Process Conditions for Separating Fatty Acid Esters by Supercritical CO_2

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The solubilities of ethyl palmitate, ethyl oleate, ethyl eicosapentaenoate (EPA) and ethyl docosahexaenoate (DHA) in supercritical carbon dioxide were determined by a continuous flow method. The solubilities of fatty acid ethyl esters increased with pressure and decreased as the temperature was increased. An empirical equation, similar to Chrastil's equation, was used to describe the relationship between solute solubility and the density of carbon dioxide. The empirical equation was further used to qualitatively estimate the separation efficiency of isolating EPA and DHA ethyl esters from fatty acid esters. The operating conditions yielding high solubility gave fast extraction rate but resulted in low separation efficiency. Experiments were conducted to separate ethyl EPA and ethyl DHA from a model mixture containing four fatty acid ethyl esters and from esterified squid visceral oil. The experimental data compared closely with the calculated values.

KEY WORDS: Fatty acid ethyl esters, separation efficiency, supercritical carbon dioxide.

Supercritical fluid extraction (SFE) has several advantages, such as high separation efficiency and low oxidation susceptibility, and has been used for isolating flavors, caffeine and lipids since 1970 (1). Carbon dioxide has several advantages, such as its non-toxicity, non-flammability, no chemical residue problem and low critical temperature (304.2°K), over other solvents used for SFE. Thus it is the most widely used solvent in SFE. Solubilities of pure materials in supercritical carbon dioxide ($SC\text{-}CO_2$ are the prerequisite data to evaluate the separation efficiency of a process).

There are many studies reporting solubilities of fatty acids and esters of fatty acids in $SC\text{-}CO_2$. Bamberger and co-workers (2) measured the solubilities of lauric acid, myristic acid, palmitic acid and triglycerides in $SC\text{-}CO_2$; Brunetti *et al.* (3) reported the solubilities of four fatty acids in $SC\text{-}CO_2$ at the pressure range of 2901–4531 psig (20–30 MPa) and the temperature range of 313.2–333.2°K. Chrastil (4) investigated the solubilities of certain fatty acids and triglycerides in $SC\text{-}CO_2$ within the pressure range of 1160–3626 psig (8–25MPa) and the temperature range of 313.2–353.2°K, and mathematically described the relationship between solute solubility and density of $SC\text{-}CO_2$. Several other investigators have also reported the results on extracting fatty acid esters from esterified fish oil (5,6; Suzuki, Y., M. Shimazu, K. Arai and S. Saito, private communication). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are considered as health products that can reduce atherosclerosis, diabetes, ulcerative colitis and arthritis (7–9). Data on the solubilities of EPA, DHA and/or their ethyl esters in $SC\text{-}CO_2$ are limited. Also, investigations on relating the solubility to the separation efficiency are limited.

The objective of this work was to investigate whether the solubility data can be used to select an appropriate process condition for separating ethyl eicosapentaenoate (ethyl EPA) and ethyl docosahexaenoate (ethyl DHA) from a mixture of fatty acid esters. First, an empirical equation was built based on the solubility data of pure fatty acid ethyl esters. Then the total solubility and separation efficiency were estimated from the equation. The experiments for extracting EPA and DHA ethyl esters from a model mixture and from esterified squid visceral oil were conducted to validate the theoretical concept.

MATERIALS AND METHODS

Four kinds of ethyl esters of fatty acids were used in this study. Ethyl palmitate and ethyl oleate (purity >99%) were purchased from NuChek Prep, Inc. (Elysian, MN). EPA (purity 90.9%) and DHA (purity 90.3%) were bought from Nippon Oil & Fats Co., Ltd. (Tokyo, Japan). Squid visceral oil was obtained from Fung-I Co. (Kaohsiung, Taiwan). All the materials were stored at −20°C until use. EPA, DHA, and squid visceral oil were esterified with absolute ethanol (10).

