Fatty Acid and Phosphorus Contents of Canola Seed Extracts Obtained with Supercritical Carbon Dioxide

Michael Fattori, N. Ross Bulley,[1] and Axel Meisen*

Samples of canola seed (*Brassica campestris*) were extracted using supercritical carbon dioxide at 36 MPa and 55 °C. The oil extracts were examined with respect to fatty acid composition and phosphorus content. The fatty acid composition was found to be invariable for most of the extraction period. However, extracts obtained after 80% of the oil was removed from the seed were found to be richer in C22 and C24 fatty acids. This is unlike the hexane extraction process where the fatty acid composition of the oil remains constant throughout the extraction. Phosphorus content of the supercritical extracts was always less than 7 ppm, the detection limits of the system.

In order to assess the feasibility and merits of using supercritical carbon dioxide as a solvent in the canola oilseed industry, basic information is required on oil solubility as a function of temperature and pressure. Changes in the chemical composition of the oil during extraction and as effected by temperatures and pressure also need to be considered.

The chemical composition of a vegetable oil can be characterized in different ways, e.g. unsaponifiables, free fatty acids, triglyceride content, fatty acid profile, phosphorus content. In the present study the fatty acid and phosphorus contents were selected to characterize the oil extracted with supercritical carbon dioxide.

The fatty acid composition was chosen because it gave an indication of the triglyceride components. It was known that different triglycerides exhibit different solubilities in supercritical carbon dioxide. This is unlike hexane, in which a wide variety of triglycerides are completely miscible. Since canola oil is comprised of several triglycerides, the possibility exists that the CO_2 extraction process would simultaneously extract and fractionate the oil. Experiments were, therefore, conducted to determine the fatty acid composition of the extracts at different stages during the extraction.

The phosphorus content of the oil extracts is also an important consideration. When canola seed is crushed, phospholipid components, which appear naturally in the seed cell membranes, are released. These phospholipids are subsequently dissolved by the seeds storage lipids (seed oil). As a result, the crude oil obtained by expelling canola seed contains 1.5–3 wt % phospholipids (Teasdale and Mag, 1983). In the conventional hexane extraction process these phospholipid gums are removed along with the seed oil. Since the gums are undesirable in the finished oil product, they are removed in a refining process and added back to the canola meal (Anjou, 1972). Previous experiments had provided some indication that CO_2 would not extract phospholipid gums from the crushed seed (Friedrich and List, 1982). Accordingly, experiments were conducted to investigate possible phospholipid removal.

MATERIALS AND METHODS

Experimental Equipment. The equipment used for the experiments was developed in house by adapting an existing high-pressure liquid chromatograph. The modifications that were made to the equipment allowed liquid carbon dioxide from a storage cylinder to be pumped through an extraction vessel at a maximum temperature and pressure of 99 °C and 40 MPa, respectively. The system also allowed the CO_2 and the collected extracts to be quantified.

The extraction vessel used in this work had interior dimensions of 12.7-mm i.d. and 82-mm length. The volume of the vessel was 10.4 mL. During operation, the vessel typically contained 4 g of seed material.

A more detailed description of the equipment and its operation is described elsewhere (Bulley et al., 1984).

Materials. Flaked and cooked samples of *Brassica campestris* were obtained from a commercial seed processor (CSP Foods, Saskatchewan). The same processor also supplied samples of crude canola oil. The refined oil was acquired from a local retailer. The carbon dioxide extract was obtained by extracting cooked/flaked canola seed at 36 MPa and 55 °C, at a CO_2 flow rate of 0.7 g/min. The hexane extract was produced from cooked/flaked seed with hexane at 55 °C.

The CO_2 used in the experiments was USP siphon grade, drawn as a liquid from pressurized steel cylinders.

FATTY ACID ANALYSIS

The fatty acid analysis was performed in two steps. In the first step the oil was transesterified. In the second step, the resulting methyl esters were identified and quantified by gas chromatography.

