Supercritical CO_2 Extraction of Flaxseed

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ABSTRACT: Extraction of flaxseed oil was performed with supercritical carbon dioxide (SC-CO_2). To investigate the effects of pressure and temperature on the solubility of oil and oil yield, three isobaric (21, 35, and 55 MPa) and two isothermal (50 and 70°C) extraction conditions were selected. Although the maximal solubility of flaxseed oil, 11.3 mg oil/g CO_2, was obtained at 70°C/55 MPa, the oil yield obtained after 3 h of extraction at this condition was only 25% (g oil/g seed × 100), which represented 66% of the total available oil of the flaxseed. Lipid composition and FFA and tocol (tocopherol and tocotrienol) contents of the oils obtained by both SC-CO_2 and petroleum ether extraction were determined. The α-linolenic acid content of the SC-CO_2-extracted oil was higher than that obtained by solvent extraction.

Paper no. J10081 in *JAOCS 79,* 231–235 (March 2002).

KEY WORDS: Extraction, flax oil, α-linolenic acid, supercritical CO_2.

The consumption of vegetable oils rich in n-3 PUFA has increased over the last decade due to the undesirability of saturated fats associated with a higher risk of heart disease and cancer (1). n-3 PUFA, which are believed to be the beneficial agents in oils, are now added to infant formula and various food products in some countries. They are also available in many countries as nutraceutical supplements (2).

Among the n-3 FA, α-linolenic acid (ALA, 18:3) has a broad spectrum of potential health benefits associated with its consumption. ALA has been shown to reduce blood pressure in hypertensives and to lower serum TG and cholesterol (3). The predominant sources of ALA in the diet are vegetable and seed oils and fish. Flax oil is the richest source of ALA, which makes up 55–60% of total FA. Flax oil has therefore received increasing attention as a specialty oil (4). Similar to other commercial vegetable oils, flax oil is produced by either cold pressing or solvent extraction. Cold pressing results in only partial recovery of the oil from seeds; therefore, pressing of the seeds is often followed by solvent (usually hexane) extraction at relatively higher temperatures to achieve higher oil recovery. However, n-3 FA may also be subject to thermal degradation under solvent extraction conditions.

The supercritical fluid extraction technique has been studied extensively as an alternative to conventional methods of oil extraction (5). Supercritical fluids have gas-like diffusivities but liquid-like densities. These properties vary as a function of pressure and temperature. Supercritical carbon dioxide (SC-CO_2) has been the most frequently used supercritical fluid for oil extraction, since it is nontoxic, nonflammable, inexpensive, and easily separated from the extract. Furthermore, the low critical temperature of CO_2 (31°C) allows extraction of thermolabile compounds without degradation (6).

SC-CO_2 extraction of various oil seeds, such as soybean, safflower, and cottonseed, has been reported (7,8). Other researchers have studied the solubilities of various vegetable oils including canola, millet bran, and rice bran in SC-CO_2 (9–11). Extraction of an oil rich in n-3 FA, such as fish oil, was also investigated (12,13), and further fractionation and concentration of n-3 FA from fish oil has also attracted attention. More recent studies involving SC-CO_2 extraction of lipids have focused on specialty oils such as extraction of oil from tomato seeds (14), grape seeds (15), and evening primrose seeds (16). Even though there are numerous reports on SC-CO_2 extraction of oil from various seeds, the literature is lacking on flaxseed extraction.

Therefore, the objectives of this study were to investigate SC-CO_2 extraction of flaxseed using a laboratory-scale extraction unit and to study the influence of pressure, temperature, and flow rate on oil solubility and recovery. The quality of the SC-CO_2-extracted oil was also ascertained in terms of its FA composition and tocol and FFA contents and then compared to those factors in oil obtained by petroleum ether extraction.

MATERIALS AND METHODS

Materials. Flaxseeds (*Linum usitatissimum*) were purchased from a local market in Konya, Turkey, and kept below –20°C until used. The seeds were ground by using a coffee grinder (Philips, Model HD5112) for 10 s. Moisture content was determined according to AOCS Method 2-54 (17). Oil was extracted by Soxhlet extraction using petroleum ether (40–60°) for 5 h followed by solvent removal under vacuum at 40°C.

SC-CO_2 extraction. A laboratory-scale supercritical-fluid extraction system (Newport Scientific Inc., Jessup, MD) was used for this study. Carbon dioxide (99.95% purity, Praxair, Edmonton, AB) was compressed to the desired pressure by using a diaphram compressor with a maximum rating of 69 MPa. The extraction vessel (300 mL) was heated with a heating jacket, and temperature was controlled by a thermostat (±1°C). Pressure was controlled by a back-pressure regulator. A basket that fitted into the extraction vessel was fabricated to facilitate loading and unloading of the sample.

