Selective Extraction of Phosphatidylcholine from Lecithin by Supercritical Carbon Dioxide/Ethanol Mixture

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ABSTRACT: Selective extraction of phosphatidylcholine (PC) from deoiled soybean lecithin using supercritical fluid (SCF) mixtures of carbon dioxide (CO_2) and ethanol was studied at moderate pressures. Temperature was varied between 60 and 80°C at pressures of 17.2 and 20.7 MPa. Ethanol was added as co-solvent to supercritical CO_2 at the levels of 10 and 12.5 wt%. Constant rate of extraction of the individual phospholipids (PL) was observed for 150 min during which the extractions were carried out. Pressure and ethanol fraction had a positive effect on the selective extraction of PC, whereas temperature had a negative effect. Under all the conditions studied, the extracts were mainly composed of PC while the extraction of the other PL was very low. Extraction at 60°C and 20.7 MPa with 10 wt% ethanol/90 wt% CO_2 SCF mixture resulted in 95% selectivity to PC.

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KEY WORDS: Carbon dioxide, ethanol, lecithin, phosphatidylcholine, phospholipids, selective extraction, supercritical fluid extraction.

Lipids that are composed of two fatty acids and a phosphorus-containing region joined by ester linkages to a glycerol backbone are called phospholipids (PL). Since phosphate groups can ionize, PL have both polar and nonpolar characteristics (1). Phosphatidylcholine (PC), a major PL, has gained special attention during the last few decades owing to various health benefits it provides. Choline and its primary source in diet, PC, play important roles in cardiovascular and liver health, and in reproduction and development. Several studies suggested a possible therapeutic use for choline and PC in certain neurological disorders and liver cirrhosis. Choline has also been shown to improve memory in humans (2).

Lecithin, a by-product of the vegetable oil refining process, is the major source of PC along with some other PL, neutral lipids, carbohydrates, glycolipids, and some other impurities. Several methods such as solvent extraction, solvent treatment after chemical modification, precipitation, ultrafiltration, and chromatographic methods have been used for fractionation of deoiled lecithin in order to obtain high-purity PC (3). Ethanol has been the choice of solvent for the first PC enrichment due to both toxicological reasons and high solubility of PC in ethanol, but ethanol extraction is not sufficient to obtain high-purity PC, as phosphatidylethanolamine (PE) is also soluble in ethanol to a significant extent. Today, chromatographic separation seems to be the method of choice to prepare both high-purity PC and other PL. However, the major problem with chromatographic methods is the large volume of solvent involved in separation, making the process very expensive.

Various studies have shown that supercritical carbon dioxide (SC-CO_2) is very effective in removing oil from different seed matrices. Oil extracted from soy flakes with SC-CO_2 was characterized for free fatty acid, phosphorus, tocopherol and iron contents, neutral oil loss, and color, and the product was found to be similar to a hexane-extracted degummed oil (4). However, better oxidative stability was observed with the hexane-extracted degummed oil. This was attributed to the low levels of phosphorus content indicating low levels of PL, although the function of PL in protecting crude oil from oxidation is unclear. This feature was used for SC-CO_2 degumming of hexane-extracted crude soybean oil in which the phosphorus content of the crude oil was reduced from 620 ppm to less than 5 ppm (5).

SC-CO_2 does not dissolve PL effectively, but recovery of PL can be achieved by addition of a polar entrainer (or co-solvent) to SC-CO_2. Presence of an entrainer enhances the solubility in the supercritical fluid (SCF) at the same temperature and pressure, making it possible to conduct the extraction at lower pressures. Addition of 10 wt% ethanol to SC-CO_2 increased the solubility of palm oil from 0.25 to 5% at 20.3 MPa and 70°C (6). Choice of an entrainer, especially for food applications, must take into account not only the desired properties based on thermodynamic fundamentals but also some regulatory aspects such as food safety. It has to be a Generally Recognized As Safe (GRAS) solvent. Temelli (7) qualitatively demonstrated the extraction of PL from canola meal using SC-CO_2 after impregnation of the seeds with ethanol, which is accepted GRAS in the USA. Dunford and Temelli (8) added ethanol into the SCF phase continuously and observed a positive effect of ethanol fraction on the PL extraction from canola meal. In another study, in addition to ethanol, methanol and isopropanol were also found to be efficient in extracting more than 90% PC in a few stages of countercurrent extraction (9). Montanari *et al.* (10) carried out ex-

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traction of PL from soybean flakes, after extraction of oil with neat CO_2, with SC-CO_2/10 wt% EtOH for 30 min varying the pressure from 16.6 to 68.9 MPa and the temperature from 60 to 80°C. They observed PC enrichment at low pressures (80.1% PC, relative to the other PL, at 19.4 MPa and 80°C) although total yields increased with increasing pressure.

