An Evaluation of Supercritical Fluid Extraction as an Analytical Tool to Determine Fat in Canola, Flax, Solin, and Mustard

Véronique J. Barthet* and James K. Daun

Canadian Grain Commission, Grain Research Laboratory, Winnipeg, Manitoba, R3C 3G8, Canada

ABSTRACT: Supercritical fluid extraction (SFE) with carbon dioxide was used to extract oil from soft oilseeds (flax, solin, canola, and mustard). Oil content determinations from the SFE method AOCS Am 3-96, with and without ethanol as a modifier, were compared to results obtained with an exhaustive extraction using petroleum ether (FOSFA as in AOCS Am 2-93). Without the modifier, oil recoveries using SFE were 10 to 15% lower than oil contents by the FOSFA method for the flax and canola samples. For mustard, the oil recoveries by SFE were about 20 to 30% lower than oil contents by the FOSFA method. In the presence of the modifier, oil recoveries for flax and canola were about 3% lower than the FOSFA recoveries. Varying the time, temperature, and amount of modifier (ethanol) showed that recoveries increased with time, pressure, temperature, and amount of modifier independently of the oilseeds tested. Kinetics of the SFE extraction showed that the oil recoveries increased with the extraction time and reached a plateau after 60 min. Multiple extractions (2 × 30 min), however, gave better recoveries than a single extraction for the same amount of time (60 min). The best results were obtained using multiple extractions without modifier or a combination of multiple extractions first without and then with 15% modifier. Under these last two conditions, oil recoveries were close to 100% for flax, solin, and canola, but mustard oil recoveries were still 10% lower than recoveries using the FOSFA method. Mustard samples gave the lowest oil recovery from SFE when compared to FOSFA method recoveries whatever conditions were tested, suggesting a matrix effect on the oil recovery. The acyl lipid content of the various extracts was studied using the sum of all FA expressed as TAG as a measure of acyl lipid extraction. The acyl lipid contents of the extracts were close to 100% when no modifier was used during the SFE. In the presence of modifier, the acyl lipid contents of the extracts were 10 to 15% lower than the results obtained without modifier. The amount of acyl lipid in the extract decreased as the quantity of modifier increased. This suggests that increasing the ethanol modifier increased the amount of polar compounds extracted without significantly increasing the total amount of lipids. The FA profiles were constant throughout the various extraction procedures.

Paper no. J10059 in *JAOCS 79,* 245–251 (March 2002).

KEY WORDS: Canola, flax, *juncea, linum,* mustard, *napus,* oil extraction, *rapa,* solin, supercritical fluid extraction.

Oilseeds are usually extracted analytically with an organic solvent designed to remove the neutral lipid. A popular official method uses multiple grinds and extractions with petroleum ether (FOSFA method, AOCS Am 2-93). In routine laboratory analysis, this method requires up to 10 h to complete and consumes a significant amount of solvent. Over the last decade, supercritical fluid extraction (SFE) has been investigated as a possible replacement for hexane-based oil content determinations for oilseeds (1–6).

Liquid CO_2 is the most commonly used solvent for analytical SFE and mimics petroleum ether in many of its characteristics. In addition, its polarity can be changed by the addition of modifiers such as ethanol or methanol (7). CO_2 reverts to a gas at room temperature and pressure and has only minor toxicity. It was therefore easy to conclude that SFE with CO_2 would offer clear advantages over organic solvents for oil analysis. Canola and soybeans have been analyzed for oil content by SFE (1,2,4), but no studies have reported the use of SFE to analyze the oil content of soft oilseeds such as flax, solin, and mustard.

The purpose of this work was to study the potential of SFE for determining oil content in soft oilseeds (flax, solin, canola, and mustard). The approach was to work from a routine analysis approach for various types of seeds and to study the effect of the extraction method on the FA content and composition of the extracted oil. Oil contents from a reference method based on exhaustive extraction with hexane (FOSFA) were compared to the oil contents from the SFE methods.

MATERIALS AND METHODS

Samples. Four types of oilseeds (flax, solin, canola, and mustard) were tested. Flax (*Linum usitatissimum*) samples of food grade were donated by Pizzey's Milling & Baking Company (Angusville, Manitoba, Canada) and Prairie Flax (Portage La Prairie, Manitoba, Canada). Solin (*L. usitatissimum*), canola (Parkland a *Brassica rapa* and Legend a *Brassica napus*), and mustard (*Brassica juncea* and *Sinapis alba)* were obtained from farm samples through the Canadian Grain Commission survey of harvested oilseeds.

Reagents and standards. Methanolic base and triheptadecanoin were purchased from Sigma (Sigma-Aldrich Canada Ltd., Mississauga, Ontario). A GC reference standard, GLC 549, designed for this project was obtained from Nu-Chek-Prep, Inc. (Elysian, MN). Phosphorus standards were purchased from the American Oil Chemists' Society (Champaign, IL). Leco-dry (diatomaceous earth) was provided by the Leco Corporation (St. Joseph, MI).