The flow diagram of the extraction system is shown in Figure 1. The system was purchased from Newport Scientific Inc. (Jessup, MD). The heart of the system is a 15,000 psi (103.4 MPa) double-ended, diaphragm-type compressor. The pressure was controlled by a back pressure regulator. Compressed CO_2 was pumped through high pressure tubing (stainless steel 304) into the extractor, which was wrapped with heating tape to maintain the desired temperature. The extraction vessel was 25.4 cm high with an inside diameter of 6.5 cm and had a volume of 845 mL. The extractor, packed with glass wool, had an effective volume of 500 mL. A temperature controller was used to control the temperature in the extractor. The flow rate of CO_2 was adjusted to 6 L (ambient condition) per min by a variable speed controller and the volumetric flow was recorded by a flow totalizer. The volume of CO_2 in liters at ambient condition is herein designated as NL. In a typical experiment, the pressure and temperature in the extractor were adjusted to the set points. The system was stabilized for one hour after the feed materials were put into the extractor. The extract samples were eluted continuously by CO_2 and collected in a cold trap. The collected samples were weighed. The solubilities of esters were calculated as the initial slope of the weight of collected samples *vs.* the eluted volume of CO_2. Three types of feed materials were used. They were 100 g pure esters; 20 g model mixtures containing 28.14% ethyl palmitate, 25.7% ethyl oleate, 24.8% ethyl EPA and 21.36% ethyl DHA; and thirdly, 100 g esterified squid visceral oil containing 2.01% ethyl myristate, 10.96% ethyl palmitate, 1.63% ethyl palmitoleate, 3.29% ethyl stearate, 15.74%

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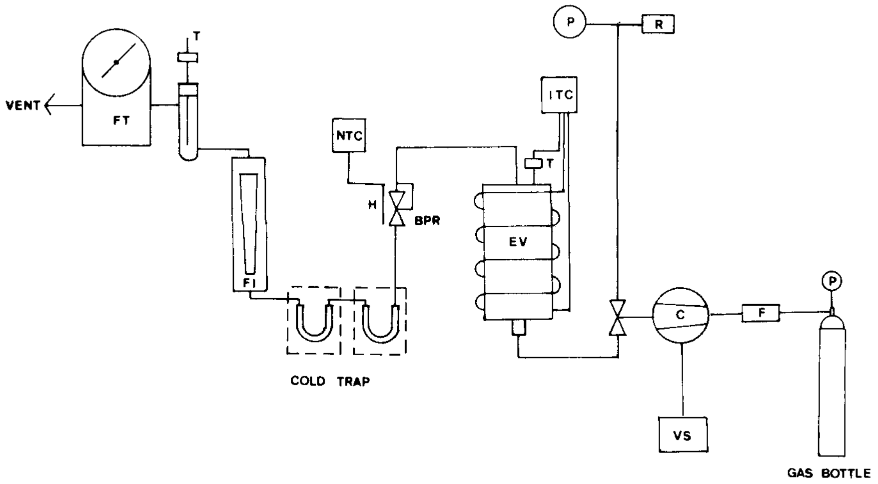

FIG. 1. Flow diagram of the extraction system. Abbreviations: BPR, back pressure regulator; C, diaphragm compressor; EV, extraction vessel; F, filter; FI, flow indicator; FT, flow totalizer; H, heater; ITC, indicating temperature controller; NTC, nonindicating temperature controller; P, pressure gauge; R, rupture disc; T, thermocouple; VS, variable speed controller.

ethyl oleate, 0.66% ethyl linoleate, 0.37% ethyl linoleneate, 15.84% ethyl eicosenoate, 4.16% ethyl EPA, 12.01% ethyl DHA, cholesterol (2,867 mg/100g) and trace amount of fatty acid esters. All the percentages were in area percentages determined by a gas chromatograph (GC).

The samples collected were analyzed by a gas chromatograph (Varian 3400, Varian Associates, Palo Alto, CA). The temperature of the FID detector was 250°C. A SP-2330 fused silica capillary column of 30 m × 0.25 mm i.d. was used. The column temperature was maintained at 160°C for four minutes and then raised to 200°C at a rate of 2°C/min. The injector temperature was 250°C. The flow rate of the carrier gas, hydrogen, was 1.5 mL/min. The split ratio was 100:1. The percentage composition of individual ethyl ester was determined *via* the area percentage method. Ten milligrams of samples were mixed with 1 mL n-hexane containing 1 mg of ethyl tricosanoate. One microliter of the mixture was injected into the GC.

