Extraction of Phospholipids from Canola with Supercritical Carbon Dioxide and Ethanol

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ABSTRACT: The potential use of supercritical (SC)–CO_2/ethanol mixture for the extraction and fractionation of phospholipids (PL) from flaked canola seeds, canola meal, and acetone insolubles (AI) was investigated. PL extraction was possible when ethanol was used as a cosolvent in SC–CO_2. PL recovery of 20.8% was achieved when canola flakes were extracted at 70°C and 55.2 MPa with SC–CO_2/10% EtOH after oil removal with neat SC–CO_2. Soaking of canola meal with ethanol prior to SC–CO_2/EtOH extraction increased PL recovery to 30.4%. PL content of the extracts increased with decreasing triglyceride concentration in the feed material and increasing amounts of ethanol added to SC–CO_2 or used for soaking. Fractionation of AI gums resulted in extracts containing 50% PL, of which 90% was phosphatidylcholine (PC); but yields were low, even after soaking treatment, due to caking. SC–CO_2/EtOH mixture may be used to extract PC-enriched PL from flaked canola seeds, canola meal, and AI. However, further research is needed to improve extraction efficiency.
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KEY WORDS: Canola, ethanol, phosphatidylcholine, phospholipids, supercritical carbon dioxide.

The term "lecithin" refers to a by-product of the edible oils industry, consisting of a mixture of phospholipids (PL) mixed with vegetable oil. Lecithins produced from egg yolk and soybeans are commercially available. Although canola has slightly higher PL concentration than soybeans (1.5–3.6 wt% vs. 1.1–3.2 wt.%), it is not used for commercial lecithin production. Lecithin is an important natural emulsifier, which is used in food, feed, and pharmaceutical industries. Crude lecithin has both weak water-in-oil (w/o) and oil-in-water (o/w) emulsifying properties due to the constituent PL. To improve its properties for specific product applications, crude lecithin is modified, refined, and/or fractionated.

Demand for lecithin with a high phosphatidylcholine (PC) content has increased in the cosmetic, pharmaceutical, food, and other industries (1). High-PC lecithin has better o/w emulsifying properties, and it functions over a wider pH range. PC is also reported to have beneficial therapeutic effects, such as lowering cholesterol levels and for the treatment of neurological disorders (2). Industrial-scale modification of crude lecithin is performed by physical, chemical, and enzymatic methods (3). Commercially deoiled lecithin is obtained by treating crude lecithin with acetone. The disadvantages of this process are the high temperatures required for drying the product as well as incomplete solvent removal.

Supercritical fluid extraction (SCFE) of crude lecithin is a relatively new process. Crude lecithin, when extracted with supercritical (SC)–CO_2, produces a deoiled lecithin without any residual solvent. This product can be used directly for medicinal purposes, and its physical properties, such as aroma and color, are better than those of the products obtained by conventional methods (4). Heigel and Hueschens (4) have patented an SC–CO_2 extraction process for the production of deoiled lecithin from crude soybean lecithin, which contained 30% (wt%) oil. When crude lecithin was extracted with CO_2 at 60°C and 40 MPa for 4 h, a solid lecithin was obtained with a light yellow color (4). However, when commercial liquid lecithin was extracted with SC–CO_2 at 30–40 MPa and 32–60°C by Castera (5), a product was obtained that was darker in color but had less soybean odor than their product from acetone insolubilization. Alkio *et al.* (6) obtained a crystalline or semiplastic product from crude oat lecithin after extracting the oat oil with SC–CO_2. This product had acceptable flow properties and appearance, eliminating the need for any additives. The PL concentration of oat lecithin, obtained by SC–CO_2 extraction (30–35%, wt%), was lower than that of soybean lecithin (50%, wt%) obtained by the same technique. However, oat lecithin exhibited better antioxidant properties than soybean lecithin (6).