*To whom correspondence should be addressed at Canadian Grain Commission, Grain Research Laboratory, 1404-303 Main St., Winnipeg, MB R3C 3G8, Canada. E-mail: vbarthet@grainscanada.gc.ca

Moisture. Moisture was determined prior to lipid analysis using the method ISO 665. Approximately 2.0 to 2.5 g of seed was weighed into a moisture tin. The tin was placed in an oven at 103°C for 3 h, cooled in a desiccator, and moisture was determined gravimetrically. Oil content determinations were reported on a dry basis.

Sample grinding. Seeds were ground using a Retsch mill (Brinkmann Instruments Canada Ltd., Mississauga, Ontario, Canada) equipped with a 1.0-mm size sieve.

Fat content reference method. The solvent extraction was performed according to AOCS Am 2-93 (FOSFA method) using a Soxtec extraction unit (Foss-Tecator, Höganäs, Sweden).

SFE. The extraction was performed on ground seed (approximately 1–2 g) using a Model TFE 2000 Fat/Oil Determinator (Leco Corporation) with dual pumps for fluid and modifier. The AOCS Am 3-96 method without modifier was performed at 7500 psi for 30 min, 100°C extraction and restrictor temperatures. The method with 15% ethanol as dynamic modifier used 7500 psi for 60 min, extraction temperature of 100°C, and a restrictor temperature of 110°C. The SFE conditions were optimized by extractions using 7500 or 9000 psi, extraction temperature 80 or 100°C, restrictor temperature 100°C without modifier and 110°C with modifier, extraction durations ranging from 5 to 99 min and with or without 0 to 15% ethanol as dynamic modifier. The CO_2 flow rate was kept at 2 mL/min during all extractions. Multiple extractions with different combinations were also tested. The oil was quantitatively trapped on glass wool in removable collection vials. The amount of extract was obtained gravimetrically. To allow further analysis, the oil was extracted from the glass wool by hexane extraction.

FAME. Triheptadecanoin (2 mg/mL in toluene) was used as the internal standard for the GC analysis. Approximately 0.05 g (± 0.005 g) of extracted oil was weighed into a glass tube; after addition of 0.5 mL of internal standard, 5 mL of isooctane was added. After mixing, 0.5 mL of methanolic base was added. After incubation (30 min) at room temperature, 2 drops of bromothymol blue (0.1%, wt/vol in methanol); 0.4 mL HCL (1 M), and 0.6 mL sodium carbonate (0.15 M) were added in this sequence. After each solvent addition, the tube contents were mixed on a vortex mixer for a few seconds. Finally, 7 mL of deionized water was added to the mixture. After waiting for 1 h to allow the mixture to clear, the top layer was transferred into a GC vial.

GC analysis. The reference solution and samples were analyzed under the same operation conditions on a Hewlett-Packard 5890 gas chromatograph (Agilent Technologies, Mississauga, Ontario, Canada) equipped with an FID and a 7673A injector tower. Methyl esters were separated on a Supelco 10 silica column (Sigma-Aldrich Canada Ltd.) (15 m × 0.3 mm, 5 μm). Hydrogen was the carrier gas (52 cm/s); injection port and detector temperatures were 250°C. A two-step temperature program was used, 125 to 175°C at 25°C/min, then to 220°C at 6°C/min. The temperature was held at 220°C for 4 min.

Phosphorus analysis. Oils tested were diluted with *n*-butanol (1:3, wt/wt) before analysis by graphite furnace atomic absorption spectrophotometry using palladium/magnesium nitrate modifier samples. AOCS reference oils were used as quality control samples.

Statistical analysis. The statistical analysis of the results was done using Origin® 6.0 (Microcal Software Inc., Northampton, MA) and InStat 3.05 (GraphPad Software Inc., San Diego, CA).

RESULTS

The SFE method (AOCS Am 3-96) previously tested with canola and soybean oils was used as the starting point to measure the performances of the method in comparison to the FOSFA method. The AOCS Am 3-96 method without modifier gave oil recoveries 10, 15, and 25% lower than the results obtained by the FOSFA method for flax and solin (P = 0.0024, very significant), canola (P = 0.0003, extremely significant), and mustard (P = 0.0001, extremely significant), respectively (Table 1). When 15% modifier was introduced and the extraction time increased from 30 to 60 min, oil recoveries were still lower than those obtained with the FOSFA method for the flax samples (Table 1). The results were statistically significant and extremely significant for canola (Legend, P = 0.0008, and Parkland, $P < 0.0001$), whereas no real statistical difference was observed with flax (P = 0.3985).

Time, pressure, temperature, and sample particle size have been shown to affect the oil solubility during SFE (8). Experiments were carried out to optimize time, temperature, pressure, grinding, and the number of extractions to increase the oil recoveries to match FOSFA results and to study the effect of the extraction method on the composition of the lipids removed.