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Five grams of ground sample was loaded into a 25-mL basket and placed into the main extraction cell for each extraction. The extracts were collected in glass tubes attached to the depressurization valve, which were held in a circulating refrigerated bath at –20°C. SC-CO_2 extraction of oil was performed at temperatures of 50 and 70°C, pressures of 21, 35, and 55 MPa, and CO_2 flow rates of 1 and 3 L/min (measured at the ambient conditions) for 3 h. The extracted oil fractions were collected at time intervals of 30, 60, 90, 120, 150, and 180 min, and the amount of each fraction was determined gravimetrically. The collected fractions were combined and stored at –20°C for analysis.

Analysis of extracts. (i) FA composition. FA compositions of oil samples were determined using a modified FAME method (18). The oil extracts obtained after 3 h extraction time were transmethylated to FAME by heating (90°C for 45 min) with a mixture of 35% (vol/vol) BF_3/MeOH (12% BF_3/MeOH; Supelco, Oakville, Ontario, Canada), 45% (vol/vol) MeOH, and 29% (vol/vol) hexane. The FAME were then analyzed by GC (Varian 3600 GC; Mississauga, Ontario, Canada). The system was equipped with an autosampler (Model 8200; Varian) and an FID. The resultant data were processed by a computer using Class VP data processor (Shimadzu Corporation, Columbia, MD). Helium was used as the carrier gas. Tricosanoic acid was used as an internal standard at the concentration of 1 mg/mL. The FAME were separated on a fused-silica capillary column (50 m × 0.32 mm, BPx-70, SGE Column, Pty. Ltd., Victoria, Australia) with the film thickness of 0.25 μm. The detector temperature was set at 230°C. Initial injector temperature was held 70°C for 3 min, then increased at 150°C/min to 230°C and held for 17 min. Initial column temperature was 50°C for 0.1 min and increased to 170°C at the rate of 25°C/min, held at 170°C for 1 min, then increased to 180°C at the rate of 2°C/min, and then increased to 230°C at the rate of 10°C/min and held for 3 min.

(ii) FFA analysis. FFA content of the extraction products was determined by supercritical fluid chromatography (SFE/GC Series 600; Dionex, Mississauga, Ontario, Canada). The SFC system was equipped with a fused-silica column (10 m × 50 μm i.d.) with 0.25 μm film (SB-100 methyl-silicone) and a timed-split injector. The FID was maintained at 350°C. SC-CO_2 was the mobile phase. The column temperature and pressure programs applied were identical to those used by Rezaei and Temelli (19).

The samples were dissolved in petroleum ether/diethylether (1:1, vol/vol). Oleic acid was used as the standard to represent FFA and hexadecane was used as internal standard for quantification of FFA. The amount of FFA in the oil samples was calculated using the internal standard calibration curve ($r^2 = 0.998$).

(iii) Tocol analysis. Tocols (tocopherols and tocotrienols) were analyzed by HPLC. The Varian 9010 HPLC system (Varian, Mississauga, Ontario, Canada) was equipped with HP 1050 series auto injector. The detector used was a Shimadzu-RF 535 fluorescence detector (Shimadzu Corporation) with wavelengths set at 330 nm for emission and 298 nm for extinction. Tocols were separated on a normal-phase column (Supelcosil-LC-Diol, 25 cm × 4.6 mm i.d., 5 μm particle size, Supelco) with the mobile phase flow rate at 1 mL/min. The mobile phase was a mixture of *n*-hexane/isopropanol (99.4:0.6, vol/vol). The data were integrated and analyzed using Shimadzu Class-VP Chromatography Laboratory Automated Software system (Shimadzu Corporation). Standards of tocopherol α, β, γ, and δ isomers (Sigma Chemical Co., St. Louis, MO) and tocotrienol α, β, γ, δ isomers (Merck, Darmstadt, Germany) were dissolved in hexane and used for identification and quantification of peaks. The amount of tocols in the extract samples was calculated as mg tocol per 100 g oil sample using external calibration curves, which were obtained for each tocol isomer standard.

Statistical analysis. SC-CO_2 extraction of flaxseed at each temperature and pressure condition was carried out in duplicate. FA composition and FFA and tocol analysis of each extract were performed in duplicate and means were reported. ANOVA of the results was performed using General Linear Model procedure of SAS Statistical Software, Version 6 (20). Multiple comparison of the means was performed by a LSD test at the $\alpha = 0.05$ level (20).