A continuous near-critical fluid extraction process with CO_2 has been developed to remove oil from crude soybean lecithin (7), yielding a solvent-free powder, yellow-white in color. In this and another study (8), design and operation of a continuous SCFE pilot-plant system for deoiled lecithin have been described. Improving contact between the solvent and viscous lecithin under high pressure, by adding crude lecithin on an inert carrier material, or the addition of organic solvents was advocated. The use of stirred extractors or the development of a high-pressure jet injection system for introducing the crude lecithin into the solvent stream were other options discussed (8). Eggers and Wagner (9) developed a semi-industrial-scale apparatus to bring viscous lecithin into contact

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with SC–CO_2 in a special spraying device. While the above-mentioned studies (4–9) focused on oil removal from crude lecithin, List *et al.* (10) developed a countercurrent process to degum crude soybean oil. The phosphorus content of crude oil was reduced from 620 ppm to <5 ppm with SC–CO_2 at 55 MPa and 70°C (10).

The SC solvent of choice for food applications, CO_2, exhibits a low solubility for the relatively polar PL. It has been reported that neat SC–CO_2 does not extract PL from crushed oilseeds (11,12). Soybean and canola oils extracted with SC–CO_2 contain less than 100 ppm PL, while a hexane-extracted oil contains approximately 1% (wt%) PL (11,12). Thus, when SC–CO_2 is used for oil extraction, valuable PL are left behind in the meal. However, addition of a polar cosolvent would enhance the selectivity of the SC solvent toward PL. Temelli (13) has qualitatively shown the recovery of PL from canola flakes and press cake with an SC–CO_2/EtOH mixture after SC–CO_2 extraction of oil. Bulley *et al.* (14) demonstrated that SCFE of PL from freeze-dried egg yolk with methanol or ethanol as a cosolvent (at 3–5%, wt%) in SC–CO_2, yielded extracts containing 6.8–17% (wt%) PL. More recently, there have been simultaneous efforts focusing on the recovery of PL from oilseeds: canola (15), soybean (16), and cottonseed (17). Montanari *et al.* (16) used a consecutive two-step SCFE process to deoil soybean flakes and to isolate PL-enriched fractions. These studies (13–17) demonstrated that the addition of ethanol as a cosolvent into SC–CO_2 allows selective recovery of polar PL from oilseeds, but optimization of processing parameters and fractionation of PL mixtures need further investigation.

The objectives of this study were to investigate the use of SC–CO_2/EtOH mixtures to extract PL from flaked canola seeds, canola meal, and the acetone-insoluble (AI) fraction obtained from crude canola lecithin, and to examine the effects of processing parameters, such as temperature, pressure, and ethanol concentration, on PL recovery and the PC content of the extracts.

MATERIALS AND METHODS

Crude canola (*Brassica napus* and *B. campestris*) lecithin, flaked canola seeds, and canola meal were obtained from Canamera Inc. (Fort Saskatchewan, Alberta, Canada) and kept below –20°C until used. Headspace of the crude canola lecithin containers was flushed with nitrogen before storage.

Oil content of the flaked canola seeds was determined according to the American Oil Chemists Society Official Method Ac 3-44 (18). Flaked canola seeds and canola meal were extracted with chloroform/methanol (2:1, vol/vol) mixture according to the method of Sosulski *et al.* (19) to determine PL content.

