Supercritical Carbon Dioxide Extraction of Evening Primrose Oil[1]

Fabio Favati[a], Jerry W. King[b,*] and Monica Mazzanti[b]

[a]Istituto di Industrie Agrarie dell'Universita, Via S. Michele, 4 - 56123 Pisa, Italy and [b]Food Physical Chemistry Research, National Center for Agricultural Utilization Research, USDA, Agricultural Research Service, Peoria, IL 61604

The oil extracted from the seeds of *Oenothera biennis* L. (evening primrose) is a major commercial source of gamma-linolenic acid, a fatty acid having potential therapeutic value in the treatment of several diseases. This fatty acid is prone to oxidation and thermal rearrangement; therefore, the conventional recovery of the oil via mechanical expression and hexane extraction must be carried out under very mild and controlled conditions.

In this study, supercritical fluid extraction with carbon dioxide has been employed as an alternative method to recover evening primrose oil (EPO). Extractions were performed over the pressure range of 20–70 MPa and at temperatures from 40 to 60°C, with a CO_2 mass flow rate of 18 g/min. The experimental data permitted the determination of EPO solubility in supercritical CO_2 at the tested extraction conditions. Supercritical fluid chromatographic analysis of fractions collected during the extraction showed a subtle shift in the triglyceride composition. Fatty acid methyl ester analysis on similar fractions indicated that the fatty acid content was invariant with respect to extraction time.

KEY WORDS: Carbon dioxide, evening primrose, extraction, gamma-linolenic acid, *Oenothera biennis* L., seed oil, supercritical fluid.

Gamma-linolenic acid (*cis*-6,9,12-octadecatrienoic acid) (GLA) is an important intermediate in the human metabolic pathway that converts dietary linoleic acid (LA) into prostaglandins. The bioconversion of LA to GLA is catalyzed by the enzyme delta-6-desaturase; but several factors, including stress, diabetes, consumption of alcohol and ageing, can reduce or inhibit the activity of this enzyme, leading to a variety of ailments (1–3). Dietary supplementation of GLA with naturally derived oils has been reported to be of value in treating several pathological conditions (4–9).

Natural sources of GLA are found in some plants, predominantly belonging to the Aceraceae, Boraginaceae, Oenagraceae, Saxifragaceae and Scrophulariaceae families, as well as some fungi and yeasts (3,10–15). At present one of the most important commercial sources of GLA is the oil extracted from the seeds of the evening primrose (*Oenothera biennis* L).

Evening primrose oil (EPO) has a high retail market price, approximately $0.48 (U.S.) per gram of oil, and the technology presently used to extract EPO is similar to that applied in processing conventional seed oil crops. While cold pressing allows only a partial recovery of the oil from the seeds, much higher yields are obtained by solvent extraction with hexane. However, since the therapeutic value of the oil is based on its content of GLA, considerable care must be taken to avoid or minimize any possible physicochemical change of GLA during extraction and refining. For this reason, the seeds are usually cold extracted by percolation with hexane and the solvent is then removed under reduced pressure at low temperatures. Subsequent refining of extracted oil is also conducted under low temperature conditions (3,12).

The solvent properties of supercritical carbon dioxide ($SC\text{-}CO_2$) are well documented (16–20). Applications of supercritical fluid extraction (SFE) include the recovery of natural pigments, processing of hops, decaffeination of coffee and extraction of lipids or cholesterol (21–27).

SFE utilizing CO_2 is a potentially milder alternative processing method for the recovery of EPO. Because of the low critical temperature of CO_2 (31°C), the evening primrose seeds can be extracted at a relatively low temperature. Furthermore, by using CO_2, no harmful solvent residue is left in the extracted oil. To date there have been several citations in the literature concerning the extraction of EPO by $SC\text{-}CO_2$ (20,28–30), although basic processing data are limited. The purpose of this study was to ascertain the influence of processing parameters (pressure and temperature) on the extraction yields and quality of derived EPO, as well as to acquire basic solubility data for optimizing the SFE process.

EXPERIMENTAL PROCEDURES

Two different batches of evening primrose seeds were utilized in this study. The first was supplied by the Istituto di Agronomia, University of Pisa, Italy and was composed of seeds harvested in Tuscany in 1988. The oil content was 21.9% by weight (b.w.) on a dry basis, and GLA represented 7.9% b.w. of the total fatty acids. The second batch was kindly provided by Efamol Ltd. (Guilford, England) and the seeds, harvested in England in 1988, were of a selected variety having a higher total oil content (26.1% b.w.) and GLA content (10% b.w.).