RESULTS AND DISCUSSION

The solubilities of four esters, ethyl palmitate, ethyl oleate, ethyl EPA and ethyl DHA, in SC-CO_2 were determined over the temperature range of 298.2-328.2°K and pressure range of 1,000-2,500 psig (6.89-17.24 MPa). As shown in Figure 2, the solubilities of esters increased with pressure and decreased as the temperature increased. For example, the solubility of ethyl EPA was 15 g/L at 1,500 psig (10.34 MPa) and increased to 46.5 g/L at 2,500 psig (17.24 MPa) when the temperature was 313.2°K. However, when the temperature was raised to 328.2°K, the solubility decreased to 1.7 g/L at 1,500 psig (10.34 MPa). This phenomenon where the solute solubility decreased as the temperature increased is called retrograde behavior (11). In addition, it appeared that, in general, the solubility of fatty acid ethyl esters decreased with the increase of the carbon number in a molecule. Under the experimental conditions, ethyl palmitate with sixteen carbons had the highest solubility and ethyl DHA with twenty-two carbons had the lowest solubility among the compounds tested. This concurs with the results reported by Schultz and Randall (12) for solute solubilities in liquid CO_2.

A comparison of (a), (b), and (c) in Figure 2 shows that the change in fatty acid ester solubility with pressure at 298.2°K is different from the trends recorded at 313.2 and 328.2°K. This is because the data were collected at 298.2K below the critical temperature (304.2°K) of carbon dioxide. Consequently, only the results obtained under the supercritical conditions were fitted to the mathematical equation. An equation similar to Chrastil's (4) was found to fit the experimental data pretty well.

$$\mathrm{SOL} = \varrho^{k} \exp\,(a/T + b) \qquad [1]$$

where SOL is the solubility in g/L; k, a, and b are coefficients; T is the temperature in °K; and ϱ is the density of carbon dioxide. For Equation [1], the density of CO_2 was taken from the report of Angus *et al.* (13). Table 1 lists the correlated coefficients, k, a and b, in Equation [1] for four fatty acid esters.

From Equation [1], the solubility (SOL) of individual ester in SC-CO_2 can be estimated. Let the summation of the individual solubility be the total solubilities of esters and designated as $\mathrm{SOL_t}$:

$$\mathrm{SOL_t} = \mathrm{SOL(ethyl\ palmitate} \\ \mathrm{+ethyl\ oleate+ethyl\ EPA+ethyl\ DHA)} \qquad [2]$$

where SOL(esters) represents the summation of the solubilities of esters in the parenthesis. And the separation efficiency, S, is

$$\mathrm{S} = \mathrm{SOL(ethyl\ palmitate+ethyl\ oleate)/} \\ \mathrm{SOL(ethyl\ EPA+ethyl\ DHA)} \qquad [3]$$

Herein, the prefix CAL indicates that the value is calculated from Equation [1]. Figures 3 and 4 show the