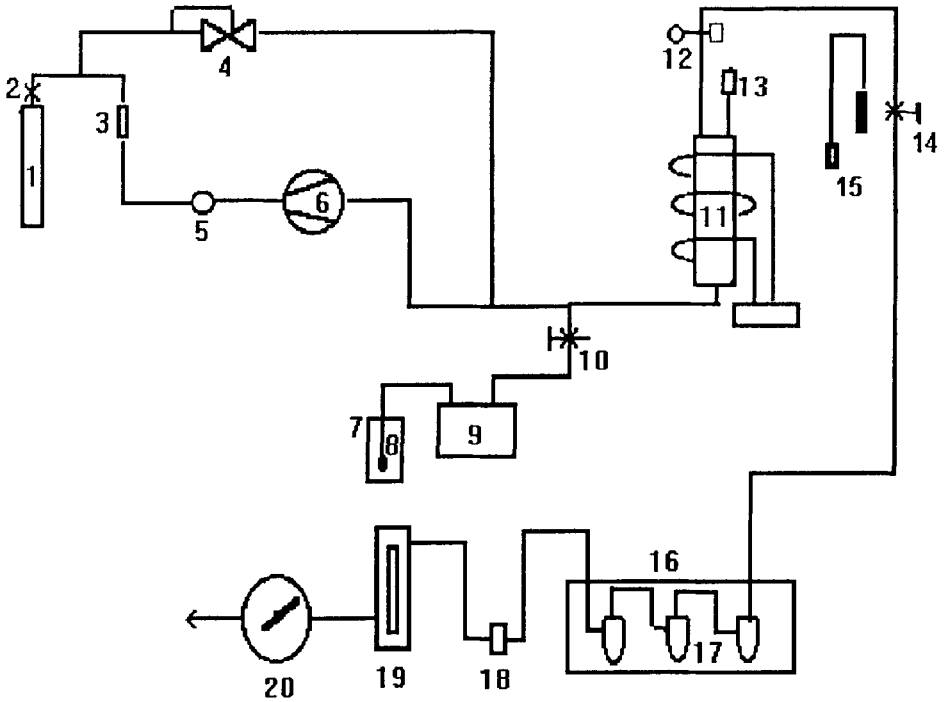
AI was obtained by treating 25 g of crude canola lecithin with acetone (99.5% purity; Omni Solv., BDH Inc., Toronto, Ontario, Canada) at room temperature. The oil was removed from crude lecithin by three consecutive extraction steps, consisting of the addition of 150, 100, and 100 mL acetone, followed by 1 h of stirring for each stepwise addition. AI were filtered after each step, pooled, and dried in a vacuum oven at 40°C for 24 h. All dried samples were stored at <0°C.

SCFE. A laboratory scale SCFE unit (Newport Scientific Inc., Jessup, MD), previously described by Temelli (13), was used. The system was modified for cosolvent introduction into the supercritical solvent [99.9% (wt%) pure CO_2; Medigas Alberta Ltd., Edmonton, Alberta, Canada] in this study. A flow diagram for the modified SCFE unit is shown in Figure 1. Ethanol of 99.9% purity (Commercial Alcohol Ltd., Montreal, Canada) was pumped into the system by a high-pressure piston pump (Model 305; Gilson Inc., Middleton, WI).

Although the maximum pressure obtainable on the SCFE unit was 69.0 MPa, a maximum pressure of only 55.2 MPa was used for the described experiments, due to the lower pressure limit of the cosolvent addition pump. The mole percent of ethanol continuously pumped into the SC–CO_2 during the extraction period was reported as the average based on the total amount of CO_2 passed through the system for each run because of slight fluctuations in the CO_2 flow rate as ethanol separates in the depressurization valve. The volume of CO_2 used was recorded by a dry gas meter. The extract and ethanol mixture was collected in glass tubes, which were attached after the depressurization valve and held in a refrigerated circulating bath (Lauda, Model RMT-6; Brinkmann, Rexdale, Ontario, Canada) at –15°C (see Fig. 1). The quantities of the various extracts were determined gravimetrically. For the cosolvent-based runs, ethanol was removed from the final extract by rotary evaporation under vacuum at a temperature of 45 ± 3°C before gravimetry. All experiments were performed in duplicate.

Canola flakes. The extractor cell was loaded with 45 g flaked canola seeds for each experiment. SCFE experiments were performed at temperature and pressure ranges of 45–70°C and 41.1–62.1 MPa, respectively. Two sets of experiments were performed with canola flakes. Full-fat canola flakes were used for the first set of experiments. SC–CO_2 extractions of full-fat canola flakes were performed, with and without the addition of ethanol (8%, mole %) as cosolvent. The second set of experiments was performed with canola flakes of a reduced oil content. To affect this oil reduction, full-fat canola flakes were extracted with SC–CO_2 at 62.1 MPa and 70°C until the oil content was reduced to <15% (wt%), prior to the addition of ethanol into the system at 5 or 10% levels. These extraction conditions were determined to give maximum oil solubility in SC–CO_2 with the same unit (13). All canola flake extractions were done for 3 h.