The extractions were performed with the apparatus shown in Figure 1, which has been described previously (24). Preliminary tests were conducted on approximately 25 g of seed, with subsequent tests utilizing samples of approximately 50 g. The seeds were ground to pass a 0.355 mm sieve before being packed into a tubular extraction vessel (1.75 cm i.d. × 30 cm). The experiments were run at pressures of 20, 30, 50 and 70 MPa, and temperatures of 40, 50 and 60°C. A standard amount of CO_2 (2.5 kg) was used in each test at an average CO_2 mass flow rate of 18 g/min. The extracted oil was collected at discrete intervals, after 125, 250, 375, 500, 750, 1,000, 1,500 and 2,500 g of CO_2 were passed through the extractor.

Small amounts of water were coextracted with the lipid fraction; therefore, the extracts were diluted in diethyl ether and dried over anhydrous sodium sulfate. The solvent was then removed at room temperature under reduced pressure and the extraction yields were determined gravimetrically. The collected fractions were stored under nitrogen at low temperature until they were analyzed. Extraction of EPO by percolation with n-hexane (EM

[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

*To whom correspondence should be addressed.

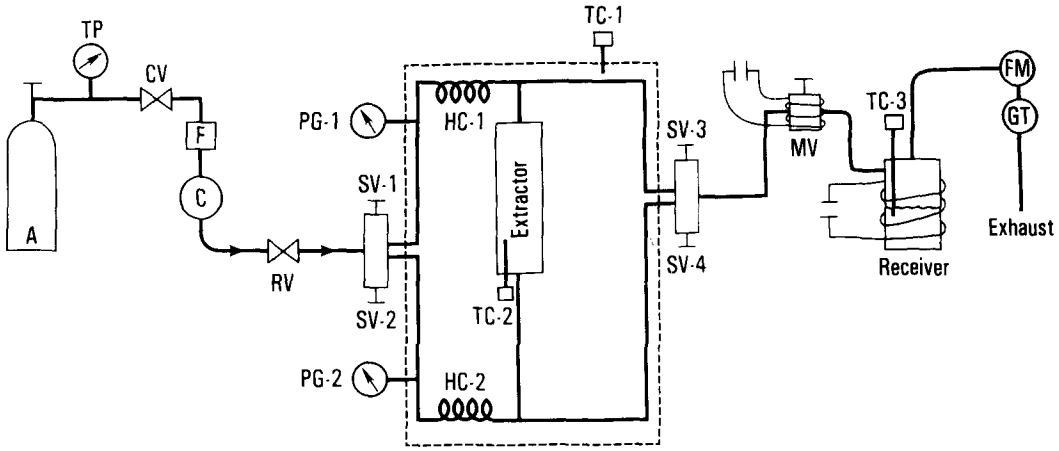

FIG. 1. Supercritical fluid extraction system. Dashed lines indicate thermostatted region. (A = CO_2 cylinder; TP = tank pressure gauge; CV = check valve; F = particulate filter; C = air-driven gas booster compressor; RV = relief valve; SV-1,2,3,4 = valves; PG-1,2 = pressure gauges; HC-1,2 = thermal equilibrating coils; TC-1,2,3 = thermocouples; MV = micrometering valve; FM = flow meter; GT = gas totalizer).

Science, Gibbstown, N.J.) was also performed on the seeds in order to compare the two processes.

Moisture and oil content of the seeds were determined according to standard methods (31). The fatty acid composition of each collected fraction was determined by gas-liquid chromatography analysis of the fatty acid methyl esters (FAME). FAME were obtained by transmethylation of the oil with sodium methoxide in methanol and injected onto a SP-2330 fused silica capillary column (30 m × 0.32 mm i.d., 0.2 μm film thickness) (Supelco Inc., Bellefonte, PA) at a split ratio of 100:1. The analyses were performed with the injector and FID detector temperatures set at 230°C; the chromatograph oven temperature was held at 150°C for 3 min and then programmed at a rate of 4°C/min to 200°C, where it was held constant for an additional 15 min. The chromatographic peaks were tentatively identified by comparing their retention times to those of injected standards and by spiking the extracts with known standards. The relative amount of each fatty acid was then calculated by internal normalization.