Effect of time, pressure, and temperature. Oil recovery increased with extraction time in isobaric and isothermic conditions without modifier (Fig. 1). However, there was an inverse relationship between oil recovery and extraction rate; the oil extraction rate decreased with time (Fig. 1). Increasing the time indefinitely might have increased the oil recovery to eventually match the FOSFA results, but CO_2 consumption and extremely long extraction times made this approach unrealistic for routine laboratory analysis. The benefits of SFE—speed and economy—would be lost. This is a general observation based on studies performed by other researchers (8–10).

Oil recovery increased with temperature; in isobaric conditions there was a statistically significant difference (P = 0.0346) between performing the extraction at 80 or 100°C (Table 2). These results are in agreement with the findings of other researchers (8,9,11). Even with these increased recoveries, the oil recoveries from the FOSFA method could not be matched. The temperature increase maximum was limited by the need to use the oil recovered from the analytical extraction for FA composition and other analyses.

The SFE apparatus used in this study did not allow pressures exceeding 9000 psi; the two tested pressure parameters

TABLE 1
Percent Oil Content (Mean ± SD, $n = 3$) on a Dry Basis in Seeds Obtained with SFE[a] and Reference Method (FOSFA)

Sample	FOSFA (triple hexane extraction)	SFE-AOCS Am 3-96 (30 min, no modifier)	SFE-AOCS Am 3-96 (60 min + 15% ethanol)	Multiple extractions (5 × 20 min, no modifier)	Triple extraction (2 × 30 min then 30 min + 15% ethanol)
Flax *(Linum usitatissimum)*					
Food flax I	41.34 ± 0.06	37.59 ± 0.14		40.61 ± 0.07	41.65 ± 0.67
Food flax II	44.50 ± 0.27	40.46 ± 0.15		44.55 ± 0.08	44.42 ± 0.15
Normandy I	42.89 ± 0.05	39.81 ± 0.33	42.54 ± 0.64	42.90 ± 0.16	43.04 ± 0.22
Normandy II	43.58 ± 1.05	37.84 ± 0.04		42.61 ± 0.06	43.02 ± 0.27
Solin	43.52 ± 0.89	41.06 ± 0.34		42.52 ± 0.39	43.71 ± 0.45
Canola					
Legend (*Brassica napus*)	47.26 ± 0.05	40.32 ± 0.09	46.27 ± 0.18	46.37 ± 0.05	47.18 ± 0.23
Parkland (*Brassica rapa)*	44.03 ± 0.05	38.32 ± 0.34	42.34 ± 0.17	43.45 ± 0.15	44.25 ± 0.28
Canola 1	44.36 ± 0.03	38.00 ± 0.26		43.29 ± 0.32	44.67 ± 0.18
Canola 2	43.40 ± 0.07	36.57 ± 0.11		42.08 ± 0.39	43.08 ± 0.05
Canola 3	50.68 ± 0.01	45.41 ± 0.27		49.38 ± 0.79	48.39 ± 0.06
Canola 4	42.97 ± 0.03	38.60 ± 0.44		41.51 ± 0.33	43.41 ± 0.29
Canola 5	48.19 ± 0.07	38.70 ± 0.67		47.49 ± 0.09	47.80 ± 0.28
Canola 6	46.06 ± 0.40	39.06 ± 0.67		45.60 ± 0.21	46.11 ± 0.20
Brassica juncea					
Brown mustard	39.41 ± 0.27	31.64 ± 0.35		38.72 ± 0.28	36.10 ± 0.06
Oriental mustard	43.22 ± 0.17	33.84 ± 0.67		40.50 ± 0.57	38.36 ± 0.25
Sinapis alba					
Yellow mustard	31.35 ± 0.27	21.59 ± 0.29		28.63 ± 0.57	28.60 ± 0.49

[a]All supercritical fluid extractions (SFE) extractions were performed at 7500 psi and 100°C.

(7500 and 9000 psi) did not have any significant effect on the oil recoveries ($P = 0.9379$). Moreover, Molero Gomez and Martinez de la Ossa (9) showed that increasing the pressure was not the best approach to increase oil recovery.

Effect of multiple extractions and sample particle size. Particle size affects oil recovery during fat extraction of oilseeds by conventional methods (12), and the same was observed by SFE with grape seeds (9). Two 30-min extractions produced higher extraction rates than a single 60-min extraction ($P = 0.009$, very significant), whatever the temperature and the pressure (Table 2). In isothermal conditions, the pressure had no effect ($P = 0.9679$) on the oil recovery (Table 2). Taylor *et al.* (3) also reported that two extractions were necessary to obtain a good oil recovery for canola extraction by SFE.