Transesterification. The transesterification reaction converts triglycerides directly to their component fatty acid methyl esters (Knapp, 1979). The procedure used in this work is based on a method developed by Shehata et al. (1970). The reaction was carried out at room temperature with use of sodium methoxide in a single-phase mixture of methanol, petroleum ether, and diethyl ether. When the reaction was complete, the mixture was forced to separate into two phases (by the addition of a small amount of water), with the methyl esters dissolving in the petroleum ether phase. The petroleum ether and the dissolved methyl esters were then removed from the reaction vial by pipet.

Gas Chromatography Procedure. The fatty acid ester solution was analyzed on a Perkin-Elmer (PE) Sigma 2 gas chromatograph, connected to a PE Sigma 10 data system. The esters were separated with a 6 ft × $^1/_8$ in. (i.d.) stainless steel column packed with SP-2330 on 100/120-mesh Chromosorb WAW. The column was obtained prepacked from Supelco Corp. All GC analyses were performed in accordance with the conditions shown in Table I.

PHOSPHORUS ANALYSIS

The procedure by which oil samples were analyzed for their phospholipid content was based on the work of

Departments of Bio-Resource Engineering (M.F., N.R.B.) and Chemical Engineering (A.M.), University of British Columbia, Vancouver, BC, Canada V6T 1W5.

[1]Present address: Department of Agricultural Engineering, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

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Table I. Gas Chromatographic Parameters for the Fatty Acid Methyl Ester Analyses[a]

column	SP-2330 on 100/120 mesh Chromosorb WAW
column temperature	200 °C isothermal
detector temperature	250 °C
injector temperature	250 °C
carrier gas	helium
carrier gas flow rate	20 cm^3/min
sample size	1 μL
detector	flame ionization

[a] Under the above column and conditions, base-line separation of each of the fatty acid esters could be obtained. The integration of the peaks was thus from base line to base line.

Table II. Major Phospholipid Components of Canola Oil (Sosulski et al., 1981)

phospholipid	mol formula	mol wt	occurrence, %
phosphatidylcholine	$C_{44}H_{80}O_8NP$	781	48
phosphatidylinositol	$C_{47}H_{70}O_{13}P$	873	20
phosphatidylethanolamine	$C_{43}H_{80}O_8NP$	769	9

Duck-Chong (1979). The method was conducted in two parts. In the first part the sample was ashed at high temperature in the presence of magnesium nitrate. In the second step the residual phosphate was determined by a standard colorimetric procedure and known concentration reference standards (Fattori, 1985).

Phospholipid Calculations. The above procedure yields only total phosphorus content of samples. In order to calculate the amount of phospholipid present, the ratio between the phosphorus and the phospholipids must be known. In principle, this ratio can be calculated from the molecular weights of phosphorus and phospolipids. However, since many types of phospholipids occur in biological materials, the ratio in practice is between the molecular weight of phosphorus and an "average" molecular weight of phospholipids.

The average molecular weight of phospholipids in the canola oil was calculated from the concentration and molecular weight of the major phospholipids known to occur in the oil (see Table II):

$$\text{mol wt } (x) = [0.48(781) + 0.2(873) + 0.09(769)]/0.77 = 800$$

The ratio of phosphorus to phospholipids is, therefore, 31/800 = 0.0386; i.e., 3.86 wt % of phospholipids in canola oil is phosphorus. Thus, the mass of phospholipids in the oil can be estimated by multiplying the mass of phosphorus as determined by the aforementioned procedure by 1/0.0386 or 25.9.

Detection Limits of the Procedure. The minimum amount of phosphorus determined by the above procedure was found to be 0.05 μg. Since the typical oil sample size was 7 mg, the limits of detection were 0.05 μg/7 mg or 7 ppm of phosphorus. This value corresponds to a minimum phospholipid detection limit of approximately 0.02%.