Supercritical fluid chromatography (SFC) was also employed to ascertain the triglyceride composition of the collected fractions. The samples were dissolved in hexane and the triglycerides were separated on a SB-octyl-50 capillary column (15 m × 50 μm i.d., 0.25 μm film thickness) (Dionex, Lee Scientific Div., Salt Lake City, UT) with a Lee Scientific 501 supercritical fluid chromatograph controlled by an IBM PC-AT computer. The separation of the triglyceride moieties was achieved with SFC-grade CO_2 (Scott Specialty Gases, Plumsteadville, PA) as the mobile phase, utilizing density-based programming of the eluent. In this case, the carrier (CO_2) density was held at 0.28 g/cm^3 for 15 min, then linearly increased at the rate of 0.006 g/cm^3/min to 0.5 g/cm^3, where it was held constant for an additional 5 min. The temperature of the column was held at 200°C during the analysis, while the FID was maintained at 350°C. The relative amount of each triglyceride was calculated by internal normalization.

RESULTS AND DISCUSSION

Tests were performed initially to ascertain the effect of extraction conditions on the composition of the oil. These experiments were conducted with the seeds harvested in Italy as the substrate.

Over the range of pressures and temperatures investigated, the FAME analysis did not reveal any major variation in the fatty acid profile of the fractions collected at discrete intervals. The amount of GLA detected in the extracted fractions varied from 6.5 to 8.4% b.w. of the total fatty acid, and in over 93% of the analyzed samples the GLA content ranged between 7.3 and 8.4% by weight. These values are very close to the percentage of GLA (7.9% b.w.) determined in the oil extracted with n-hexane.

In Table 1 the triglyceride composition of the oil extracted by n-hexane was tabulated in comparison with that of specific fractions collected during SFE tests conducted at different pressures and temperatures. The analytical data show that the composition of oil recovered at 50°C and 70 MPa was virtually identical to that of the

TABLE 1

Normalized Triglyceride Composition of Evening Primrose Extracts as Determined by Capillary Supercritical Fluid Chromatography

Extraction type	% Triglycerides			
	C_{50}	C_{52}	C_{54}	C_{56}
Soxhlet (hexane)	1.23	16.95	74.89	6.93
SC-CO_2 at 70 MPa and 50°C (first fraction)	1.27	16.92	75.01	6.80
SC-CO_2 at 20 MPa and 40°C (first fraction)	1.55	18.83	74.80	4.82
SC-CO_2 at 20 MPa and 40°C (last fraction)	0.67	13.04	75.20	11.09

oil extracted with the organic solvent. A similar triglyceride composition for EPO has been reported in the literature (29).

A slight fractionation of the oil seems to occur when using SC-CO_2 at the lower pressures and temperatures. However, there was no significant enrichment of GLA in the fractions having a higher percentage of C_{50} triglyceride. This is because in EPO, the level of myristic acid is small (0.1% b.w.), and over 96% of GLA occurs in combination with at least one other C_{18} fatty acid (32). Furthermore, GLA has been found to be mainly concentrated in the 3-position of the triacylglycerol structure, while the