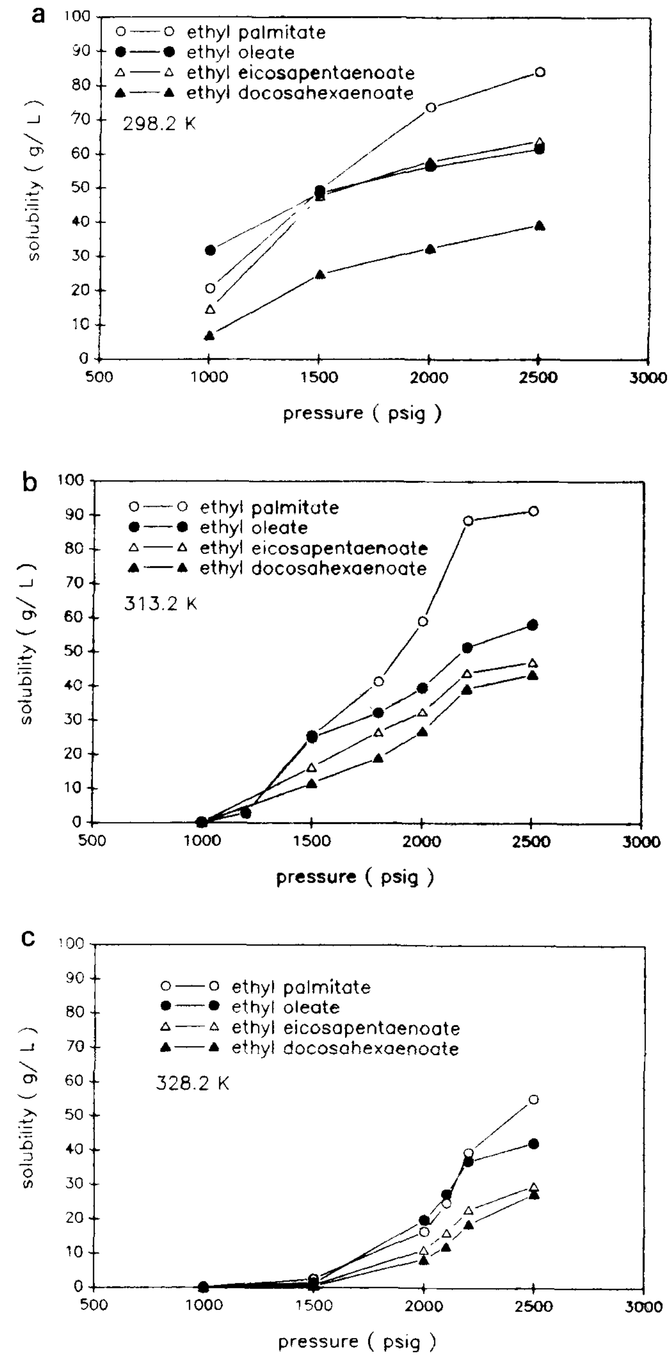

FIG. 2. The solubilities of four fatty acid esters in carbon dioxide at different conditions. (a) at 298.2°K, (b) at 313.2°K, and (c) at 328.2°K.

computed results for $CALSOL_t$ and CALS, respectively. The numerical values on the curves are the values of $CALSOL_t$ and CALS for Figures 3 and 4, respectively. In Figure 3, $CALSOL_t$ increased with the density of SC-CO_2. At constant density of SC-CO_2, temperature had only a slight effect on the solubility. $CALSOL_t$ increased with the temperature. However, as shown in Figure 4, CALS decreased as the density of SC-CO_2 increased, and increased with the temperature. This indicated that high solubilities were associated with low separation efficiency.

TABLE 1

Coefficients in Chrastil's Equation (ref. 4) for the Solubilities of Esters in Supercritical Carbon Dioxide at Temperature Range of 313.2–328.2 K and Pressure Range of 1500–2500 psig[a]

Compound	k	a	b
Ethyl palmitate	4.48	−533.4	−23.72
Ethyl oleate	2.94	−1109.0	−12.06
Ethyl eicosapentaenoate	3.51	−75.6	−19.37
Ethyl docosahexaenoate	4.60	−1073.3	−23.56

[a] SOL = $\varrho^k \exp(a/T + b)$. SOL, solubility (g/L); ϱ, density of carbon dioxide (g/L); and T, temperature (K).

True activity coefficients are needed to accurately calculate the separation efficiency. However, the selection of process conditions could probably be made on the basis of solubility data exhibited by the pure solute. To check the feasibility of this concept, Table 2 lists the eight operating conditions selected. As discussed previously, the operating condition with high $CALSOL_t$ was associated with low value of CALS. Run 1 [2000 psig (13.79 MPa) 313.2°K] had the highest value of $CALSOL_t$ and lowest value of CALS. Run 8 [1500 psig (10.34 MPa) 328.2°K] had the highest value of CALS and lowest value of $CALSOL_t$. In other words, Run 1 would have the fastest extraction rate but the least separation efficiency. Run 8 would have the slowest extraction rate but the best separation efficiency. Obviously, extraction rate and separation efficiency govern process economics.