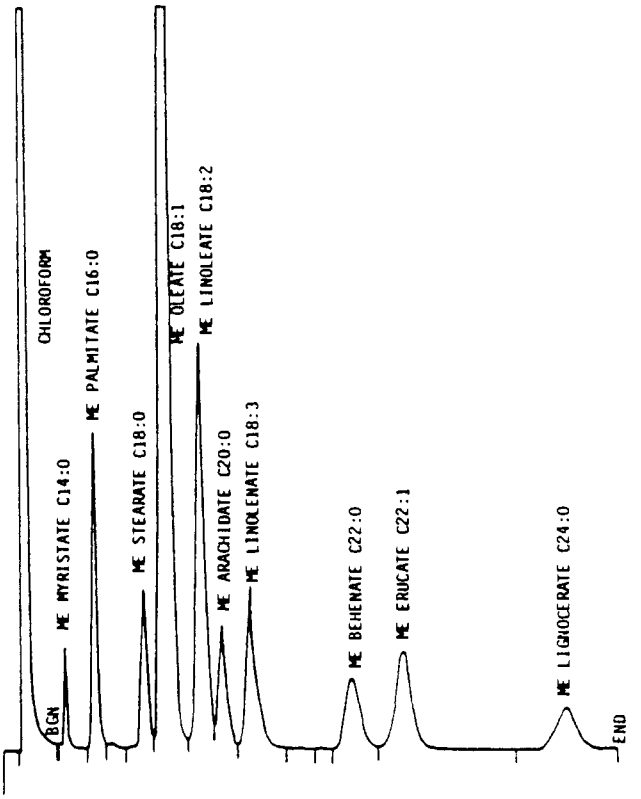

Figure 1. Chromatogram of the fatty acid methyl esters in an esterified sample of a typical CO_2 extract of canola seed (36 MPa, 55 °C, flow 0.7 g/min). Analysis conditions: column, SP-2330 on 100/120-mesh Chromosorb WAW; detector (fid) and injector temperature, 250 °C; isothermal 200 °C.

RESULTS AND DISCUSSION

Fatty Acid Composition of Canola Oil. The various triglycerides that make up canola oil contain both saturated and unsaturated fatty acid moieties ranging in carbon number from 14 to 24. The fatty acid profile can thus be used as a means of characterizing the oil and is often the measure by which oils from different varieties of canola seed are compared (Table III) (Ackman, 1983).

In Table IV, the fatty acid composition of canola oil from four sources is shown. The determinations were made by the procedure discussed above.

A typical chromatograph showing the elution sequence of each fatty acid ester and the peak resolution is given in Figure 1. The identity of each major peak in the

Table III. Fatty Acid Composition (%) of Triglycerides in Canola Oil Obtained from Three Varieties of Canola Seed (Ackman, 1983)

variety	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1
Andor	3.9	0.2	1.3	58.2	21.6	12.6	0.5	1.6	0.4	0.0
Tobin	3.8	0.1	1.2	58.6	24.0	10.3	0.6	1.0	0.1	0.3
Jet Neuf	4.9	0.4	1.4	56.4	24.2	10.5	0.7	1.2	0.3	0.0

Table IV. Fatty Acid Composition (%) of the Triglycerides in Four Samples of Canola Oil[a]

canola oil	14:0	16:0	18:0	18:1	18:2	18:3	20:0	22:0	22:1	24:0
refined oil	0.2	3.9	1.8	58.0	22.0	11.0	0.8	0.8	1.3	0.3
CSP crude	0.2	4.4	1.8	58.2	21.5	10.9	0.7	0.3	1.0	0.2
CO_2 extract	0.1	4.7	2.0	56.9	21.9	11.2	0.8	0.5	0.6	0.3
hexane extract	0.2	4.8	2.1	57.4	22.0	11.0	0.9	0.4	1.0	0.2
absolute error	±0.1	±0.2	±0.2	±1.4	±0.3	±0.2	±0.2	±0.2	±0.2	±0.2

[a] For a description of each sample refer to the text. The fatty acid C 20:1 and C 18:3 were not resolved by the chromatographic procedure.