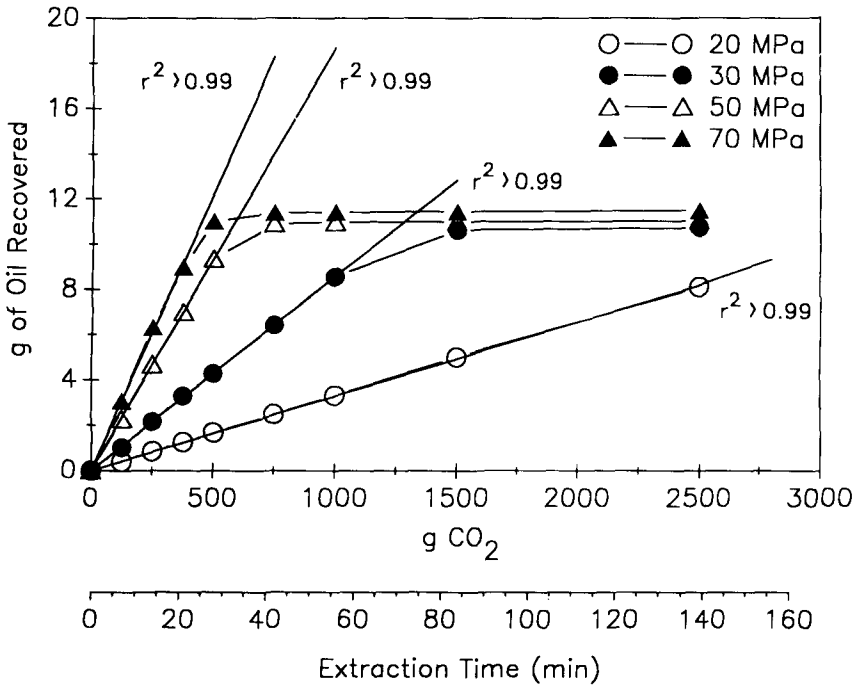

FIG. 2. Effect of pressure on the extraction of evening primrose oil at 40°C as function of the amount of CO_2 passed through the extractor and the elapsed extraction time.

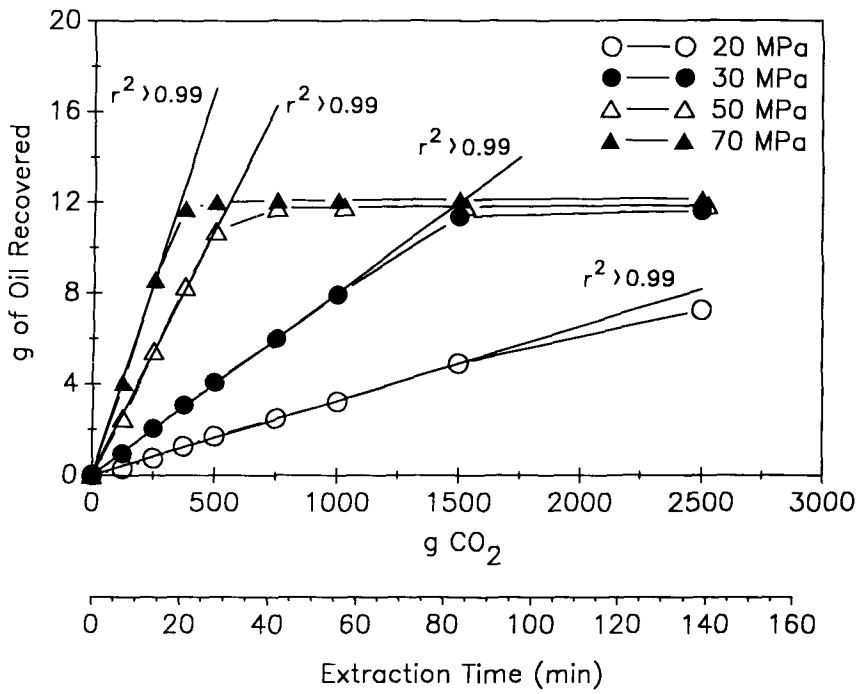

FIG. 3. Effect of pressure on the extraction of evening primrose oil at 50°C as function of the amount of CO_2 passed through the extractor and the elapsed extraction time.

palmitic acid moiety is nearly excluded from the 2-position, being mostly concentrated in the 1-position (14).

Experiments were also conducted to study the influence of the extraction conditions on the oil solubility in SC-CO_2 and the extraction yield. For these studies, a second set of extractions was carried out with the seeds of higher oil content. In Figures 2-4 the amount of EPO recovered at different extraction pressures and temperatures is plotted *vs* the weight of CO_2 passed through the seeds and the elapsed extraction time. Over the temperature range of 40-60°C, only a limited amount of oil could be removed at the lowest extraction pressure (20 MPa). After passage of 2.5 kg of CO_2, the calculated extraction yields at 40, 50 and 60°C were 65.6, 59.1 and 34.2%, respectively, of the theoretically available oil. Recoveries above 90% were obtained when the gas pressure was increased to 30 MPa, and even higher yields were recorded when operating at 50 and 70 MPa (Table 2).

The shape of the resultant curves indicates that the extraction process can be divided into three phases—an equilibrium-controlled phase, a transitional phase and a diffusion-controlled phase (20). For the equilibrium-controlled phase, the concentration of the oil in the SC-CO_2 remained nearly constant, as represented by the linear portion of the curves. This indicates that the amount of oil recovered is ultimately limited by the equilibrium solubility of the solute (oil) in the extraction gas.