Regrinding the ground sample with Leco-Dry reduced the particle size of the sample and increased oil recovery compared to the same extraction conditions with no regrinding (Table 2). However, the results are not quite statistically significant ($P = 0.0840$). The amount of sample extracted had no effect on the oil recovery; extractions performed with 1 or 2 g of sample in the same conditions gave statistically the same results (data not shown). There was no statistical difference ($P = 0.5449$) between regrinding then extracting the sample compared to the yield obtained with two consecutive extractions of 30 min (Table 2). It is possible that the compression–decompression changed the sample matrix, producing an effect similar to the use of an expander in oilseed extraction. With each compression–decompression cycle of SFE, the sample matrix was opened further to allow the solvent from the next extraction to reach the lipid.

Effect of modifier. Oil recovery increased with the amount of ethanol introduced in the CO_2 as modifier (Fig. 2). At the same time, multiple extractions gave more oil recovery than a

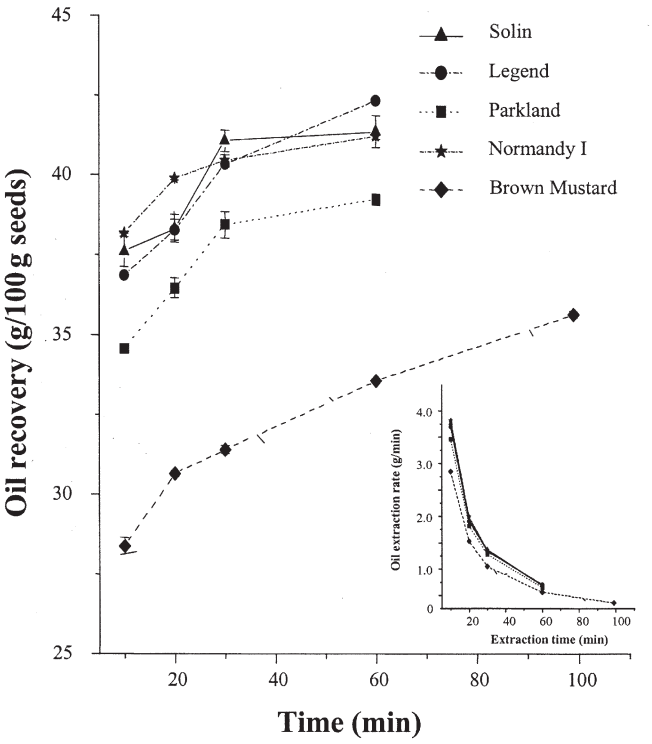

FIG. 1. Effect of time on oil recovery and extraction rate, in isobaric (7500 psi) and isothermal (100°C) conditions.

TABLE 2
Effect of Time, Temperature, and Pressure on the Percent Oil Content (mean ± SD, *n* = 3) on a Dry Basis in Seeds

Extraction parameters			Parkland	Legend	Normandy I
Extraction time	Temperature (°C)	Pressure (PSI)	(*Brassica rapa*)	(*Brassica napus*)	(*Linum usitatissimum*)
Extraction performed with 2 g of sample: mix Leco-Dry/ground seed (1:1, w/w)					
Once 30 min	100	7500	40.52 ± 0.38	44.53 ± 0.69	42.36 ± 0.09
Two times 30 min	100	7500	42.12 ± 0.45	45.47 ± 0.17	42.69 ± 0.06
Extraction performed with 2 g of seed sample					
Once 30 min	100	7500	38.32 ± 0.34	40.32 ± 0.09	39.81 ± 0.33
Two times 30 min	100	7500	42.77 ± 0.58	45.75 ± 0.16	42.09 ± 0.38
Once 60 min	100	7500	39.45 ± 0.50	42.33 ± 0.06	41.22 ± 0.09
Once 30 min	100	9000	38.41 ± 0.07	40.88 ± 0.20	40.42 ± 0.12
Two times 30 min	100	9000	42.79 ± 0.07	45.51 ± 0.17	42.51 ± 0.17
Once 60 min	100	9000	40.07 ± 0.10	43.30 ± 0.26	41.31 ± 0.08
Once 30 min	80	7500	36.44 ± 0.34	37.68 ± 0.53	38.95 ± 0.67
Two times 30 min	80	7500	39.11 ± 0.05	43.22 ± 0.90	41.30 ± 0.51
Once 60 min	80	7500	37.55 ± 0.57	40.03 ± 0.41	39.79 ± 1.14
Once 30 min	80	9000	36.33 ± 0.22	39.00 ± 0.67	38.72 ± 0.15
Two times 30 min	80	9000	39.60 ± 0.04	43.06 ± 0.32	41.07 ± 0.12
Once 60 min	80	9000	37.56 ± 0.08	40.81 ± 0.21	39.16 ± 0.16

single extraction of the same duration. These results agree with the results of Cocero and Calvo (13), who showed that increasing the ethanol content increased the oil solubility of sunflower seed, facilitating the extraction. However, increasing the ethanol concentration also increased the green color of the extracted oil, especially with canola, suggesting that other more polar material was co-extracted. Even with modifier, the FOSFA results could not be matched.