The experimental results obtained from extracting the model mixture agreed with the concept discussed above. In Figure 5, the extraction rate of Run 1 [2000 psig (13.79 MPa) 313.2°K] was the fastest and Run 8 [1500 psig (10.34 MPa) 328.2°K] was the slowest. In Run 1 [Fig. 5(b)], only 260 NL CO_2 was used to collect 17.5 g extract. While in Run 8 [Fig. 5(a)], 3600 NL CO_2 was needed to collect 6.8 g extract. The order of the extraction rate of eight runs followed the order of $CALSOL_t$ as listed in Table 2. The experimental S was compared closely to the calculated value. Although the experimental SOL_t deviated from the $CALSOL_t$, the calculated and experimental data show similar trends. This implies that the calculation can be used as a qualitative prediction.

Figure 6 shows the percentage composition change of four esters *vs.* extract percentage for Runs 1 [2000 psig (13.79 MPa) 313.2°K] and 8 [1500 psig (10.34 MPa) 328.2°K]. The total weights of the extracts were taken from Figure 5. In Figure 6(a) (Run 8), the percentage composition of ethyl palmitate was 53.5% initially and was the highest among four esters. When the extract percentage was greater than 40%, the percentage composition of ethyl palmitate dropped rapidly and eventually became nearly zero. As the ethyl palmitate dropped, the percentage of ethyl oleate increased. The percentage of ethyl docosahexaenoate was the last to increase when the total extract percentage was higher than 50%. Therefore, the high molecular weight compounds, ethyl EPA and ethyl DHA, can be left as residues in the extraction vessel.

However, the percentage composition change in the esters was not apparent under the conditions in Figure

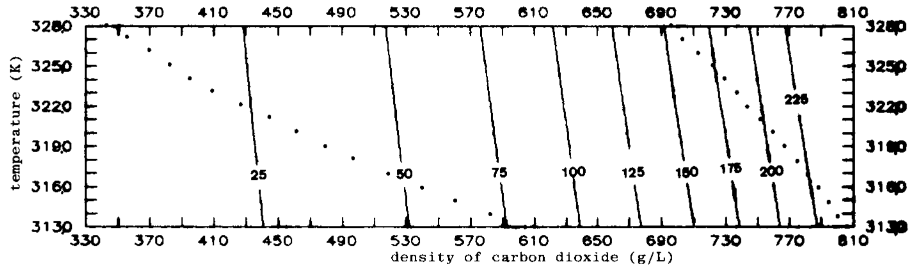

FIG. 3. The contour map for CALSOL$_t$ at various temperatures and densities of carbon dioxide.

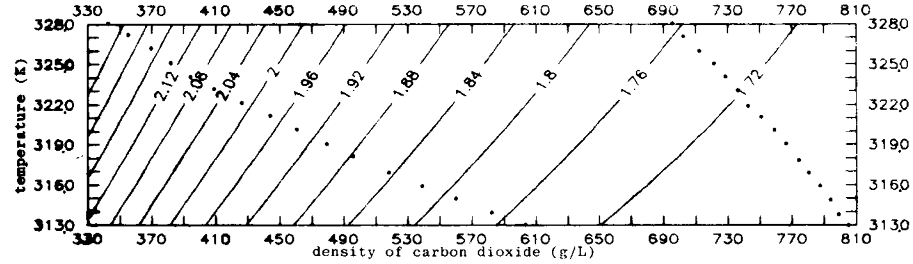

FIG. 4. The contour map for CALS at various temperatures and densities of carbon dioxide.

TABLE 2

The Calculated and Experimental Values of SOL$_t$ and S at Different Operating Conditions for Separating the Model Mixture

Run no.	Pressure (psig)	Temperature (°K)	Density of CO$_2$ (g/L)	CALSOL$_t$ (g/L)	CALS	Experimental data	
						SOL$_t$ (g/L)	S
1	2000	313.2	723.4	161.5	1.69	87.23	1.46
2	2000	318.2	655.8	114.4	1.74	45.54	1.60
3	1500	313.2	602.4	79.8	1.75	20.72	1.95
4	2000	323.2	588.5	78.3	1.82	29.41	1.85
5	2000	328.2	527.7	53.8	1.91	16.63	2.05
6	1500	318.2	494.4	39.4	1.88	6.90	2.81
7	1500	323.2	408.1	20.2	2.06	2.02	3.30
8	1500	328.2	342.3	11.2	2.29	0.59	4.36

