koga, Y., Maruyama, H. and Arai, Y., "Solubilities of Stearic Acid, Stearyl Alcohol, and Arachidyl Alcohol in Supercritical Carbon Dioxide at 35 °C," *J. Chem. Eng. Data*, **38**, 506 (1993).
- Karmali, R. A., Marsh, J. and Fuchs, C., "Effect of w-3 Fatty acid on Growth of Rat Mammary Tumor," *J. Nat. Cancer Inst.*, **75**, 457 (1984).
- Kim, E. M., Jo, J. H., Oh, S. W. and Kim, Y. M., "Characteristics of Squid Viscera Oil," *J. Korea Fish. Soc.*, **30**, 595 (1997).
- Kosal, E., Lee, C. H. and Holder, G. D., "Solubility of Progesterone, Testosterone and Cholesterol in Supercritical Fluids," *J. Supercritical Fluids*, **5**, 169 (1992).
- Lands, W. E. M., *Fish and Human Health*, Academic Press, Inc. Orlando (1986).
- Mansoori, G. A., Schultz, K. and Martinelli, E. E., "Bioseparation Using Supercritical Fluid Extraction/Retrograde Condensation," *Bio/Technology*, **6**, 393 (1988).
- McHugh, M. A. and Krukonis, V. J., *Supercritical Fluid Extraction Principles and Practice*, Butterworth-Heinemann (1986).
- Mohamed, R. S., Neves, G. M. and Kieckbusch, T. G., "Reduction of Cholesterol and Fractionation of Butter Oil Using Supercritical Carbon Dioxide with Adsorption on Alumina," *Int. J. Food Sci. Technol.*, **33**, 245 (1998).
- Nilsson, W. B., Gauglitz, E. J., Hudson, J. K. and Stout, V. F., "Fractionation of Menhaden Oil Ethyl Esters Using Supercritical Fluid CO₂," *J. Am. Oil Chem. Soc.*, **65**, 109 (1988).
- Noh, M. J., Kim, T. G., Hong, I. K. and Yoo, K. P., "Measurements and Correlation of Effect of Cosolvents on the Solubilities of Complex Molecules in Supercritical CO₂," *Korean J. Chem. Eng.*, **12**, 48 (1995).
- Rambjor, G. S., Walen, A. I., Windsor, S. L. and Harris, W. S., "Eicosapentaenoic Acid is Primarily Responsible for Hypotriglyceridemic Effect of Fish Oil in Humans," *Lipids*, **31**, 45 (1996).
- Stahl, E., Quirin, K.-W. and Gerard, D., *Dense Gases for Extraction and Refining*, Chap. IV. Springer-Verlag, New York (1988).
- Valderrama, J. O. and Silva, A., "Modified Soave-Redlich-Kwong Equations of State Applied to Mixtures Containing Supercritical CO₂," *Korean J. Chem. Eng.*, **20**, 709 (2003).