All these results suggested that the best approach to extract oilseeds by SFE would be to use multiple extractions. Two approaches—multiple extractions with no modifier and a combination of multiple extractions with and without a modifier—were tested. The highest oil recoveries were obtained by a triple extraction (two consecutive extractions of 30 min without modifier followed by a 30-min extraction with 15% ethanol) (Table 1). There was a tendency to obtain higher recoveries with this method than with the reference FOSFA method (Table 1). However, there were no statistical differences ($P = 0.62035$) between the results obtained by FOSFA and the triple extraction. Increasing the polarity of the solvent possibly allowed the extraction of more polar material, leading to an apparent increase in oil recovery. Five consecutive 20-min extractions without modifier produced good oil recoveries (Table 1); compared to the FOSFA method (Table 1), the results were at a maximum 3% lower for flax, solin, canola, and brown mustard. Oil recoveries for Oriental mustard and yellow mustard were still 7 to 9% lower than the FOSFA results.

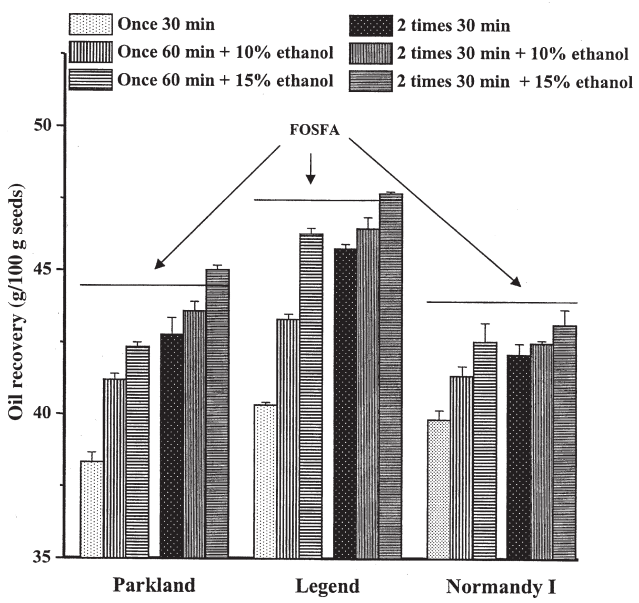

FIG. 2. Effect of modifier and multiple extractions on oil recovery.

Oil recovery appeared to be independent of the amount of oil present in the sample; yellow mustard had the lowest oil content but gave the lowest oil recovery whatever the method tested. Matrix effects seemed to be the main factor influencing oil recoveries. This hypothesis agreed with Taylor *et al.* (4), who suggested that the matrix effect, more than oil content, played an important role in oil recovery.

Effect of extraction on FA composition and acyl lipid content of the extracted oil. All extracts contained less than 1% FFA. It was decided therefore that a base-catalyzed derivatization could be used to study the FA composition and the acyl lipid content of the various extracted oils. The acyl lipid content of the samples was determined using the sum of the FA obtained by base-catalyzed methylation of the SFE and FOSFA oils, expressed as TAG. The underestimation of the acyl lipid contents of the oils by the base-catalyzed derivatization due to the FFA was negligible since this underestimation (less than 1%) was lower than the standard deviation of the results obtained with triplicate analysis (Table 3).

The acyl lipid content of the oil recovered by FOSFA was

TABLE 3
Effect of Multiple Extraction With or Without Modifier on the Percentage of Acyl Lipid Content of Extracted Oil (mean ± SD, *n* = 3)

	AOCS Am 3-96 (30 min, no modifier)	Multiple extractions (5 × 20 min, no modifier)	AOCS Am 3-96 (60 min + 10% ethanol)	AOCS Am 3-96 (60 min + 15% ethanol)	Multiple extractions with ethanol (2 × 30 min + 15% ethanol)	FOSFA (oil from first extraction)	FOSFA (combined oils)
Flax *(Linum usitatissimum)*							
Food flax I	97.99 ± 0.84	97.23 ± 1.68			91.27 ± 0.07	95.85 ± 1.55	95.62
Food flax II	97.94 ± 1.82	97.75 ± 0.47			92.68 ± 1.30	97.72 ± 0.95	97.53
Normandy I	97.15 ± 1.22	98.31 ± 0.75	97.99 ± 2.61	95.91 ± 0.27	92.43 ± 0.05	95.96 ± 0.43	95.87
Normandy II	98.29 ± 0.94	98.32 ± 0.60			89.52 ± 2.34	97.08 ± 0.17	96.86
Solin	95.47 ± 1.79	97.37 ± 0.19			83.43 ± 1.54	96.49 ± 0.55	96.21
Canola							
Legend (*Brassica napus*)	95.47 ± 2.13	95.91 ± 1.20	94.81 ± 0.63	87.16 ± 1.72	89.56 ± 0.41	94.78 ± 0.67	96.55
Parkland (*B. rapa*)	96.16 ± 1.82	95.74 ± 1.54	93.53 ± 0.70	88.48 ± 2.68	90.02 ± 1.39	93.95 ± 0.63	93.60
Canola 1	93.15 ± 0.71	94.94 ± 0.48			85.54 ± 1.93	97.9 ± 2.38	94.86
Canola 2	95.08 ± 0.71				87.90 ± 0.96	99.65 ± 0.34	101.11
Canola 3	95.68 ± 2.47	96.20 ± 1.19				102.56 ± 1.13	98.20
Canola 4	93.94 ± 0.37	95.59 ± 1.26			87.72 ± 2.02	99.09 ± 1.98	99.16
Canola 5	96.46 ± 0.79	96.15 ± 0.72			90.48 ± 6.55	101.12 ± 0.59	98.13
Canola 6	98.13 ± 0.64	95.28 ± 2.14			95.59 ± 1.02	101.31 ± 1.67	103.99
B. juncea							
Brown mustard	101.35 ± 0.58	99.27 ± 1.56			102.22 ± 0.38	99.61 ± 1.32	100.03
Oriental mustard	101.67 ± 1.05	101.26 ± 2.54			100.27 ± 0.82	99.21 ± 0.34	99.38
Sinapis alba							
Yellow mustard	102.68 ± 1.16	102.16 ± 1.48			92.53 ± 1.84	100.01 ± 0.87	99.30