Canola meal. Sixty-five grams of canola meal were used for each experiment. Initially, experiments were performed with an SC–CO_2/EtOH (5.9 mole%) mixture at 55.2 MPa and 70°C. A second set of experiments was performed by soaking the canola meal. For the solvent-soaking experiments, SC–CO_2 and ethanol were added to the system in two stages. During a pre-extraction period, CO_2 was pumped into the system until the designated extraction pressure and temperature



1-CO2 tank	8-filter	
2-valve	9-ethanol pump	15- temperature controller
3-CO2 filter	10-valve	16-cooling bath
4-pressure regulator	11-extractor	17-collection tubes
5-pressure gauge	12-extractor temperature controller	18-silica trap
6-compressor	13-rupture disc	19-flow indicator
7-ethanol reservoir	14-depressurization valve	20-flow totalizer

FIG. 1. Flow diagram of the supercritical fluid extraction unit.

were reached. During this initial pressurization, 50 mL of ethanol was pumped into the extraction cell over a 2-min period. The compressor was then allowed to run for 90 min to improve the mixing of CO_2 and ethanol. This period of the extraction was referred to as the "soaking" step because the depressurization valve was closed.

The second stage of the extraction was started after opening the depressurization valve, followed by CO_2 pumping for 1.5 h at a flow rate of 3.7 ± 0.2 L/min (as measured at room temperature and pressure). Throughout this extraction stage, ethanol was mixed into the CO_2 by the Gilson pump at a constant flow rate.

AI. SCFE of AI was performed within the temperature and pressure ranges of 45–70°C and 20.7–55.2 MPa, respectively. Twenty grams of canola AI were used in each experiment. Experiments with or without soaking were performed similarly to the canola meal experiments by using the following parameters: The quantity of ethanol used for soaking was varied from 0–60 mL for a soaking period of 60 min, keeping the CO_2 flow rate at 1.1 ± 0.2 L/min.

PL analysis. Total PL content of the samples was determined by perchloric acid digestion, followed by spectrophotometric quantitation of phosphorus according to the Bartlett method (20) as modified by Marinetti (21). A conversion factor of 25.6 was used to convert total phosphorus to PL amounts. Calculation of the conversion factor was based on the PL composition of canola AI reported by Vaisey-Genser and Eskin (22). Molecular weights of individual canola PL were taken from Fattori *et al.* (12). Separation of PL components and quantitation based on the peak areas were done by an Iatroscan TH-10 Mark II analyzer equipped with precoated silica gel chromarods SII (T.M.A. Scientific Supply, Mississauga, Ontario, Canada), according to the method of Ratnayake and Ackman (23). The chromarods were developed in two steps: acetone was used first to separate the nonpolar compounds, followed by a polar solvent system of chloroform/methanol/water (65:35:4, vol/vol/vol). Chromarods were scanned under the following conditions: hydrogen pressure 73.5 kPa, air flow rate 2 L/min. Peak areas were integrated with the Hewlett-Packard Series 3356 laboratory automation system (Hewlett-Packard, Wilmington, DE). PL standards (Sigma Chemical Co., St. Louis, MO) [PC, phos-

phatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA)], were used for peak identification. Lipid standards and samples were kept at −80°C until analysis. Analyses were performed in duplicate for each sample.

RESULTS AND DISCUSSIONS

Oil, PL, and PC content of the starting materials are given in Table 1.

Canola flakes. When full-fat canola flakes were extracted with SC–CO_2 at 55.2–62.1 MPa and 45–70°C, no phosphorus was detected in the extracts. These results were in agreement with previous findings (11,12), confirming the higher selectivity of SC–CO_2 for nonpolar triglycerides (TG) relative to polar PL in the presence of large amounts of TG.

Addition of 8% ethanol into the SC–CO_2 during the extraction of full-fat canola flakes resulted in a PL concentration of <200 ppm in the oil extracted at 55.2 MPa and 70°C. This indicates that the selectivity of SC–CO_2 for TG was not affected by the presence of ethanol at 8% level when full-fat canola flakes were extracted.

