ENRICHMENT AND FRACTIONATION OF PHOSPHOLIPID CONCENTRATES BY SUPERCRITICAL FLUID EXTRACTION AND CHROMATOGRAPHY

ABSTRACT

Supercritical fluid extraction (SFE) has been combined with supercritical fluid chromatography (SFC) in a preparative mode to develop a system for fractionating and enriching high value phospholipids (PLs) contained in soyflakes. Soyflakes were initially extracted with neat carbon dioxide (CO₂) at 680 bar and 80°C to remove the available oil. The defatted flakes

- Keywords: carbon dioxide, chromatography, extraction, phospholipids, soybean, supercritical fluid -
were then extracted with 15 mol% ethanol-modified CO₂ at 655 bar and 80°C to obtain the phospholipids. This phospholipid-enriched extract was then delivered to the head of a chromatographic column containing neutral alumina. The modifier was then changed to ethanol:water (9:1) and SFC was performed at 350 bar and 50°C, collecting fractions at equal volume intervals of CO₂. The SFC modifier was added in an increasing step-wise gradient to affect elution of the PLs. The resultant fractions (analyzed by high-performance liquid chromatography with evaporative light-scattering detection) showed PL enrichment factors relative to the de-fatted soyflakes from a 2.3 to 19.9 fold increase, depending on the individual PL.

INTRODUCTION

The term “lecithin” is often used to describe a diverse group of commercially available phospholipid (PL) mixtures including fractions containing one or more PLs, triglycerides, pigments, carbohydrates, sterols and cerebrosides in different proportions (FLIDER, 1985; SCHOLFIELD, 1985). The main commercial source is soybean lecithin which is a mixture of polar (PL) and non-polar (neutral) lipids. It is a by-product obtained by degumming soybean oil containing PLs. It finds wide use as a natural emulsifier in the stabilization of various food products and as a wetting agent.

In general, liquid lecithins are oil containing products with PL contents ranging between 30 and 65%. In contrast, solid lecithin, provided as powder or granulate, has to be de-oiled to obtain the desired consistency (HEIDLAS, 1997). Demand for lecithin with a high phos-
Phatidylcholine (PC) content is increasing by the pharmaceutical, cosmetic and food industries. In some pharmaceutical applications, lecithin with a 40-50% PC content is used, while for other formulations, lecithin containing 80-90% PC is desired (PARDUN, 1989).

The de-oiling and enrichment of lecithin has traditionally been accomplished using organic solvents such as acetone, ethanol, or ethanol-ether mixtures. However, organic solvents can contaminate the product and lead to off-flavors and unacceptable taste (WEIDNER et al., 1993). In addition, up to 50% of the PLs can be left in an oilseed matrix during the traditional hexane extraction process (MONTANARI et al., 1999).

Lecithins, i.e. PC, play a crucial physiological role as constituents of cell membranes, especially in the central nervous system (YOREK, 1993). The main nutritional value of lecithin is as a precursor of choline. In this respect, it is not the mixture of PLs in lecithin which is of value, but specifically PC (PARNHAM, 1996). Numerous other health benefits of a PC-enriched diet have been demonstrated within recent years. PLs play a part in a wide range of human metabolic processes, including: fat absorption, cholesterol metabolism, regulating serum lipid level, fat transport, blood clotting, nerve function, lung function, biosynthesis of prostaglandins and vision (KRAWCZYK, 1996). Based on the increasing health consciousness of consumers, the physiological importance of PC is often cited in the marketing of specialty lecithins as nutraceuticals and as ingredients in dietary products (HEIDLAS, 1997).

Previous studies have shown that supercritical carbon dioxide (SC-CO$_2$) is very effective in removing oils from a variety of seed matrices (SNYDER et al., 1984; LIST et al., 1989) and from crude lecithin (HEIGEL and HUESCHENS, 1983; STAHL and QUIRIN, 1985; ALKIO et al., 1991; EGGERS and WAGNER, 1993; MANOHAR et al., 1995; BEGAN et al., 1997). However, the limited solubility of PLs in SC-CO$_2$ leaves an economically valuable component in the de-oiled matrix. To solubilize PLs in SC-CO$_2$ requires addition of a polar cosolvent since PLs are relatively polar lipid compounds and are only sparingly soluble in SC-CO$_2$ (FRIEDRICH and PRYDE, 1984; FATTORI et al., 1987; TEMELLI, 1992).