Table V. Fatty Acid Composition (%) of Sequential CO_2 Extracts of CSP-Cooked Canola Seed[a]

fatty acid ester	extract number 1	2	3	4	5	6	7	error, %
C 14:0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1
C 16:0	4.7	5.1	5.2	5.1	4.6	4.1	3.4	0.2
C 18:0	2.0	1.9	1.8	1.9	2.0	2.2	2.5	0.2
C 18:1	56.9	57.3	57.3	57.9	58.8	59.6	58.5	1.4
C 18:2	21.9	22.6	22.7	22.4	21.8	20.8	19.6	0.3
C 20:0	0.8	0.6	0.6	0.6	0.7	0.9	1.3	0.2
C 18:3	11.2	11.2	11.3	11.1	10.9	10.6	10.7	0.2
C 22:0	0.5	0.3	0.3	0.3	0.3	0.4	1.0	0.2
C 22:1	0.6	0.6	0.5	0.5	0.8	0.9	2.5	0.2
C 24:0	0.3	0.2	0.2	0.1	0.1	0.2	0.6	0.2

[a] Conditions: 10-mL vessel; pressure 36 MPa; temperature 55 °C; CO_2 flow rate 0.7 g/min.

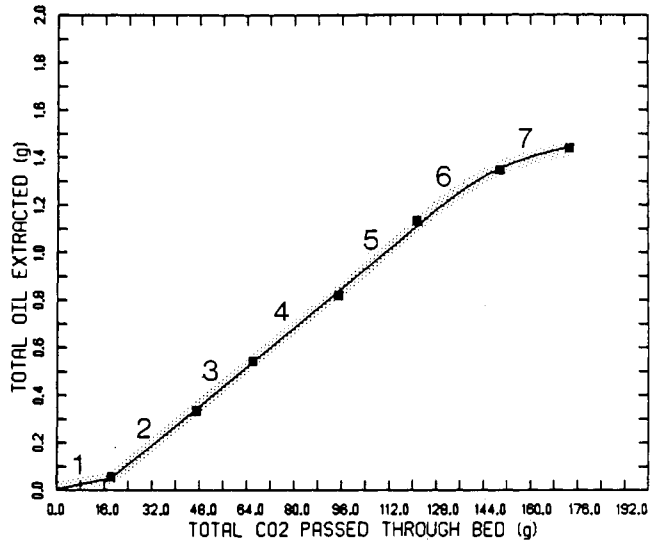

Figure 2. Extraction curve for a 4 ± 0.5 g sample of commercially cooked canola seed. The extraction was carried out at 36 MPa and 55 °C at a CO_2 flow rate of 0.7 g/min. The numbered intervals on the curve indicate the regions over which oil samples were collected for fatty acid analysis.

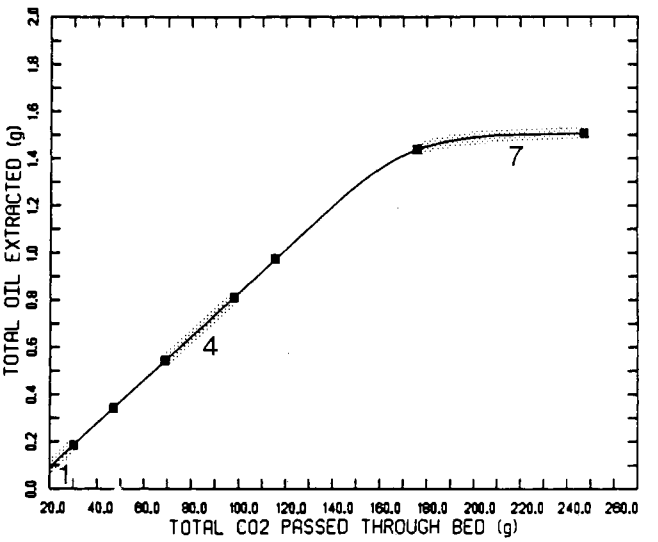

Figure 3. Extraction curve for a 4 ± 0.5 g sample of commercially cooked canola seed (36 MPa, 55 °C, CO_2 flow rate 0.7 g/min). The dotted areas on the curve represent the regions over which samples were collected for fatty acid analysis.

chromatogram was obtained initially by comparing retention times with analytical standards and this later confirmed by GC/mass spectrometer techniques. The absolute error in each case is the sum of the total measurement error involved with the integration of the chromatographic peaks and the error associated with the analytical standards.

It can be seen from Table IV that the hexane and carbon dioxide extract of the same seed are virtually identical in all of the fatty acids with the exception of erucic acid (C 22:1). In the hexane extract this fatty acid appeared in a slightly higher concentration.