Currently, we are working on the extraction of PC from a PL concentrate obtained as the retentate of a membrane oil refining process in order to produce high-purity, pharmaceutical-grade PC. Here, the first part of that study is being reported in which the objective was to investigate the effect of pressure, temperature, ethanol fraction in the supercritical phase, and extraction time on the selective extraction of PC at low-to-moderate pressures using a commercially-available deoiled lecithin as a model mixture in order explore the optimal conditions.

EXPERIMENTAL PROCEDURES

The soybean lecithin used for selective extraction of PC was CENTROLEX®F with a composition of 23% PC, 20% PE, 14% phosphatidylinositol (PI), 8% phosphatidic acid (PA), 8% minor PL, 15% glycolipids, 8% complexed sugars, 3% triglycerides, and 1% water. It was obtained from Central Soya Company, Inc. (Gibson City, IL), who also provided the data on composition. Ethanol (99.8%) was purchased from Omni Solv-EM Industries (Gibbstown, NJ). CO_2 was obtained from Brazos Valley Welding Supply, Inc. (Bryan, TX).

The experimental apparatus used for PL extraction is shown in Figure 1. The co-solvents, CO_2 and ethanol, were delivered by two separate syringe pumps (Isco, Inc., Lincoln, NE). The flow rates of CO_2 and ethanol necessary to achieve the desired composition of the SC-CO_2/ethanol mixture were calculated from a mass balance. Density values of SC-CO_2/ethanol were taken from Pohler and Kiran (11). After bringing the isothermal chamber to the desired temperature *via* the immersion heater 6, the system pressurized with CO_2 slowly to the desired pressure. Ethanol flow was then started, and CO_2 and ethanol were mixed and passed through an equilibration coil. The flow was first passed through a by-pass line to determine the ethanol breakthrough, which was monitored by an on-line Variable Wavelength Absorbance detector (Isco, Inc.).

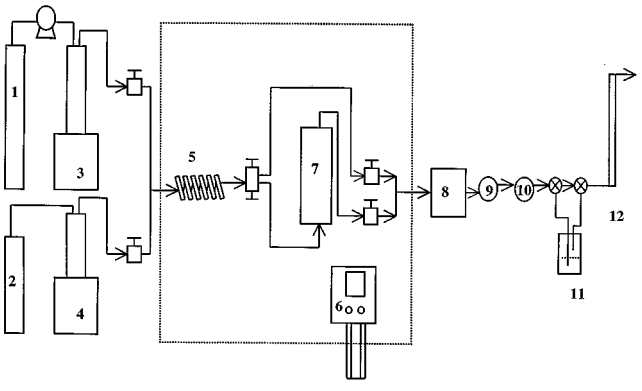

FIG. 1. Supercritical fluid extraction system: (1) carbon dioxide cylinder; (2) co-solvent reservoir; (3) syringe pump for carbon dioxide; (4) syringe pump for co-solvent; (5) equilibration coil; (6) heater; (7) fixed-bed column; (8) ultraviolet/visible detector; (9) backpressure regulator; (10) backpressure regulator; (11) sampling vial; (12) flowmeter.

The absorbance detector served only for qualitative purposes. After the steady state was observed through the breakthrough curve, outlet ethanol and CO_2 flow rates were measured to close the mass balance. Ethanol flow rate was determined by measuring the volume of ethanol collected in the sampling vial with respect to time after expansion. The sampling vial contained activated carbon and was cooled in an ice bath in order to capture all the ethanol. CO_2 flow rate was measured with a flow meter. Once the steady state was reached and the mass balance was confirmed, the flow was switched to the extraction column. The PL breakthrough curve was monitored with the on-line absorbance detector. The effluent was bubbled through chloroform after expansion *via* the two backpressure regulators placed in series, in order to capture the extracted PL. Two backpressure regulators were used in order to obtain a smooth flow in the system and eliminate back pulsing in the extractor. Extracts were then dried under nitrogen and redissolved in chloroform for further analysis of individual PL fractions.