In contrast to SC-CO$_2$, WEIDNER et al. (1993) and HEIDLAS (1997) have reported the de-oiling of lecithin with propane. Both investigators used a countercurrent process to produce a de-oiled fine powder containing small amounts (<3%) of residual oil and found the process applicable to a broad spectrum of lecithins of different PL compositions. WEIDNER et al. (1993) called it LPGE (low pressure gas extraction) and HEIDLAS (1997) called it near-critical fluid extraction.

Prior work (TEMELLI, 1992; DUNFORD and TEMELLI, 1995; MONTANARI et al., 1999) has shown the possibility of extracting PLs from canola and soybean flakes using a SC-CO$_2$/ethanol mixture. Ethanol is a logical choice for a cosolvent as it enjoys GRAS (Generally Regarded As Safe) status in the United States for its use in food processing. All of these studies were performed using a two-step extraction sequence. First, the oil seed matrix was extracted with SC-CO$_2$ to remove as much oil as possible; then in the second supercritical fluid extraction (SFE) step, ethanol was introduced as an entrainer to extract residual lipids containing phospholipids. TEMELLI'S (1992) research employed static SFE for the second extraction step. The equipment utilized would not allow continuous mixing of ethanol with CO$_2$ before it entered the extraction cell. Thus, a known amount of ethanol was added to the canola, left overnight to equilibrate, and then extracted with SC-CO$_2$. MONTANARI et al. (1996, 1999) utilized dynamic SFE.
A second pump continuously added ethanol to the flowing SC-CO$_2$ stream prior to its introduction into the soyflake-filled extraction cell. DUNFORD and TEMELLI (1995) combined static and dynamic extraction during their second EtOH-modified step.

In this study, we have expanded upon earlier research (MONTANARI et al., 1996) that had shown the selective extraction of PLs using a SC-CO$_2$/EtOH mixture. We have developed an integrated procedure for the removal of oil and then phospholipids from soybean flakes using SC-CO$_2$ and ethanol and combined it with on-line supercritical fluid chromatography to obtain fractions enriched in one or more of the major phospholipids. Processing parameters such as pressure, temperature, modifier and sorbent type were examined.

MATERIALS AND METHODS

Materials

Phospholipid standards of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidic acid (PA) were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). A soybean-based lecithin sample was acquired from Central Soya (Fort Wayne, IN). Flaked soybeans were provided by Archer Daniels Midland Company (Decatur, IL).

Supercritical fluid chromatography

A Hewlett-Packard 1082 supercritical fluid chromatograph (Hewlett-Packard, Wilmington, DE) fitted with a packed column (Econosphere silica, 5 μm, 250x4.6 mm. Alltech Associates, Inc., Deerfield, IL) was utilized for initial studies designed to establish a suitable solvent system, sorbent and extraction parameters. An Alltech Model 500 evaporative light-scattering detector (ELSD) at 90°C and 2 SLPM of N$_2$ (Alltech Associates, Inc., Deerfield, IL) was used for detection. Stainless steel capillary restrictors (1.0 and 1.5 mL/min) from Isco, Inc. (Lincoln, NE) were utilized to connect the column to the detector. Various pressures (100-350 bar), temperatures (30°-80°C) and modifiers (ethanol, 2-propanol, water) were studied to optimize separation of the phospholipids. Once the chromatographic parameters were optimized (350 bar, 50°C, 25 vol% modifier of ethanol:water, 9:1), they were applied to an analytical-scale extraction unit for supercritical fluid fractionation studies.

Supercritical fluid fractionation

Supercritical fluid fractionation (SFF) studies were performed with an Isco, Inc. Model SFX 3560 automated extractor (Lincoln, NE) and utilized a commercially-available organic solvent-extracted lecithin. The sorbents used for SFF were as follows: silica gel (60-200 mesh) and Celite 545 (J.T. Baker Chemical Co., Phillipsburg, NJ), amino-bonded silica (Varian Associates Inc., Harbor City, CA), neutral and acidic alumina (60-325 mesh, Fisher Scientific, Milwaukee, WI), alumina C (Universal Scientific, Inc., Atlanta, GA), Chem Tube-Hydromatrix (Varian Associates Inc., Harbor City, CA) and bentonite (200-325 mesh, Oil Dri Corporation of America, Chicago, IL). Sorbents were added to a 10 mL extraction vessel and lecithin (0.5 g) was manually applied to the top of the sorbent bed. The extraction/fractionation procedure was then commenced with fractions being collected at 60 min (120 mL solvent) intervals. The first 2-3 fractions effectively resulted in removal of any residual oil from the lecithin. The parameters for subsequent fractions were then designed to enrich the collection of individual PLs. Parameters for a typical SFF run would be as follows:
Fraction 1-2: 650 bar; 80°C; 60 min; 2 mL/min; CO₂
Fraction 3: 350 bar; 50°C; 60 min; 2 mL/min; 10 vol% modifier/CO₂
Fraction 4-6: 350 bar; 50°C; 60 min; 2 mL/min; 20 vol% modifier/CO₂
Fraction 7-9: 500 bar; 50°C; 60 min; 2 mL/min; 20 vol% modifier/CO₂
Fraction 10-12: 500 bar; 50°C; 60 min; 2 mL/min; 25 vol% modifier/CO₂
Fraction 13-14: 650 bar; 50°C; 60 min; 2 mL/min; 30 vol% modifier/CO₂