Fatty Acid Composition of Sequential CO_2 Extracts. One of the objectives of this study was to determine how the composition of the supercritical carbon dioxide extracts varied as a function of extraction time. In addition, the compositions of these extracts were compared with those obtained by conventional hexane extraction.

In the first experiment 4.2 g of cooked CSP seed was extracted at 36.0 MPa and 55 °C at a CO_2 flow rate of 0.7 g/min. Seven consecutive extract fractions were collected (see Figure 2).

In Table V the fatty acid ester composition of each extract is given. As can be seen, only small variations occur in the oil composition during the extraction. In the final extract sample, however, obtained after about 80% of the oil had been removed from the seed bed, the proportion of the heavier fatty acid esters (C20–24) was higher than in the previous fractions. In an effort to verify this and to collect extracts after longer times, the experiment was repeated under identical conditions. At the completion of this second experiment the restricter valve was also washed with $CHCl_3$ and the oil analyzed. In Figure 3 the extraction curve is shown along with the positions where the extracts were collected for analysis. In this case the last extract was obtained after 90% of the oil had been removed from the sample. In Table VI the compositions of the various extracts are listed.

Table VI. Fatty Acid Composition (%) of Sequential CO_2 Extracts of Commercially Cooked Canola Seed[a]

fatty acid ester	extract number 1	4	7	oil from valve	error, %
C 14:0	0.1	0.1	<0.1	<0.1	0.1
C 16:0	5.4	5.1	3.3	4.5	0.2
C 18:0	1.9	1.9	2.7	2.8	0.2
C 18:1	56.5	56.9	55.2	54.5	1.4
C 18:2	23.0	21.5	19.9	19.8	0.3
C 20:0	0.5	0.5	1.1	1.3	0.2
C 18:3	11.3	11.4	11.3	10.9	0.2
C 22:0	0.3	0.3	1.3	1.4	0.2
C 22:1	0.6	0.6	4.3	4.3	0.2
C 24:0	0.1	0.1	0.9	0.9	0.2

[a] The analysis of oil extracts obtained from the restricter valve is also provided. Conditions: 10-mL vessel; pressure 36 MPa; temperature 55 °C; CO_2 flow rate 0.7 g/min.

Extracts 1 and 4, which were obtained early and midway through the extraction, were similar in composition to the previous extracts. The proportions of the heavier fatty acid

Table VII. Profile by Carbon Number of the Various Triglycerides Present in Canola Oil from Two Different Sources (%)

seed sample	triglyceride carbon number								
	C51	C53	C55	C57	C59	C61	C63	C65	C67
1	2	6	18	61	7	2	1	1	
2	3	4	18	70	5	1	<1		

Table VIII. Fatty Acid Composition of Sequential Hexane Extracts of Commercially Cooked Canola Seed[a]

fatty acid ester	hexane extract number									
	1	2	3	4	5	6	7	9	12	error, %
C 14:0	0.2	0.2	0.2	0.2	0.5	0.6	1.0	0.5	2.0	0.2
C 16:0	4.8	4.7	4.8	5.0	4.9	5.3	5.4	5.1	5.3	0.2
C 18:0	2.1	2.0	2.1	2.1	2.1	2.1	2.2	2.0	2.1	0.2
C 18:1	57.4	57.6	57.7	57.1	57.2	57.0	57.0	57.1	56.4	1.4
C 18:2	22.0	22.1	22.1	22.2	21.9	22.0	21.7	22.0	21.4	0.2
C 20:0	0.9	0.8	0.9	0.9	1.0	0.9	0.9	0.9	0.9	0.2
C 18:3	11.0	10.9	11.6	11.0	10.7	10.7	10.5	10.8	10.2	0.2
C 22:0	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.2
C 22:1	1.0	1.0	1.0	1.0	1.0	0.9	0.8	0.9	0.9	0.2
C 24:0	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2

[a] Conditions: 10-mL vessel; pressure 1.5 MPa; temperature 55 °C; flow rate 0.7 g/min.

esters were significantly higher in the last extract and in the oil collected from the restricter valve.