Freidrich *et al.* (24) reported that the phosphorus concentration of SC–CO_2-extracted soybean oil increased in the final oil fractions. While earlier fractions (first 10–20%) contained less than 20–25 ppm phosphorus, last fraction (last 10%) had ~95 ppm phosphorus. Similar results have been obtained on freeze-dried egg yolk (14). When canola flakes of reduced oil content were extracted with SC–CO_2 in the presence of ethanol, PL were detected in the extracts. Table 2 shows that the PL concentration of the SC–CO_2/EtOH extracts increased with decreasing oil content in the canola flakes. Further deoiling of the canola flakes beyond ~14% (wt%) was not attempted because attainable extraction rates in this region were low with the available SCFE unit. However, further deoiling of the canola flakes with neat SC–CO_2 should increase PL extraction efficiency with SC–CO_2/EtOH in the second step. Experiments performed at 55.2 MPa, 70°C, and two levels of ethanol addition showed that both the amount and the PL concentration of the extracts increased with increasing ethanol percentage in SC–CO_2 (Table 2). Doubling the amount of ethanol added, from 5 to 10%, resulted in a 10-fold increase in the PL concentration of the extracts. PL concentrations of the extracts obtained at different temperatures and pressures were between 4.0–5.4% (Table 3). There appears to be an anomaly in PL content of extracts obtained at 55.2 MPa and 55°C. The amount of extract collected varied between 1.5–2.7 g. No consistent trend was observed in either the amount or the PL concentration of the extracts with extraction pressure; however, PL concentration increased with temperature.

Canola meal. Although the composition of canola lecithin (22) is similar to that of soybean lecithin (3), it has not been previously utilized and is added back to the meal, which is used as animal feed. During commercial processing of canola flakes, the flake oil content is reduced to 1–2% by hexane extraction. However, addition of crude canola lecithin back into the residue increases the total lipid content of the meal up to 8% (Table 1). To study SC–CO_2/EtOH extraction of PL, hexane-extracted canola flakes were used as a solid support for crude lecithin. Such a solid support would provide better contact between the SC solvent and the lecithin because solvent-feed contact (due to the gummy consistency of crude lecithin) had been a major problem during SCFE of crude lecithin. When canola meal was extracted with SC–CO_2/EtOH mixture, PL concentration of the extracts increased with increasing ethanol percentage in the SC–CO_2 (Table 4).

TABLE 1
Oil, Phospholipid (PL), and Phosphatidylcholine (PC) Concentrations of the Feed Material for Extraction

	Oil content (wt%)	PL content (wt%)	PC content (area %)[a]
Flaked seeds	43.0[b]	3.7	n.d.[c]
Meal	8.0[d]	46.0	41.5
Acetone insolubles	n.d.[c]	72.0	41.6

[a]Based on the total Iatroscan peak area of all PL fractions.
[b]Determined according to AOCS Official Method (Ref. 18).
[c]Not determined.
[d]Total lipids determined according to Sosulski *et al.* (Ref. 19).

TABLE 2
Effect of the Triglyceride Content of Canola Flakes and Ethanol Concentration on the SC–CO_2/EtOH Extracts[a]

Pressure (MPa)	55.2[b]	55.2[b]	55.2[b]	55.2[b]	55.2[c]
Temperature (°C)	70	70	55	55	70
Oil content of flakes (wt%)	14.0	14.0	12.0	23.0	43.0
Ethanol % in SC–CO_2 (mole %)	5.0	10.1	10.3	10.1	8.3
Amount of extract (g)	1.0	2.7	2.3	6.4	3.5
PL content of extracts (wt%)	0.5	5.4	1.3	0.7	n.d.[d]

[a]SC–CO_2, supercritical–CO_2. See Table 1 for other abbreviation.
[b]CO_2 flow rate = 3.0 L/min.
[c]CO_2 flow rate = 1.1 L/min.
[d]Not detected.

TABLE 3
Effect of Extraction Conditions on the SC–CO_2/EtOH Extracts of Canola Flakes at a CO_2 Flow Rate of 3.0 L/min[a]

Pressure (MPa)	55.2	55.2	55.2	41.1	41.1
Temperature (°C)	45	55	70	45	55
Oil content of flakes (wt%)	13	12	14	11	12
Ethanol % in SC–CO_2 (mole%)	9.5	10.3	10.1	9.2	9.1
Amount of extract (g)	1.5	2.3	2.7	2.0	1.8
PL content of extracts (wt%)	4.0	1.3	5.4	4.4	5.1

[a]See Tables 1 and 2 for abbreviations.