Supercritical fluid extraction

The soyflakes were defatted prior to supercritical fluid extraction/supercritical fluid chromatography (SFE/SFC). They were extracted with neat CO₂ (13.63 kg) at 680 bar and 80°C using a SFE pilot plant (FRIEDRICH et al., 1988). The defatted flakes were stored at -4°C until they were used for the SFE/SFC studies.

Supercritical fluid extraction/supercritical fluid chromatography

The experimental apparatus employed in this study is shown in Fig. 1. It is identical in design and scale to a previously described unit (KING et al., 1996) with only a few modifications. The extraction vessel (containing 60 g of defatted soyflakes) and chromatographic column were identical to those described by KING et al. However in this study, chromatography was performed with 45 g neutral alumina (Brockman activity 1, 60-230 mesh, Fisher Scientific, Fair Lawn, NJ). The supercritical fluid extraction step was conducted at 655 bar, 80°C, 15

Fig. 1 - Schematic of the SFE/SFC unit. PG = pressure gauge. GT = gas totalizer. MMV = micro-metering valve. PRR = pressure reducing regulator.
mol% EtOH, using 4 L/min CO₂ (NTP) for 100 min. The extract-laden stream proceeded through the pressure reducing regulator prior to its deposition on the chromatographic column. This allowed the pressure to be reduced to 62 bar from 655 bar and allowed the PLs to be concentrated at the top of the column. Because of the 15 mol% ethanol modifier, some extract did elute through the alumina column and collect in the receiver (250 mL round bottom flask). However, this fraction did not contain any phospholipids as shown by high-performance liquid chromatographic (HPLC) analysis. Supercritical fluid chromatography was performed at 345 bar, 50°C, 4 L/min CO₂ (NTP) with fractions collected at equal volume intervals of CO₂ (400 L). The modifier was changed to EtOH:H₂O (9:1) and introduced at 5 mol% for three fractions, 10 mol% for five fractions, 20 mol% for two fractions and 30 mol% for one fraction. The fractions were concentrated under reduced pressure, transferred to tared vials, and diluted with chloroform for analysis via HPLC.

High-performance liquid chromatography

All analyses for phospholipids were 15 μL injections of 10 mg/mL solutions. They were performed using a Spectra-Physics SP8800 pump (Thermo Separation Products, Fremont, CA), a Rheodyne 7125 loop injector (200 μL), a Bio-Rad Model 1250424 column heater (Bio-Rad, Inc., Richmond CA) @ 35°C, an Alltech Model 500 ELSD evaporative light-scattering detector (ELSD) at 90°C and 2 SLPM of N₂ (Alltech Associates, Inc., Deerfield, IL), and an SP Datajet integrator (Thermo Separation Products, Fremont, CA). The HPLC column was a Licrosphere Si 60/11, 3 μm particle size, 250x4 mm (EM Separations, Gibbstown, NJ). The mobile phase was a linear gradient of A:B (80:20) to A:B (20:80) over 30 min at a flow rate of 0.7 mL/min. The composition of solvent A was chloroform:tert-butyl methyl ether (750:150, v:v), and solvent B was methanol:ammonium hydroxide:chloroform (920:70:10, v:v:v).

RESULTS AND DISCUSSION

Supercritical fluid chromatography

Studies using packed column SFC (pSFC) and PL standards were performed to establish conditions which could be translated for preparative SFC. The initial chromatographic parameters that were tested (135 bar, 40°C, 5-10 vol% modifier, 1.8 mL/min) were obtained from SNYDER et al. (1998). The modifier used by SNYDER et al. was ethanol:water (9:1); the water in the eluent controlled the activity of the silica column, i.e., to yield better resolution between chromatographic peaks and reduce retention times. With 100% EtOH as a modifier, it was found that PE and PI had retention times approximately four times longer than with the EtOH:H₂O modifier and PC did not elute at all. Isopropyl alcohol was also investigated as a modifier, but the PLs did not elute from the column when this cosolvent was used. Some experimental difficulties were experienced when using these eluents due to the fused silica restrictors connecting the column to the ELSD fracturing. This was overcome by using stainless steel capillary restrictors in place of fused silica.