It is of interest to note that the concentration of the C 24:0 fatty acid in the final extract fraction, exceeds 0.2%, i.e. the maximum specified for edible low erucic acid rapeseed (LEAR) oil by the Codex Alimentarius Commission (Ackman, 1983). The concentration of fatty acids in the final fraction does, however, conform to the specifications of the Canada Agricultural Products Standards Act (Boulter, 1983).

A possible reason why the composition of the oil extracts remains constant during most of the extraction may be obtained by considering the distribution of the triglycerides that appear in canola oil. In Table VII the triglyceride carbon number is listed for two samples of canola oil. The table shows that a large proportion of the oil is composed of triglycerides having 55 or 57 carbons, indicating that the molecular weight range of the triglycerides in the oil will be small.

Since the various triglycerides are also very similar chemically, it might be expected that they exhibit similar solubilities in the carbon dioxide. This suggests that a large portion of the triglycerides would be extracted at the same rate and consequently the composition of the extract remains constant for much of the extraction. However, toward the end of the extraction, the small amounts of higher molecular weight triglycerides would be expected to constitute a larger fraction of the CO_2 extracts since their mole fractions in the residual oil are higher.

Fatty Acid Composition of Hexane Extracts. In an effort to determine whether the hexane extracts obtained with cooked canola seed were similar to the CO_2 extracts, the above experiments were repeated with hexane at 55 °C and 1.5 MPa in place of CO_2. At the completion of the extraction, each collection vial was heated to 55 °C for 2 h to facilitate the removal of hexane from the extracts. Figure 4 is a plot of the total oil collected during the experiment vs. the total amount of hexane used. The figure also indicates the regions over which each of the extracts was collected. In Table VIII the fatty acid composition of each extract is presented. It is evident that the fatty acid composition of the hexane extracts is similar to that of the carbon dioxide extracts (Tables V and VI). It can be seen, however, that the late hexane extracts, unlike the corresponding CO_2 extracts, do not show an increased proportion of the high molecular weight fatty acids. One explanation for this could be that both high and low molecular weight triglycerides are equally soluble in the hexane and therefore neither is preferentially removed.

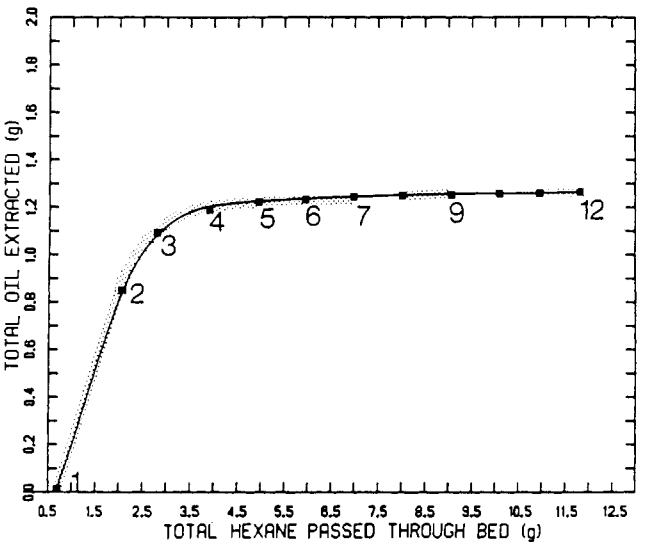

Figure 4. Extraction curve for a 4 ± 0.5 g sample of cooked canola seed. The extraction was carried out with hexane at 1.5 MPa and 55 °C. The dotted areas on the curve represent the regions over which samples were collected for fatty acid analysis.

Phosphorus in Commercially Produced Canola Oil. Prior to studying the phosphorus content of the CO_2 extracts of canola seed, the phosphorus content of commercially produced crude and refined canola oil was determined. The results from these experiments indicated that the crude oil contains 1.87 ± 0.03 wt % phospholipids while the refined oil contains less than 0.02 wt % phospholipids.