[a]All extractions were performed at 100°C, 7500 psi.

analyzed for the three extraction steps (FOSFA1, FOSFA2, and FOSFA3), and the results were combined to give the acyl lipid content of the oil obtained by FOSFA analysis (Table 3). There was no difference between the acyl lipid content of the FOSFA1 extract and the combined oil ($P = 0.68347$).

With SFE, extraction duration (time varying from 5 to 20 min) had no effect on the acyl lipid content or the FA composition of the recovered oil from flax, solin, canola, and mustard (data not shown). Without modifier, the acyl lipid contents of the oils extracted by a single 30-min extraction ($P = 0.29526$) or multiple extractions ($P = 0.32408$) were not statistically different for any seed type compared with the FOSFA1 results (Table 3). When 10% modifier was used, there was no difference in the acyl lipid content of the tested oils ($P = 0.08392$) compared to FOSFA1. However, with 15% modifier, a decrease in acyl lipid content of the oil was observed ($P = 0.000424$) compared with FOSFA1 (Table 3). The combination of multiple extractions and 15% modifier produced almost a 10% acyl lipid decrease in the extracted oil for all seeds except brown and Oriental mustard compared to FOSFA1 (Table 3). Increasing the polarity of the CO_2 by adding ethanol did not appreciably improve the oil extraction, since more polar substances were extracted, as witnessed by the increased green chlorophyll color, increasing the total extraction yield but not the acyl lipid content when compared to FOSFA ($P = 0.0000345$). These results agree with the results obtained by King (14) and Taylor *et al.* (4).

Oriental and brown mustard are color variants of the *Brassica juncea* family. They exhibited the same behavior during SFE. The extraction was incomplete whatever the method tested (Tables 1 and 3), and the modifier had no effect on the acyl lipid content of the oil (Table 3). For yellow mustard (*S. alba),* the extraction was also incomplete. However, ethanol decreased the acyl lipid content of the extracted oil (Table 3). As with canola, flax, and solin, there was a decrease in acyl lipid content of the oil when ethanol was added to the CO_2 (Table 3). Again, these results suggest that differences in seed matrix had a significant effect on the amount of oil recovered by SFE.

The relative FA composition of the various oils (Table 4) did not change with the SFE method. They were comparable to the oils obtained during the first and second step of the FOSFA method. The oil of the third extract from FOSFA had a different relative FA composition (Table 4) than the other oils. For canola and mustard (Table 4), there were increases in the 18:1n-7 FA. Earlier studies performed in this laboratory (15) showed that this 18:1 isomer was found in TAG and not in phospholipids. The isomer was also found in the third FOSFA extract of Oriental mustard (data not shown). This FA was not obtained by SFE if no modifier was added during the extraction (Table 4). Even with 15% modifier in the CO_2, the percentage of 18:1 isomer was lower than the result obtained in the third FOSFA extract. This suggested that some tightly bound or otherwise inaccessible TAG were never extracted by SFE.