TABLE 4
Effect of Ethanol Concentration in SC–CO_2 on the SC–CO_2/EtOH Extracts of Canola Meal[a]

Pressure (MPa)	55.2	55.2
Temperature (°C)	70	70
Ethanol % in SC–CO_2 (mole%)	5.0	9.0
Amount of extract (g)	0.8	2.9
PL content of extracts (wt%)	0.4	1.2

[a]See Tables 1 and 2 for abbreviations.

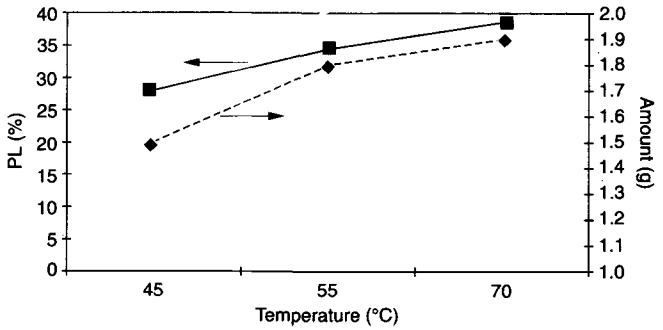


FIG. 2. Effect of temperature on the amount (---) and phospholipid (PL) concentration (—) of the canola meal extracts obtained with supercritical–CO_2/7.5 ± 0.5% EtOH mixture at 55.2 MPa and CO_2 flow rate of 3.7 ± 0.2 L/min. Samples were soaked with 50 mL of ethanol under 55.2 MPa for 90 min prior to extraction.

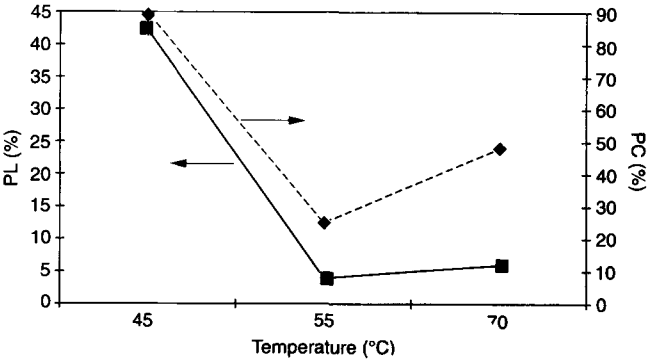


FIG. 3. Effect of temperature on the PL (—) and phosphatidylcholine (PC) (---) concentrations of supercritical–CO_2/13 ± 2% EtOH extracts of canola acetone insolubles at 55.2 MPa. See Figure 2 for other abbreviations.

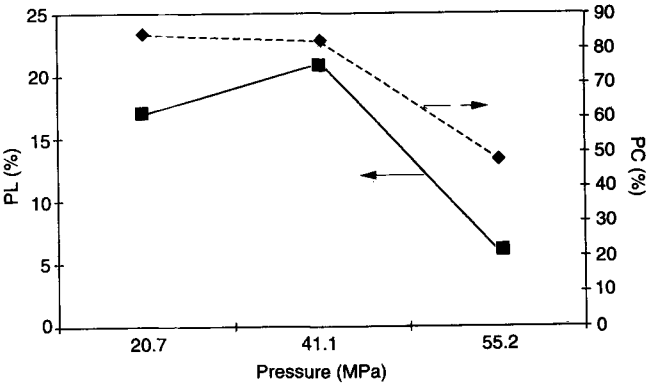


FIG. 4. Effect of pressure on the PL (—) and PC (---) concentrations of supercritical–CO_2/13 ± 2% EtOH extracts of canola acetone insolubles at 70°C. See Figure 3 for abbreviations.