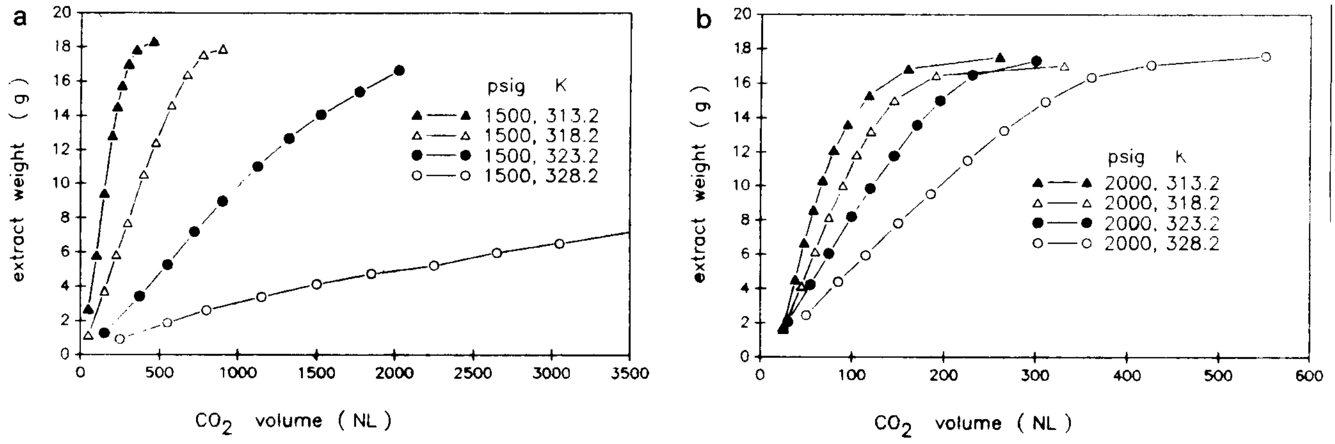

FIG. 5. Extract weight against volume of carbon dioxide used for separating the model mixture at eight specific conditions. (a) at 1,500 psig, and (b) at 2,000 psig. NL, the volume of CO$_2$ in liters at ambient condition.

6(b) [for Run 1 (2000 psig, 13.79 MPa, 313.2°K)]. The percentage of ethyl palmitate decreased gradually from 40% to 25%, while the relative percentages of the other three esters did not change during the extraction. Thus, the operating conditions producing high solubilities did not assure good separation of the individual esters.

A relative separation efficiency, R, is used to eliminate the effect of feed composition on separation efficiency.

$$R = \text{S of the extract/S of feed material} \qquad [4]$$

where S of feed material represents the calculated value from the feed composition. If the value of R is greater than unity, then the extract is rich in ethyl palmitate and ethyl oleate. Figure 7 shows the plots of R against extract percentage for eight individual runs. In general, the initial relative separation efficiency increased with temperature at constant pressure, and the initial R values at 1,500 psig (10.34 MPa) were found to be higher than those at 2,000 psig (13.79 MPa). This agrees with the order of the values of CALS listed in Table 2. The relative separation efficiency at 1,500 psig (10.34 MPa) and 328.2°K was 2.95 initially, 3.63 when the extract percentage was 35%, dropped rapidly to unity, and finally to zero when the extract percentage was higher than 70%. In Figure 7(b), the R of Run 1 [2000 psig (13.79 MPa) 313.2°K] was 1.43 initially, and attained a value of 0.97 at the end of extraction. Run 8 [1500 psig (10.34 MPa) 328.2°K] exhibited the best separation among the eight runs; however, it yielded the smallest solubility.