Effect of modifier on phospholipid extraction. The concentration of phosphorus was determined by atomic absorption. It was assumed that the only phosphorus present in the oil was due to phospholipids. Phosphorus was not detected at a concentration equal to or above 0.02 mg/kg in oils extracted without modifier either with single 30-min extractions or multiple 20-min extractions (data not shown), suggesting that no

TABLE 4
Effect of SFE Extraction Method on Relative FA Composition of Extracted Oils, Comparison with Reference Method (FOSFA)[a]

	SFE (100°C, 7500 PSI)				FOSFA extraction		
Sample	30 min	5 × 20 min	60 min +10% ethanol	60 min + 15% ethanol	1st extract	2nd extract	3rd extract
Normandy I (*Linum usitatissimum*)							
C18:1	18.87 ± 1.10	18.03 ± 0.05	18.19 ± 0.24	18.13 ± 0.01	18.35 ± 0.00	18.57[b]	
C18:2	14.47 ± 0.44	14.87 ± 0.03	14.65 ± 0.07	14.71 ± 0.09	14.74 ± 0.01	15.13[b]	
C18:3	56.94 ± 0.56	56.58 ± 0.09	56.80 ± 0.12	55.67 ± 0.14	57.01 ± 0.00	55.14[b]	
Legend (*Brassica napus*)							
C18:1	61.09 ± 1.00	61.45 ± 0.14	60.31 ± 1.54	59.97 ± 1.41	61.74 ± 0.22	55.48 ± 1.25	34.55 ± 4.67
C18:1n-7	0.53 ± 1.19	0.00	0.89 ± 1.54	0.94 ± 1.63	0.00	4.55 ± 0.53	22.83 ± 2.75
C18:2	20.26 ± 0.09	20.21 ± 0.38	20.43 ± 0.16	20.69 ± 0.12	20.40 ± 0.07	21.85 ± 1.13	23.22 ± 0.71
C18:3	8.29 ± 0.08	8.37 ± 0.06	8.42 ± 0.13	8.33 ± 0.06	8.39 ± 0.02	7.83 ± 0.18	4.72 ± 0.66
Parkland (*B. rapa*)							
C18:1	56.78 ± 0.00	56.67 ± 1.56	55.71 ± 1.37	55.85 ± 1.22	56.96 ± 0.13	52.91 ± 0.02	38.16 ± 2.59
C18:1n-7	0.00	0.00	0.00	0.00	0.00	3.39 ± 0.08	17.10 ± 1.25
C18:2	22.10 ± 0.02	21.82 ± 0.42	22.33 ± 0.02	22.44 ± 0.09	22.10 ± 0.06	22.84 ± 0.04	24.19 ± 1.36
C18:3	13.35 ± 0.02	11.86 ± 1.22	13.05 ± 0.03	13.06 ± 0.08	12.90 ± 0.04	12.57 ± 0.03	8.02 ± 0.20
Brown mustard (*B. juncea)*							
C18:1	19.15 ± 0.38	18.99 ± 0.21			19.07 ± 0.51	18.56 ± 0.05	12.27[b]
C18:1n-7	1.70 ± 0.10	0.87 ± 0.92			1.59 ± 0.03	1.86 ± 0.01	16.41[b]
C18:2	21.01 ± 0.12	20.90 ± 0.24			20.67 ± 0.55	21.16 ± 0.05	26.17[b]
C18:3	13.40 ± 0.05	13.75 ± 0.21			13.34 ± 0.36	13.44 ± 0.02	8.89[b]
C20:1	11.87 ± 0.11	11.76 ± 0.11			11.81 ± 0.32	11.63 ± 0.02	7.95[b]
C22:1	22.17 ± 0.11	22.15 ± 0.18			21.71 ± 0.38	22.27 ± 0.08	3.84[b]
Yellow mustard (*Sinapis alba*)							
C18:1	23.35 ± 0.15	23.13 ± 0.34			23.20 ± 0.06	22.64 ± 0.08	16.70[b]
C18:1n-7	1.03 ± 0.02	0.11 ± 0.32			1.00 ± 0.02	1.15 ± 0.00	11.04[b]
C18:2	9.80 ± 0.17	9.79 ± 0.04			9.67 ± 0.03	11.34 ± 0.03	6.74[b]
C18:3	10.31 ± 0.18	10.54 ± 0.04			10.54 ± 0.03	10.15 ± 0.04	1.09[b]
C20:0	0.69 ± 0.01	0.68 ± 0.00			0.67 ± 0.00	0.63 ± 0.02	6.60[b]
C20:1	10.08 ± 0.03	10.03 ± 0.02			10.10 ± 0.02	9.60 ± 0.01	18.72[b]
C22:1	34.56 ± 0.16	35.12 ± 0.24			35.39 ± 0.13	34.23 ± 0.15	21.86[b]

[a]Values are reported as mean ± SD.
[b]Derivatization was done in duplicate instead of triplicate due to the small amount of oil.

phospholipids were extracted. In the presence of a modifier, the quantity of phosphorus increased with the amount of modifier, suggesting an increase in phospholipid extraction (Table 5). However, it was not possible to conclude a relation between the amount of ethanol used as modifier and the amount of phospholipids extracted (Table 5). Phospholipids were extracted from canola, flax, solin, and yellow mustard in the presence of ethanol. Nevertheless, brown and Oriental mustard oils showed no presence of phospholipids in the oil obtained with a modifier, which confirmed the previous results showing that ethanol had no effect on the fat composition of their oils. Temelli (16) showed that ethanol should be present in CO_2 to extract phospholipids from canola.