In an effort to increase the amount of extract and its PL concentration, canola meal samples were soaked with ethanol under high pressure prior to extraction. In this case, both PL concentration and the amount of extract increased with increasing extraction temperature (Fig. 2). When the canola meal was soaked for 90 min at 55.2 MPa and 70°C and then extracted with SC–CO_2/7.5% EtOH, it was possible to obtain extracts with a PL concentration of ~39% (wt% of extract) (Fig. 2). Under these conditions, 1.9 g of extract was collected. The effect of pressure on the soaking treatment was examined by treating canola meal with ethanol at atmospheric pressure for 6 h prior to SC–CO_2 extraction. Although, the amount of extract was slightly higher, 2.4 g, PL concentration of the extract was lower, 34%, for atmospheric soaking compared to that for soaking under pressure.

Canola acetone insolubles. Extraction of canola AI with SC–CO_2 in the presence of ethanol was investigated in an effort to fractionate the PL components. The effects of temperature and pressure on the AI extracts obtained without prior soaking are shown in Figures 3 and 4, respectively. The lowest PL concentration was obtained at the highest pressure studied at 70°C (Fig. 4). The highest PL concentration in the extracts, 42%, was reached at 55.2 MPa and 45°C (Fig. 3). Experiments performed with AI to study the effect of temperature did not follow the same trend as canola meal extraction. Optimum temperature and pressures were 45°C and 41.1 MPa, respectively, for AI extraction without soaking. Extraction runs performed at 45°C and 41.1 MPa might improve the extraction efficiency. The major problem with AI extractions was the formation of a cake in the extractor. This caking problem has to be solved before any further investigation of optimum processing conditions.

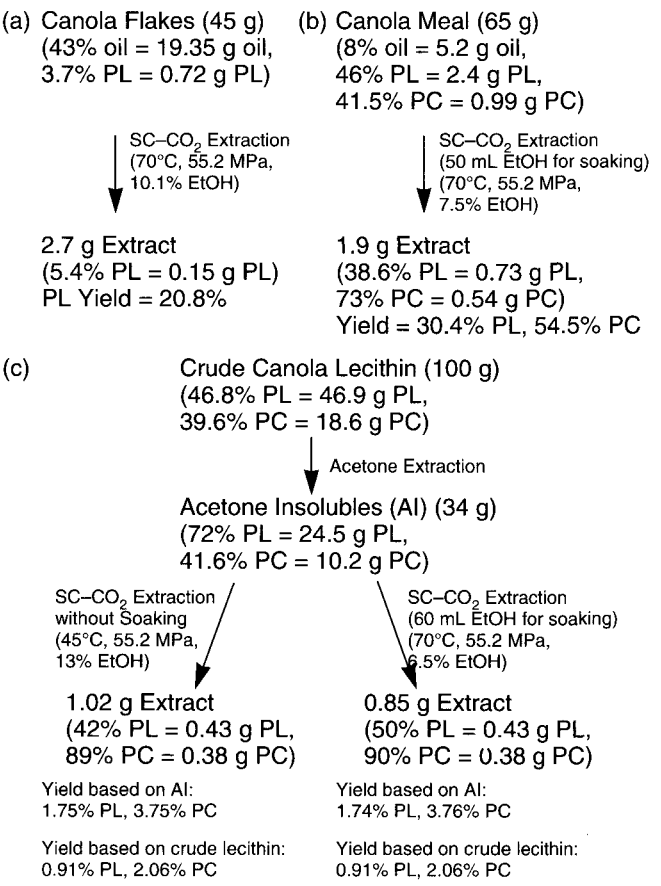
When AI samples were soaked with ethanol at 55.2 MPa and 70°C and then extracted with SC–CO_2/13 or 6.5% EtOH, the PL concentration and amount of the extract increased with an increase in the amount of ethanol used for soaking (Table 5). Extraction conditions for the soaking experiments performed with AI were based on the optimum extraction conditions (70°C and 55.2 MPa) for the previous experiments, done with canola flakes and meal. A maximum PL concentration of 50% was obtained with a SC–CO_2/6.5% EtOH mixture at 55.2 MPa and 70°C, with 60 mL of ethanol used for soaking. Higher PL and PC yields, obtained from canola meal (Fig. 2) with soaking, compared to AI also support the fact that solvent-feed contact was hindered by cake formation in the extractor. Use of a stirred extractor should improve solvent-feed contact.