Extractions were conducted for 150 min on samples of 3 g lecithin at pressures of 17.2 and 20.7 MPa. Temperature was varied between 60 and 80°C; extractor temperature was measured by a thermocouple (not shown). Ethanol fractions of 10 and 12.5 wt% were used with the SCF flow rate of 1 and 2 mL/min.

PL analyses were performed according to the high-performance liquid chromatography (HPLC) analysis developed by Hurst and Martin (12). A normal-phase silica column μPorasil (3.9 mm i.d. × 300 mm) (Waters, Milford, MA) was used. The mobile phase was acetonitrile/methanol/85% phosphoric acid (780:10:9, vol/vol/vol). The HPLC flow rate was 1 mL/min. There was a 5-min isocratic equilibration time between each injection. An injection loop of 5 μL was used. HPLC column calibration was performed using a standard mixture (obtained from Sigma, St. Louis, MO) containing L-α-PE, L-α-PC, L-α-PI, and L-α-lysophosphatidylcholine (LPC). The standard mixture had 3.0 mg PC, 2.4 mg PE, 1.8 mg PI, and 0.6 mg LPC in 2 mL chloroform solution.

RESULTS AND DISCUSSION

Extraction behavior of PL from a commercially available deoiled lecithin by supercritical mixtures of CO_2 and ethanol was investigated at pressures of 17.2 and 20.7 MPa. These pressures were selected as higher PC enrichment was observed at low pressures using 10 wt% ethanol in SC-CO_2 (10). Ethanol was chosen as the co-solvent because: (i) It is GRAS; (ii) solubility of PC is higher in ethanol than the solubility of other PL in ethanol (13); and (iii) phase behavior of CO_2/ethanol at high pressures is available (11,14,15).

An on-line ultraviolet-detector was used to obtain the breakthrough profiles. Although the breakthrough curves

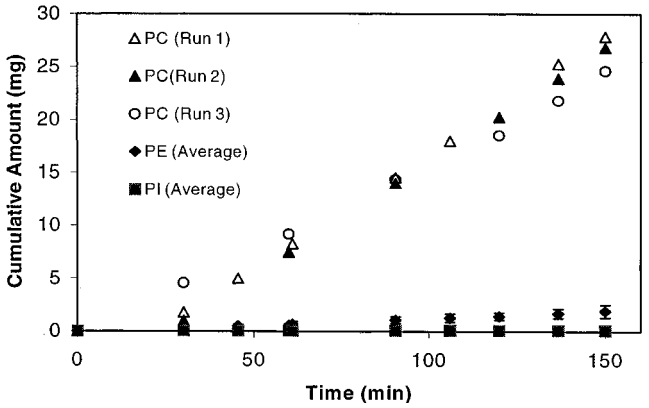

FIG. 2. Extraction of phospholipids (PL) at 20.7 MPa, 60°C with 90% CO_2/10% ethanol. PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol.

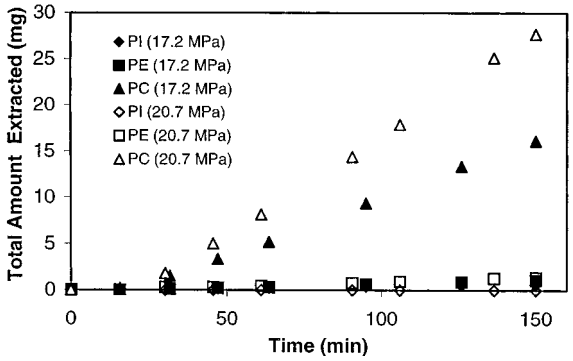

FIG. 4. Effect of pressure on the extraction of PL (T = 60°C, w_{EtOH} = 0.1, Q_{SCF} = 2 mL/min). See Figure 2 for abbreviations.

commonly showed a maximum within the first 30 min, no significant amount of PL was observed during this period. This observation was more pronounced at the lower pressure of 17.2 MPa. This suggests that PL extraction started after some other components of the mixture (probably triglycerides) were selectively removed with the SCF mixture. During extraction of full-fat canola flakes, addition of 8 mol% ethanol into the SC-CO_2 resulted in a very low PL concentration in the extracts, which indicated that presence of ethanol did not change the selectivity of SC-CO_2 to triglycerides (8). This is in agreement with our observation as the soy lecithin used in this study contained 3% triglycerides.