The above qualitative predictions were further validated by the separation of the esterified squid visceral oil. Table 3 lists the process conditions selected and the values of experimental and calculated SOL_t and S. The experimental SOL_t and S were calculated from the initial slope of the curves in Figure 8. Run 9 [1500 psig (10.34 MPa) 328.2°K] was expected to have the slowest extraction rate and the highest relative separation efficiency. The results are shown in Figure 8, where Run 14 [2162 psig (14.91 MPa) 313.2°K] had the fastest extraction rate and Run 9 had the slowest extraction rate. In Run 14, 1400 NL CO_2 were used to collect more than 93.3 g extracts; however, in Run 9, 2800 NL CO_2 were used to collect 13.3 g extracts. The order of the extraction rate followed that of the $CALSOL_t$ values listed in Table 3. Figure 9 shows the plots of R *vs.* extract percentage for various conditions. The value of R of Run 9 changed remarkably. However, the R of Run 14, which had the fastest extraction rate, was approximately unity. Although Run 14 yielded the highest extraction rate, there was little separation.

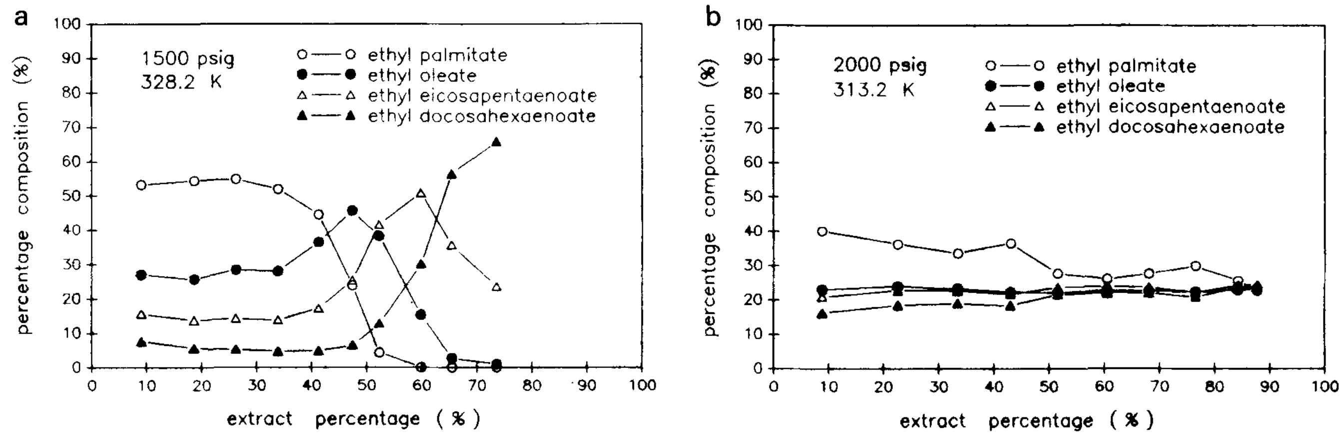

FIG. 6. The change in specific percentage composition of four esters in the extract during extraction. (a) at 1,500 psig and 328.2°K, (b) at 2,000 psig and 313.2°K.

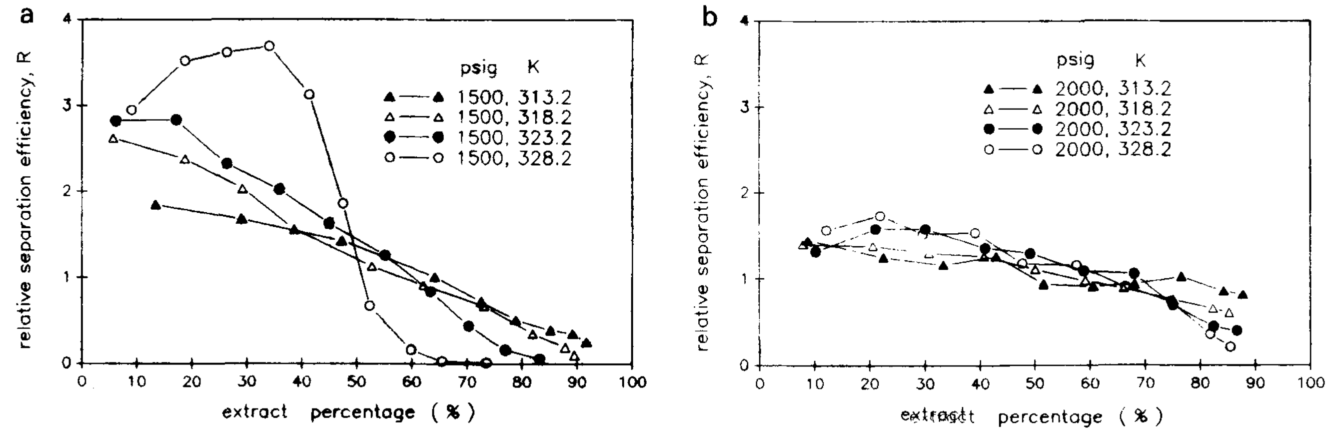

FIG. 7. The change in relative separation efficiency during extraction under the eight selected conditions. (a) at 1,500 psig, and (b) at 2,000 psig.