RESULTS AND DISCUSSION

The particle size distribution (%, w/w) of the ground seeds was as follow: 38%, >1 mm; 11.1%, 0.85–1 mm; 21.7%, 0.5–0.85 mm; and 29.2%, 0.25–0.5 mm. The seeds contained 6.2% (w/w) moisture. Oil content was 38% (w/w) as determined by Soxhlet extraction using petroleum ether (40–60°) for 5 h.

Solubility of flaxseed oil in SC-CO_2. Figure 1 presents extraction curves for oil removal by SC-CO_2 at 70°C/55 MPa at the two CO_2 flow rates studied. At low flow rates, the slope of the initial linear portion corresponded to the solubility of the oil in SC-CO_2. Then there was a transition region in which the rate of oil removal diminished, followed by an asymptotic approach as the final amount of the oil was removed from the seed. The solubility of flaxseed oil was determined from the initial linear portion for each condition at a CO_2 flow rate of 1 L/min. The solubility of oil from flaxseed under different

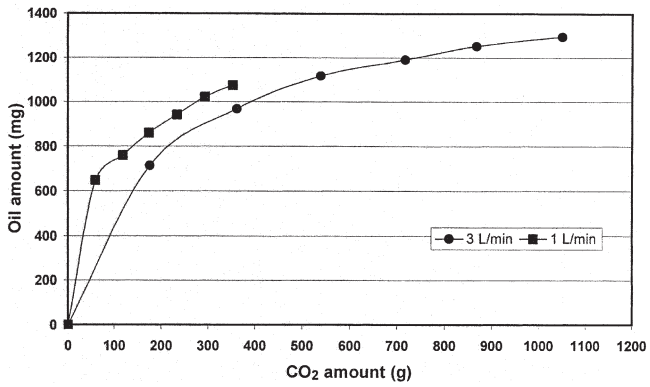

FIG. 1. Amount of flax oil extracted as a function of the amount of CO_2 used at different flow rates at 70°C and 55 MPa.

extraction conditions is shown in Figure 2. The results show that the solubility varied from 2.33 mg oil/g CO_2 at 70°C/21 MPa to 11.34 mg oil/g CO_2 at 70°C/55 MPa.

As expected, the solubility of flax oil in SC-CO_2 increased with pressure as a result of the increase in the density of CO_2. Solubility increased with temperature at higher pressures but slightly decreased at the pressure of 21 MPa as a result of the well-established crossover of the solubility isotherms (7,8). However, the solubility increase with temperature observed at 35 and 55 MPa was not statistically significant ($P > 0.05$). The fluctuation in the CO_2 flow rate (±0.1 L/min) due to the manual control of the depressurization valve contributed to the variability in the results. In addition, even though the collection tubes were held in a cold bath (–20°C) to ensure maximal recovery of extract, there was always some loss of extract with exhaust CO_2, depending on the CO_2 flow rate.

Although similar solubility behavior has been reported for other vegetable and seed oils, flax oil solubility was lower than those reported for corn, soybean, and canola oils. Corn and soybean oil solubilities were reported as 12 mg/g CO_2 at 50°C/35 MPa (21,22). Canola oil solubility from canola flakes was 11 mg/g CO_2 (23) at 36 MPa/70°C. However, in this study, flax oil solubility was 7.3 and 8.2 mg/g CO_2 at 35 MPa and temperatures of 50 and 70°C, respectively. The lower solubility of flax oil in SC-CO_2 obtained in this study may be due to the lipid composition of the flax oil, which is rich in FA with higher degrees of unsaturation. It is well established that the solubilities of FA and esters decrease with increased carbon number and molecular weight; however, the solubilities of those with higher degrees of unsaturation are reported to be both higher and lower than those with less unsaturation by different researchers (24–26). Therefore, further research is needed to better understand the relationship between the degree of unsaturation and the solubility of lipids in SC-CO_2.

The oil yield (g oil extracted/100 g dry seed) obtained from flaxseed with SC-CO_2 after 3 h at a flow rate of 1 L/min ranged from 21 to 25%, whereas the petroleum ether extraction gave a yield of 38%. In other words, SC-CO_2 extraction recovery after 3 h was only between 55 and 66% of that obtained by the conventional extraction method. There was no significant difference ($P > 0.05$) between the oil recovery obtained at 35 and 55 MPa at each temperature after 3 h. The lower solvent flow rate of 1 L/min required a longer extraction time to reach the extraction yield maximum. Maximal oil recovery increased at higher flow rate (3 L/min) from 66 to 74% of total oil. The oil yield can be enhanced further by using higher CO_2 flow rates, a higher extraction pressure, longer extraction times, and grinding the feed material to a smaller particle size. Another factor is that petroleum ether also extracts phospholipids, pigments, and unsaponifiable substances, giving a higher extraction yield. Under the conditions used in this study, the solubility of these components in SC-CO_2 is very low (27–29). The fact that the extent of refining required for SC-CO_2-extracted oils would be substantially lower than for those of crude oils obtained by organic solvents has previously been reported (30). Minimal postextraction handling is especially important for oils rich in PUFA, such as flax oil, so as to maintain its quality.