Linear regression was performed on the experimental data corresponding to the equilibrium-controlled phase of each extraction curve. As noted in Figures 2-4, the calculated correlation coefficients were all better than 0.99, indicating an excellent linear correlation between the amount of oil recovered and the amount of CO_2 used in every extraction. Therefore, the slope of the plotted straight lines was assumed to represent the EPO solubility in SC-CO_2 at the given extraction conditions (Table 2).

Data in the literature show that when SFE of EPO was carried out at 40°C and at compression levels of 30 and 50 MPa, the attained EPO concentrations in SC-CO_2 were 0.8% and 1.7% b.w., respectively (28). Analogously, Eggers and Sievers (30) reported a theoretical solubility of 0.9% b.w. for extractions conducted at 40°C and 30 MPa. These values are consistent with those listed in Table 2 under similar operating conditions.

The influence of the extraction conditions on the oil solubility is illustrated in Figure 5, where the EPO solubility in SC-CO_2 is plotted as a function of solvent density (33) for various extraction pressures and temperatures. At pressures above 30 MPa, increasing the extraction temperature resulted in a higher EPO solubility, while the opposite effect was observed at lower pressures. This inversion in the solubility behavior of triglycerides in SC-CO_2 as a function of temperature, has been previously reported (34-36). The above solubility trends can be explained by recognizing that an increase in extraction temperature affects both the solute and the density of the solvent; the vapor pressure of the solute is increased, while the density of the solvent is decreased. However, only at low pressure is the density of the gas notably affected by a change in the temperature.

An increase in the extraction pressure always generated a significant increase in triglyceride solubility in SC-CO_2. At 60°C, raising the extraction pressure from 20 to 50 MPa resulted in more than a ten-fold increase of oil concentration in the gas phase, thereby permitting a rapid extraction of lipid material from the seeds (Fig. 4). Operating the extraction system at an even higher pressure (70 MPa) permitted a more rapid removal of the oil

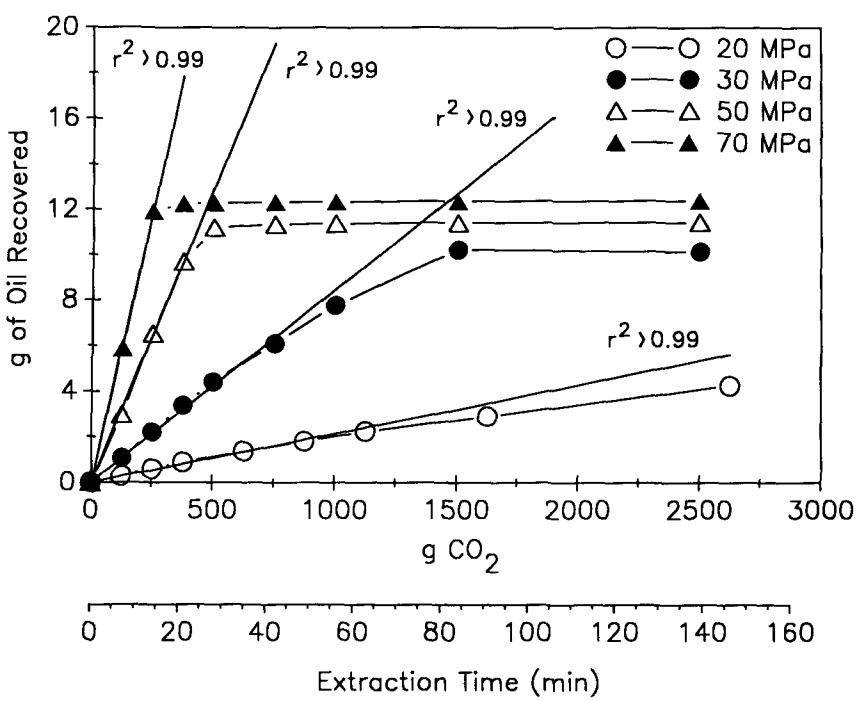

FIG. 4. Effect of pressure on the extraction of evening primrose oil at 60°C as function of the amount of CO_2 passed through the extractor and the elapsed extraction time.