The HPLC analysis used in this study was not able to detect phosphatidic acid, but no phosphatidic acid extraction was observed at low pressures in the previous studies (10,16). Figure 2 illustrates the extraction behavior of PC, PE, and PI at 20.7 MPa and 60°C with 10 wt% ethanol. It shows the cumulative amounts of the individual PL extracted with respect to time. Figure 2 also shows the reproducibility of three different extractions under the same conditions, where PC amounts are shown as scattered data and PE and PI are shown as the average of three runs with standard deviation included. Extraction curves revealed a more or less linear relationship in the first 150 min indicating a constant rate of extraction. It is seen in this figure that PE and PI were extracted to a very low extent, whereas the extracts were mostly PC. This can be observed better in Figure 3, where relative percentages of the individual PL in the extracts are plotted with respect to time. As can be seen in this figure, the percentage of PC in all the extracts, collected at different periods during the extraction time of 150 min, stayed constant around 91%. On the other hand, percentages of PI and PE were very low.

Effect of pressure on the extraction is shown in Figure 4. Increasing the pressure resulted in higher amounts of extracts. Since the amount of PE and PI were the same at both pressures, selectivity to PC was also increased with increasing pressure. Increasing the pressure from 17.2 to 20.7 MPa increased the PC selectivity (relative to other PL) from 91 to 95%.

Effect of temperature on both the total amount of PL extracted and selectivity to PC is illustrated in Figure 5. There was a considerable decrease in the total extracted amount with increasing temperature. This is attributed to a decrease in the density of the SCF, thus a decrease in the solvent capacity of the SC-CO_2/ethanol mixture when the temperature is increased. Figure 5 also reveals the negative effect of temperature on the PC content of the extracts. Increasing the temperature from 60 to 80°C decreased the selectivity to PC from

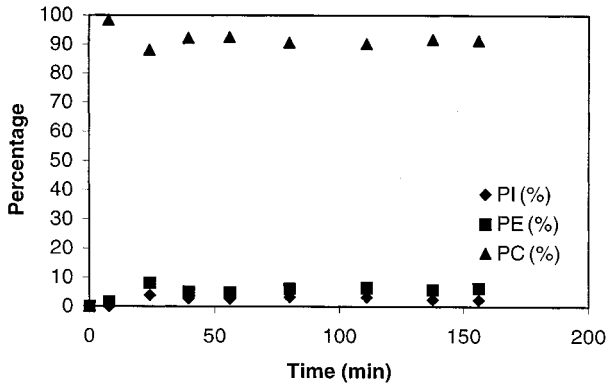

FIG. 3. Time on stream extraction selectivities of PL (P = 17.2 MPa, T =

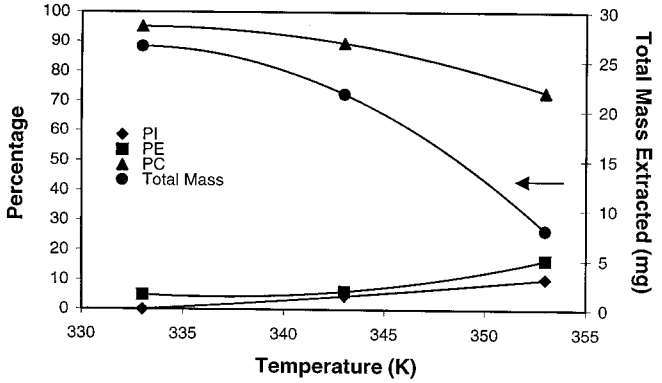

FIG. 5. Temperature dependency of PL extraction and PC selectivity (P = 20.7 MPa, w_{EtOH} = 0.1, Q_{SCF} = 2 mL/min). See Figure 2 for abbreviations.