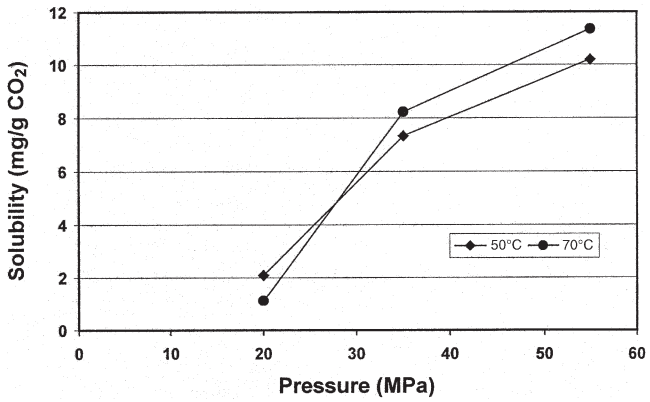

FIG. 2. Flax oil solubility in supercritical CO_2 as a function of temperature and pressure.

Lipid composition. Based on the FA compositions, the flaxseed oil obtained by SC-CO_2 under different conditions and Soxhlet extraction contained mainly palmitic acid (5.7–6.7%), stearic acid (4–4.7%), oleic acid (14.1–16%), linoleic acid (13.3–14.0%), and α-linolenic acid (56.5–61.0%). These results are similar to previously reported values (31).

Table 1 presents the FA composition of SC-CO_2- and petroleum ether-extracted oils. No significant differences were observed ($P > 0.05$) in the FA compositions among the oils obtained by SC-CO_2 under different temperature and pressure conditions. However, the FA compositions of SC-CO_2-extracted oils were different from those obtained by Soxhlet extraction using petroleum ether. The concentrations (GC area%) of saturated and monounsaturated FA obtained with petroleum ether were significantly ($P < 0.05$) higher than those obtained by SC-CO_2. The linoleic acid content of the oil obtained with petroleum ether was similar to that of SC-CO_2-extracted oils. Of the FA identified in flaxseed oil, α-linolenic acid is the dominant one, constituting >60% of the total FA, and its concentration in the SC-CO_2-extracted oils was significantly ($P < 0.05$) higher than that of solvent-extracted oil. While the α-linolenic acid contents of the SC-CO_2-extracted oils at all conditions were 60–61%, it was only 56% in the oil obtained by solvent extraction. This finding indicates minimal degradation and higher recovery of the α-linolenic acid with SC-CO_2.

Flax oil obtained with SC-CO_2 contained 904.9–914.8 mg/g oil of total FA including 91.9–93.3 mg/g oil of saturated FA, 132.8–134.0 mg/g oil of monounsaturated FA, and 679.7–689.8 mg/g oil of PUFA, whereas the total FA was only 826.9 mg/g oil in the petroleum ether-extracted oil (Table 1). These results show that the purity of the oil extracted with SC-CO_2 was higher than that of solvent-extracted oil, which is expected to contain a higher level of unsaponifiables.

FFA content is well known to affect oil quality. An increase in the FFA content of the oil should be avoided because FFA lead to heavy neutral oil losses. The removal of FFA from

TABLE 1
FA Composition (GC area% of total FA) and Content (mg/g oil) of Flaxseed Oils Obtained by SC-CO_2 at 1 L/min CO_2 Flow Rate and by Soxhlet Extraction[a,b]