TABLE 3

The Calculated and Experimental Values of SOL_t and S at Different Operating Conditions for Separating Esterified Squid Visceral Oil

Run no.	Pressure (psig)	Temperature (°K)	Density of CO_2 (g/L)	$CALSOL_t$ (g/L)	CALS	Experimental data SOL_t (g/L)	Experimental data S
9	1500	328.2	342.3	11.2	2.29	0.64	6.63
10	1500	321.2	442.6	26.8	1.97	2.57	3.03
11	1500	316.2	537.6	53.0	1.82	7.74	2.56
12	1695	313.2	644.4	103.3	1.73	21.0	2.00
13	1973	313.2	715.8	155.1	1.69	29.2	1.81
14	2162	313.2	770.5	206.5	1.67	34.1	1.73

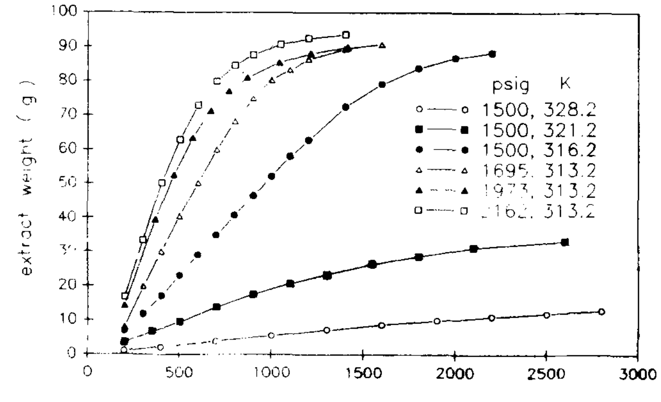

FIG. 8. The extract weight of esters obtained during the extraction of esterified squid visceral oil under the six conditions tested.

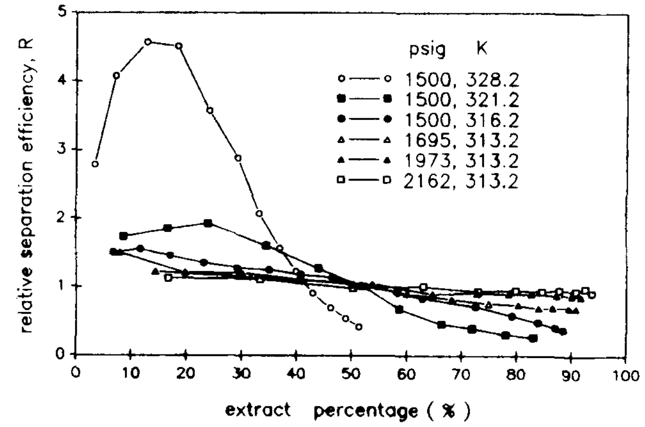

FIG. 9. The change in relative separation efficiency during the extraction of esterified squid visceral oil under different experimental conditions.

Thus the separation efficiency of Run 9 was the highest, as predicted for the experimental conditions utilized. Therefore, the qualitative prediction of process conditions was applicable not only to the model mixture but also to the esterified squid visceral oil. The choice of optimum conditions depends upon the product specification and process cost analysis.

The solubility data of fatty acid esters in SC-CO_2 can thus be correlated with an empirical equation similar to Chrastil's equation (4). These solubilities can be used to calculate total solubility (SOL_t) and separation efficiency (S), which can be used as indices of the extraction rate and separation efficiency, respectively. The operating conditions with low SOL_t yielded high separation efficiency while resulting in a low extraction rate. The process conditions can be selected according to the values of SOL_t and S.

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