To assess the potential to obtain PC-enriched extracts from various canola sources, extracts were analyzed for their PC content. Clear separation of PL fractions, other than PC, was not possible with the Iatroscan method used in this study. Therefore, PL components present in the extracts other than PC were pooled for the concentration calculation. PC concentration in each extract was calculated as the percentage of the total Iatroscan peak areas for all PL fractions in the sample. These analyses showed that PC content of the samples increased with increasing PL concentration in the extracts. The canola flake extracts with 5.4% PL (Table 2), obtained at 55.2 MPa and 70°C, had only 4.2% PC. A PC concentration as high as 72.7% was reached in the meal extracts with 39% PL obtained at 55.2 MPa and 70°C with 90 min of ethanol soaking (Fig. 2). On the other hand, extracts of canola meal soaked

TABLE 5
Effect of the Amount of Ethanol Used for Soaking and Ethanol Percentage in the SC–CO_2 During the Extraction Period on the AI Extracts[a]

EtOH in SC–CO_2 (mole%)	6.5			13.0		
EtOH used in soaking (mL)	20	40	60	20	40	60
Amount of extract (g)	0.25	0.4	0.5	0.3	0.4	0.5
PL content of extracts (wt%)	7	29	50	9	26	32
PC content of extracts (area %)[b]	66	85	90	73	86	83

[a]Samples were soaked at 55.2 MPa and 70°C for 60 min prior to extraction. See Tables 1 and 2 for abbreviations.
[b]Based on the total Iatroscan peak area of all PL fractions.



SCHEME 1

at atmospheric pressure, obtained at the same extraction conditions, had only 19.0% PC. With canola AI, the highest PC concentration was reached in the extracts with 50% PL content, which were obtained with 60 mL ethanol soaking and SC–CO_2/6.5% EtOH extraction. In these samples, 90% of the PL extracted was PC (Table 5). The extracts that had the lowest PL concentration also had the lowest PC concentration. These results can be explained with the higher solubility of PC in ethanol compared to other PL components.

Sample material balance and yield calculations are given in Scheme 1. Maximum PL yield of 30.4% was reached with meal samples soaked with 50 mL ethanol, followed by SC–CO_2/7.5% EtOH extraction at 55.2 MPa and 70°C (Scheme 1b). For the same samples, PC yield (54.5%) was higher than the PL yield, which indicates higher selectivity for PC. The highest attainable PL yield for canola flakes was 20.8% (Scheme 1a). The maximum PL recovery from soybean flakes reported by Montanari *et al.* (16) was 6.7% with SC–CO_2/10.2% EtOH at 68.2 MPa and 80°C. PL and PC yields were low for AI extracts obtained with or without soaking, because a caking problem was apparent in both (Scheme 1c).

Recovery of all valuable components is necessary if SC–CO_2 extraction of oilseeds is to be commercialized. Lecithin is a valuable by-product of the conventional process, but PL are left in the meal during SC–CO_2 extraction. This study demonstrated that PL can be recovered in a second step with SC–CO_2/EtOH mixture after oil extraction is affected with neat SC–CO_2 and processing parameters were optimized. The oil content of the seeds must be lowered as much as possible in the first step to increase the efficiency of PL extraction as well as to minimize oil in the PL extracts. Soaking of feed material with ethanol was a new approach in an effort to improve PL recovery and fractionation, inspired by the fact that coffee beans are saturated with water to release caffeine prior to caffeine extraction with SC–CO_2 (25). Soaking meal with ethanol improved PL recovery to 30.4%, compared to 20.8% from flakes without soaking. It may be possible to scale up this treatment by spraying ethanol onto the bed of flakes to saturate them with ethanol after neat SC–CO_2 extraction of oil and then extracting the PL with SC–CO_2/EtOH. Feasibility of such a soaking treatment on a larger scale needs to be further evaluated because extraction time and cost will increase while improving yield. Obviously, the soaking time between the two extraction steps needs to be minimized. When AI was the feed material, soaking prior to extraction did not improve PL recovery due to cake formation in the extractor. Design of an SCFE unit with a mixing mechanism in the extractor for better contact between solvent and the feed material should improve extraction efficiency.

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