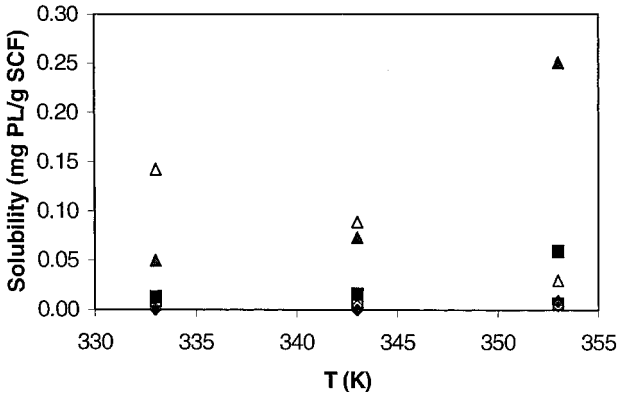

FIG. 6. Comparison of solubility data with literature. ◆, PI (Montanari *et al.,* Ref. 10); ■, PE (Montanari *et al.,* Ref. 10); ▲, PC (Montanari *et al.,* Ref. 10); ◇, PI (this work); □, PE (this work); △, PC (this work). SCF, supercritical fluid.

95 to 73%, whereas the percentages of both PE and PI increased considerably. For a 90% CO_2/10% ethanol mixture, temperatures below 60°C were not tested to make sure that the extraction fluid is in one phase, as the critical temperature of this mixture is reported to be 60°C (11). Montanari *et al.* (10) observed the highest amount of extracts at 80°C in the moderate-pressure range. This can be clearly seen in Figure 6, where the effect of temperature on the solubility of PL observed in this study and that of Montanari *et al.* (10) are compared. Basically we observed a decrease in solubility with temperature, whereas they observed an increase. Solubility data of this study are calculated from the slopes of extraction curves at 20.7 MPa, whereas solubility data of Montanari *et al.* (10) are calculated as average of reported values at 23.9 MPa, which is a slightly higher pressure (hence higher density) than the pressures used in this study. This discrepancy may be due to mass transfer effects, which become more pronounced at higher temperatures, and may indicate that we had mass transfer limitations. The discrepancy may also be due to crossover of solubility isotherms between the two pressures. Temelli (7) observed a crossover of solubility isotherms of canola lipids at about 27.6 MPa, which is slightly above the pressures used in these studies. Similar behavior of solubility isotherms (decreasing solubility with temperature at low pressures and increasing solubility with temperature at high pressures) has been reported for other solutes in SCF (17,18).

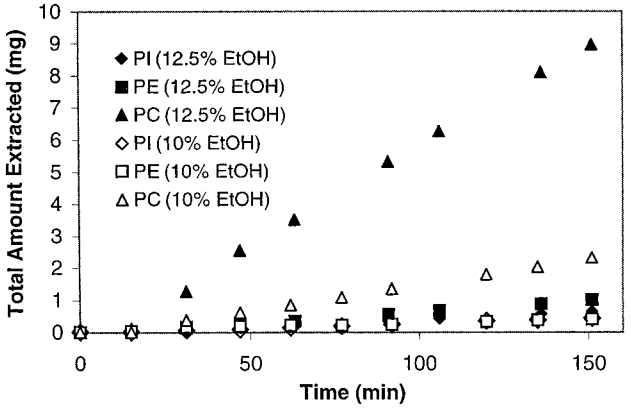

FIG. 7. Effect of ethanol fraction on the extraction of phospholipids (*P* =

Extraction of the individual PL with two different ethanol percentages in the SCF mixture is shown in Figure 7. Increasing ethanol wt% from 10 to 12.5 wt% at 17.2 MPa and 80°C resulted in about a fourfold increase in the total extracted amount. Owing to the increase in the mixture critical temperature, 12.5% ethanol extraction was carried out at 80°C. Both the amount of PC extracted and its ratio to PI and PE increased considerably with only a 2.5% increase in ethanol content of the SCF. With 10 wt% ethanol concentration at this temperature, the selectivity to PC was 73%, whereas the selectivity increased to 84% at 12.5 wt% ethanol. Although increased percentages of ethanol seem to favor selective extraction of PC, further increase was not studied as the critical temperature of CO_2/ethanol mixture increases with the increasing ethanol fraction and high temperatures can cause denaturation of PL.

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