Using single 30- or 60-min extractions gave poor oil recoveries, suggesting that SFE could not be used to match FOSFA results. On the other hand, good oil recoveries could be obtained with SFE using multiple extractions. FA derivatization showed that when no modifier was added, the extracted oils contained mostly TAG. Luque de Castro and Jiménez-Carmona (7) asked: "Where is supercritical fluid extraction going?" These results showed that SFE has potential in oilseed analysis; however, a universal method applicable to all oilseeds would not be possible. Each seed type requires a specially tailored SFE method due to the effect of the sample matrix on oil recovery.

TABLE 5
Effect of Extraction on Phosphorus Concentration[a] (mg/kg) in Oil Obtained by SFE

	Legend (*Brassica napus*)	Parkland (*Brassica rapa*)	Normandy I (*Linum usitatissimum*)
10% ethanol for 60 min	0.17 ± 0.04	1.29 ± 0.20	0.33 ± 0.51
15% ethanol for 60 min	1.43 ± 0.21	1.16 ± 0.54	1.13 ± 0.43

[a]All extractions were performed at 7500 psi and 100°C.

ACKNOWLEDGMENTS

Tricia Chornick and Barry Misener assisted with the analysis of FA composition. Eugene Gawalko carried out phosphorus analysis. We thank the Leco Corporation for the loan of the TFE2000 Fat/Oil Determinator. Partial funding was received from the Flax Council of Canada. This paper is no. 822 from the Canadian Grain Commission, Grain Research Laboratory.

REFERENCES

1. Friedrich, J.P., G.R. List, and A.J. Heakin, Petroleum-Free Extraction of Oil from Soybeans with Supercritical CO_2, *J. Am. Oil Chem. Soc. 59*:288–292 (1982).
2. Eggers, R., High Pressure Extraction of Oilseed, *Ibid. 62:*1222–1230 (1985).

3. Taylor, S.L., J.W. King, and G.R. List, Determination of Oil Content in Oilseeds by Analytical Supercritical Fluid Extraction, *Ibid 70:*437–439 (1993).
4. Taylor, S.L., F.J. Eller, and J.W. King, A Comparison of Oil and Fat Content in Oilseeds and Ground Beef—Using Supercritical Fluid Extraction and Related Analytical Techniques, *Food Res. Int. 30:*365–370 (1997).
5. Dionisi, F., B. Hug, J.M. Aeschlimann, and A. Houllemar, Supercritical CO_2 Extraction for Total Fat Analysis of Food Products, *J. Food Sci. 64:*612–615 (1999).
6. Devittori, C., D. Gumy, A. Kusy, L. Colarow, C. Bertoli, and P. Lambelet, Supercritical Fluid Extraction of Oil from Millet Bran, *J. Am. Oil Chem. Soc. 77:*573–579 (2000).
7. Luque de Castro, M.D., and M.M. Jiménez-Carmona, Where Is Supercritical Fluid Extraction Going? *Trends Anal. Chem. 19:*223–228 (2000).
8. Zhang, X.W., T. Sun, Z.Y. Sun, D.X. Gu, and X.Y. Zeng. Supercritical Carbon Dioxide Extraction of Wheat Plumule Oil, *J. Food Eng. 37:*103–110 (1998).
9. Molero Gomez, A., and E. Martinez de la Ossa, Quality of Wheat Germ Oil Extracted by Liquid and Supercritical Fluid Carbon Dioxide, *J. Am. Oil Chem. Soc. 77:*969–974 (2000).
10. Yoon, J., B.S. Han, Y.C. Kang, K.H. Kim, M.Y. Jung, and Y.A. Kwon, Purification of Used Frying Oil by Supercritical Carbon Dioxide Extraction, *Food Chem. 71:*275–279 (2000).
11. Kuk, M.S., and M.K. Dowd, Supercritical CO_2 Extraction of Rice Bran, *J. Am. Oil Chem. Soc. 75:*623–628 (1998).
12. Daun, J.K., and H. Snyder, Total Oil Analysis of Oilseeds, *Ibid. 66:*1074–1076 (1989).
13. Cocero, M.J., and L. Calvo, Supercritical Fluid Extraction of Sunflower Seed Oil with CO_2–Ethanol Mixture, *Ibid. 73:*1573–1578 (1996).
14. King, J.W., Analytical SFE Applied in Nutritional Labeling Analysis, *Proceedings of the Third International Symposium on Supercritical Fluids,* Institute National Polytechnique de Loraine, Nancy, France, 1994, Vol. 3, pp. 459–464.
15. Daun, J.K., Vaccenic Acid as a Major Component of the Triacylglycerols in Residual Oils of Canola Meals, *J. Am. Oil Chem. Soc. 66:*472 (1989), meeting abstract.
16. Temelli, F., Extraction of Triacylglycerides and Phospholipids from Canola with Supercritical Carbon Dioxide and Ethanol, *J. Food Sci. 57:*440–442, 457 (1992).

[Received August 14, 2001; accepted December 19, 2001]