Phosphorus in Carbon Dioxide Extracts of Canola Seed. The extractibility of phospholipids from canola seed by use of supercritical CO_2 was investigated. The samples obtained for analysis were collected over five intervals during the extraction (see Figure 5). Additionally, at the completion of the extraction, the extractor was taken apart and the frits and restricter valve were washed with a mixture of chloroform and methanol. The washings were then analyzed for phosphorus. No phosphorus could be detected in any of the extracts or the valve washings.

These results indicate that, under the conditions tested and within the limits of the analytical procedure, supercritical CO_2 does not extract phospholipid material from

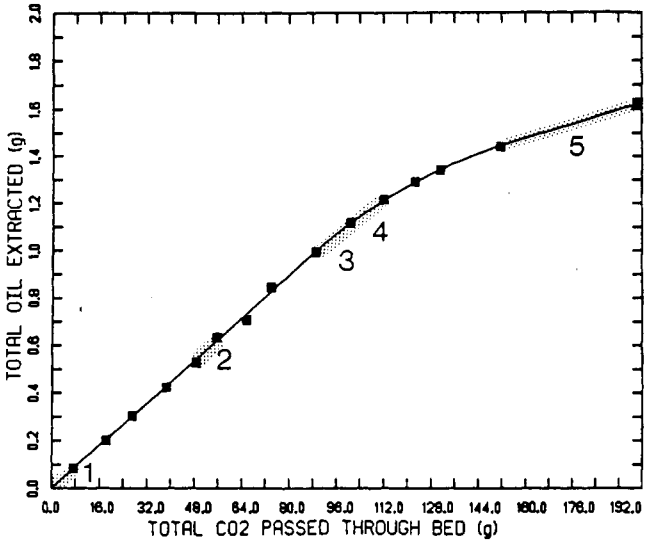

Figure 5. Extraction curve for a 4 ± 0.5 g sample of cooked canola seed showing the intervals (dotted) over which samples of oil were collected for phosphorus analysis (36 MPa, 55 °C, CO_2 flow rate 0.7 g/min).

canola seed. These findings are similar to those reported for the CO_2 extraction of corn germ (Christianson et al., 1984) and soybean (List and Friedrich, 1985). In these reports, the maximum phosphorus contents of the extracted oils were 5 and 1 ppm, respectively. In both of these studies the oils were extracted with CO_2 at 50 °C and 54 MPa.

In two additional studies, which dealt with the application of supercritical fluids to soybean meal, it was found that small amounts of phosphorus did appear in the oil extracts. In the first of these studies (Friedrich and List, 1982) was discovered that the extract obtained with CO_2 at 34 MPa and 50 °C had a phosphorus concentration of 60 ppm. In the second study (Friedrich and Pryde, 1984) it was found that the oil produced with CO_2 at 54 MPa and 50 °C had a phosphorus concentration of 45 ppm.

The difference in the phosphorus contents of the supercritical extracts from the various seeds cannot be accounted for on the basis of phospholipid concentration in the different meals since all have approximately the same value (Sonntag, 1979). However, a possible reason for the differences may be that the phospholipids in each of the seeds are of different polarities. Since it is known that a compounds solubility in a supercritical fluid is strongly dependent on the compounds polarity, it would be expected that different polarity phospholipids would also exhibit different solubilities in the CO_2 (Zosel, 1978).

CONCLUSIONS

The results of the present study indicate that little fractionation of the canola oil occurs during the supercritical CO_2 extraction process. Only at a very late stage in the extraction process does the concentration of heavier fatty acids begin to increase. This is unlike the hexane extraction process where the fatty acid composition of the oil remains constant throughout the extraction.

The experiments further indicate that the CO_2 extraction process does not extract phospholipid material from the seed. This fact could be significant for commercial seed processors. Conventional hexane extracted oil requires an acid degumming step to reduce the phosphorus levels in the oil to 5–10 ppm. Since the oil produced with supercritical carbon dioxide has a phosphorus content in this range, it should not require acid degumming. An extraction process based on supercritical extraction would in this respect be simpler and less expensive than the corresponding hexane process.

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Registry No. P, 7723-14-0; CO_2, 124-38-9; hexane, 110-54-3; erucic acid, 112-86-7.

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