TABLE 2

Evening Primrose Oil Solubility in SC-CO_2 and Percentage of Oil Recovered at Different Extraction Pressures and Temperatures After the Passage of 2,500 g of CO_2 Through the Extractor

Pressure (MPa)	Oil solubility (g/100 g CO_2)			Recovery (% available oil)		
	40°C	50°C	60°C	40°C	50°C	60°C
20	0.33	0.33	0.21	66.1	59.6	34.4[a]
30	0.86	0.80	0.85	95.1	95.3	91.3
50	1.87	2.16	2.58	96.8	97.5	97.2
70	2.44	3.40	4.76	96.0	99.2	97.7

[a]After the passage of 2,650 g of CO_2.

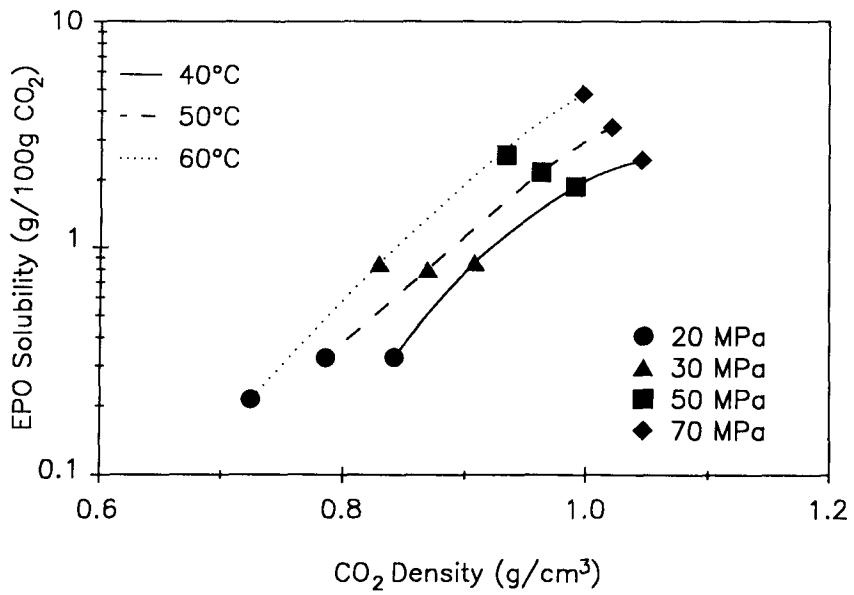

FIG. 5. Evening primrose oil solubility in SC-CO_2 as function of the solvent density at different extraction conditions.

from the seed matrix, yielding over 94% of the available oil in less than 14 min.

The color of the oil extracted via SFE was also influenced by the extraction conditions. EPO recovered by operating at 20 MPa had a light yellow color, while the samples extracted at higher pressures had a more intense hue, gradually approaching the deep yellow color of the hexane-extracted oil.

Although the moisture content of the seeds prior to SFE was less than 8% by weight, in the course of the process some water was coextracted along with the triglycerides. Despite the low solubility of water in SC-CO_2 (37), coextraction of water and lipids is not uncommon and has already been reported (28,38,39). However, when processing a large quantity of seed, separation and recovery of the oil fraction can be achieved by centrifugation of the extract.

In conclusion, the solubility data determined in this study should be of value in the design of a SFE process for EPO recovery. The experimental results indicate that EPO can be quickly and efficiently extracted via SFE at very mild conditions. Rapid extractions at high efficiencies were achieved by operating at 60°C and 70 MPa, but even extractions conducted at 40°C and 30 MPa yielded over 75% of the available oil in 55 min. Furthermore, analysis of the collected samples indicated that no appreciable compositional changes occurred in the extracted oil. However, additional studies need to be conducted on a larger extraction apparatus to determine any problems that may arise in scaling up this process.

ACKNOWLEDGMENTS

Author F. Favati is thankful to the National Research Council of Italy for financial assistance (Grant no. 203.06.42 and special project RAISA, paper no. 11).

REFERENCES

1. Traitler, H., H. Winter, U. Richli and Y. Ingenbleek, *Lipids 19*:923 (1984).
2. Helme, J.P., *Rev. Fr. Corps Gras 33*:107 (1986).
3. Uzzan, A., *Ibid. 35*:501 (1988).