	SC-CO_2 extraction				Soxhlet extraction
	50°C/35 MPa	50°C/55 MPa	70°C/35 MPa	70°C/55 MPa	
FA (%)					
C16:0	5.8 ± 0.1a	5.8 ± 0.1a	5.8 ± 0.1a	5.7 ± 0.1a	6.6 ± 0.3b
C18:0	4.0 ± 0.1a	4.2 ± 0.1a	4.0 ± 0.2a	4.2 ± 0.1a	4.7 ± 0.1b
C18:1	14.2 ± 0.1a	14.4 ± 0.1a	14.1 ± 0.3a	14.3 ± 0.2a	16.0 ± 0.5b
C18:2n-6	13.3 ± 0.0a	13.4 ± 0.1a	12.8 ± 0.8a	13.4 ± 0.1a	14.0 ± 0.4a
C18:3n-3	60.5 ± 0.1a	60.5 ± 0.2a	60.6 ± 1.1a	61.0 ± 0.4a	56.7 ± 1.4b
FA content (mg/g oil)					
C18:3n-3	565.4 ± 16.5a	561.5 ± 6.9a	556.2 ± 1.0a	559.3 ± 19.9a	477.1 ± 1.5b
SAT	92.3 ± 1.4a	93.3 ± 0.3a	93.3 ± 0.4a	91.9 ± 2.7a	90.9 ± 6.8a
MUFA	132.8 ± 1.7a	134.0 ± 1.7a	132.9 ± 0.6a	132.9 ± 2.8a	128.5 ± 8.7a
PUFA	689.8 ± 3.6a	686.0 ± 8.3a	679.7 ± 0.70a	682.9 ± 2.6a	590.5 ± 12.1b
Total FA	914.8 ± 6.4a	913.3 ± 10.4a	904.9 ± 0.5a	907.7 ± 2.6a	826.9 ± 23.3b

[a]Results are presented as means ± SD (n = 4). Values in a row followed by different letters (a,b) are significantly different ($P < 0.05$).
[b]SAT, saturated FA; MUFA, monounsaturated FA, SC-CO_2, supercritical CO_2.

TABLE 2
FFA and Tocol Contents of the Oils Obtained by SC-CO_2 and Soxhlet Extraction

	FFA	Tocol[a] (mg/100 g oil)				Total
Extract	(%, w/w)	α-T	α-T3	β-T + γ-T	δ-T	tocols
SC-CO_2, 70°C 55 MPa, 1 L/min	1.16	0.21	0.67	53.74	0.95	55.6
Soxhlet	1.02	0.46	0.42	73.90	1.64	76.4

[a]T, tocopherols; T3, tocotrienols; for other abbreviation see Table 1.

crude oils also represents the most delicate and difficult stage in the refining cycle since it is at this stage that the highest losses of neutral oil may occur and that the final quality of the refined product may be compromised (32). Table 2 shows the FFA and tocol contents in the oils obtained by SC-CO_2 and petroleum ether extraction. The FFA content of the SC-CO_2-extracted oils at 35–55 MPa and 50–70°C varied between 1.12 and 1.29% and was comparable ($P > 0.05$) to that of the petroleum ether extract (1.02%). There was no significant difference ($P > 0.05$) among the FFA contents of the oils obtained by SC-CO_2 at each condition and the solvent-extracted oil. Eggers (30) reported that generally there is less FFA in the CO_2 extract; however, the FFA content in the SC-CO_2-extracted oils varied between 0.3 and 2.6% for different oil seeds. Christianson *et al.* (21) indicated that the FFA content in the SC-CO_2-extracted corn oil (0.3%) at 50°C/35 MPa was lower than that in expeller oil (0.6%). FFA content in the soybean oil obtained by SC-CO_2 increased with pressure from 1.3 to 2.6% at 50°C and 35 and 55 MPa, respectively (33). More FFA was also reported in CO_2-extracted cottonseed oil (1.3–1.7%) than in the hexane-extracted oil (1.15%) (34).

It is known that an increased consumption of the n-3 FA leads to an even higher increase in the antioxidant requirement. As lipid-soluble antioxidants, tocopherols play an important role in the stability of n-3 FA. In the absence of an appropriate level of tocopherols as an antioxidant agent, PUFA can result in the formation of free radicals and have a significant prooxidant effect, leading to substantial depletion of tocol content and an increased level of oxidation products (2). Flaxseed oil contains α-tocopherol, α-tocotrienol, β-tocopherol, γ-tocopherol, and δ-tocopherol (Table 2). The total tocol content of the oil extracted with petroleum ether was higher than the previously reported value of 40–50 mg/100 g oil (35). Tocol content of the CO_2-extracted oil (55.6 mg/100 g oil at 70°C and 55 MPa) was somewhat lower than that of the petroleum ether-extracted oil (76.4 mg tocol/100 g oil). Similarly, tocopherol content of SC-CO_2-extracted cottonseed oil was found to be less than that of solvent-extracted oil (34); however, it was found to be higher in the SC-CO_2-extracted soybean oil when compared to the hexane-extracted oil (36).

ACKNOWLEDGMENTS

We are grateful to the Scientific and Technical Research Council of Turkey (TUBITAK) for NATO scholarship support for B. Bozan and the Natural Sciences and Engineering Research Council of Canada for financial support.

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[Received September 6, 2001; accepted December 17, 2001]