4. Glen, E., L. MacDonnel, I. Glen and J. MacKenzie, in *Pharmacological Treatments for Alcoholism*, edited by G. Edwards and J. Littleton, Crooms-Helm, London, 1984, p. 331.
5. Puolakka, J., L. Makarainen, L. Viinikka and O. Ylikorkala, *J. Reprod. Med. 30*:149 (1985).
6. Belch, J.J.F., S. Ansell, R. Madhok, A. O'Dowd and R.D. Sturrock, *Ann. Rheumatic Dis. 47*:96 (1988).
7. Biagi, P.L., A. Bordoni, A. Masi, G. Ricci, C. Fanelli, A. Patrizi and E. Ceccolini, *Drugs Exptl. Clin. Res. 14*:285 (1988).
8. Carter, J.P., *Food Technol. 42*:72 (1988).
9. Monnier, L., S. El Boustani, A. Crastes de Paulet, B. Descomps and F. Mendy, *Rev. Fr. Corps Gras 36*:3 (1989).
10. Bohannon, M.B., and R. Kleiman, *Lipids 11*:157 (1976).
11. Wolf, R.B., R. Kleiman and R.E. England, *J. Am. Oil Chem. Soc. 60*:1858 (1983).
12. Hudson, B.J.F., *Ibid. 61*:540 (1984).
13. Bottazzi, F., R. Izzo and G. Lotti, *Agrochimica 29*:331 (1985).
14. Lawson, L.D., and B.G. Hughes, *Lipids 23*:313 (1988).
15. Sakaki, K., Y. Yokoshi, O. Suzuki and T. Hakuta, *J. Am. Oil Chem. Soc. 67*:553 (1990).
16. Paul, P.F.M., and W.S. Wise, *The Principles of Gas Extraction*, Mills & Boon, London, England, 1971.
17. Bott, T.R., *Chem. Ind. 15*:288 (1980).
18. McHugh, M.A., and V.J. Krukonis, *Supercritical Fluid Extraction*, Butterworths, Stoneham, MA, 1986.
19. Rizvi, S.S.H., J.A. Daniels, A.L. Benado and J.A. Zollweg, *Food Technol. 40*:57 (1986).
20. Stahl, E., K.W. Quirin and D. Gerard, *Dense Gases for Extraction and Refining*, Springer-Verlag, New York, NY, 1988.
21. Zosel, K., U.S. Patent 4,247,570, (1981).
22. Vollbrecht, R., *Chem. Ind. 12*:397 (1982).
23. Friedrich, J.P., U.S. Patent 4,466,923, (1984).
24. Favati, F., J.W. King, J.P. Friedrich and K. Eskins, *J. Food Sci. 53*:1532 (1988).
25. Temelli, F., C.S. Chen and R.J. Braddock, *Food Technol. 42*:145 (1988).
26. Bradley, R.L., *J. Dairy Sci. 72*:2834 (1989).
27. Di Giovacchino, L., M. Solinas, V. Brandani, G. Del Re and G. Di Giacomo, *Industrie Alimentari 28*:925 (1989).
28. Quirin, K.W., D. Gerard and J. Kraus, *Fat Sci. Technol. 89*:139 (1987).
29. Tolboe, O., I.R. Hansen and V.K.S. Shukla, in *Proceedings of the First International Symposium on Supercritical Fluids*, October 17-19, 1988, Nice, France.
30. Eggers, R., and U. Sievers, in *Supercritical Fluid Science and Technology*, ACS Symposium Series 406, edited by K.P. Johnston and J.M.L. Penninger, ACS, Washington, D.C., 1989, p. 478.
31. *Official and Tentative Methods of the American Oil Chemists' Society*, 3rd edn., American Oil Chemists' Society, Champaign, IL, 1964 (revised to 1976).
32. Ratnayake, W.M.N., D.G. Matthews and R.G. Ackman, *J. Am. Oil Chem. Soc. 66*:966 (1989).
33. Kennedy, G.C., *Am. J. Sci. 252*:225 (1954).
34. Friedrich, J.P., and E.H. Pryde, *J. Am. Oil Chem. Soc. 61*:223 (1984).
35. Quirin, K.W., *Fette Seifen Anstrichmittel 84*:460 (1982).
36. Stahl, E., K.W. Quirin and D. Gerard, *Ibid. 85*:458 (1983).
37. Evelein, K.A., R.G. Moore and R. Heidemann, *Ind. Eng. Chem. Process Des. Dev. 15*:423 (1976).
38. Dakovic, S., J. Turkulov and E. Dimic, *Fat. Sci. Technol. 91*:116 (1989).
39. Eggers, R., and U. Sievers, *J. Chem. Eng. of Japan 22*:641 (1989).

[Received November 15, 1990; accepted March 29, 1991]