However, when using SNYDER et al.'s (1998) chromatographic parameters, the PLs were not detected. By adjusting the pressure (100-350 bar), temperature (30°-80°C) and modifier percentages (10-50 vol%), the separation of the PL standards was optimized. The modifier concentration was the most influential parameter for eluting the PL moieties. Below 20 vol% modifier, the PL standards were strongly retained. When the chromatographic parameters were optimized (350 bar, 50°C, 25 vol% modifier of
Supercritical fluid fractionation

A variety of sorbents other than silica gel were studied. These sorbent screening experiments allowed a more accurate assessment of the ability to separate/fractionate PL-containing extracts as these small-scale columns mimicked future preparative columns which would also be manually-packed in our laboratory.

The first SFF experiments were performed with silica gel as the sorbent to reproduce the results obtained on the pSFC studies. An extraction cell was filled with sorbent and a soybean-based lecithin was applied to the top (inlet) of the cell. Fig. 2 shows representative HPLC chromatograms from this SFF experiment. Chromatogram 2a shows the profile of the starting lecithin, while chromatogram 2b (from the fifth fraction) displays a decreased amount of PE when compared to the original matrix. The results from the collected seventh fraction are shown in chromatogram 2c and consist mainly of PC; PI is only a minor constituent of the fraction, and PE and PA are absent.

SFF (with silica gel) was also attempted with 100% EtOH modifier, and the results corroborated our results from the pSFC studies. The PLs were strongly retained and elution did not occur within a reasonable time or volume of eluent fluid. Thus, the use of water as a secondary modifier is necessary to affect PL elution. In this regard, we attempted SFF with an increased percentage of water in the modifier (EtOH:H₂O; 7:3). However, this mobile phase composition did not produce any fractionation of PLs; it only affected a more rapid elution of the PLs. It was found that all four PLs eluted in a single fraction when the ethanol:water, 9:1), they were transferred to an analytical-scale extraction unit for additional fractionation studies prior to scaling up to preparative SFC.

Fig. 2 - HPLC-ELSD-chromatograms of: (A) lecithin, (B) the fifth fraction, and (C) the seventh fraction of the supercritical fluid fractionation of lecithin using silica gel as the sorbent.
extraction fluid concentration was 20 vol% modifier/CO₂.

By contrast to adjusting the modifier in the mobile phase with water, we attempted to deactivate the silica gel by addition of water to the sorbent. This was done at two different levels (5 and 20 weight %). With addition of water to the silica gel, it was hoped that 100% EtOH could be used as the modifier. However, addition of water to the stationary phase (silica gel) did not work as well as having water in the mobile phase. Interestingly, the silica gel deactivated with 5 weight % water retained the PLs until a 40 vol% ethanol-modified CO₂ fluid was passed over the sorbent. Further, a concentration of 30 vol% EtOH/CO₂ was needed to elute PLs from the silica gel deactivated at 20 weight % with water. This may be compared to the results obtained when adding 20-25 vol% of modifier to CO₂ with the modifier being EtOH:water (9:1), and using neat silica gel.

Sorbents other than silica gel were also investigated. All of the SFF conditions and fractionation sequences were kept identical to those reported above so that just the influence of the sorbent type could be examined. Chem-Tube Hydromatrix and Celite 545 did not fractionate the PLs at all, but their elution occurred at lower modifier concentrations than those required using silica gel. Amino-bonded silica did fractionate the PLs, but required almost 40 vol% modifier/CO₂ to elute PC. Bentonite clay exhibited such strong retention properties that PC did not elute even at 40 vol% modifier.

Four different types of alumina were also examined in the sorbent screening experiments. Alumina C was found to be inadequate for PC enrichment. Only two of the fractions were enriched with PC. Early fractions contained both PE and PC, while later fractions indicated a carryover of PC into PI-containing fractions. Basic alumina proved capable of fractionating the PL moieties as well as silica gel. PE and PI were separated into the early and late eluting fractions, respectively, but PC again eluted over a broad range of modifier concentration (10-30 vol%) and was found in every fraction. Acidic and neutral aluminas behaved similarly and provided a slight fractionation improvement over silica gel. There was some minor co-elution of PC with PE, but PC eluted individually with no cross-contamination from PI or PA. Therefore, alumina (either acidic or neutral) would be the preferred chromatographic sorbent because of its enhanced capability to fractionate the PLs. Neutral alumina was chosen for the scaled-up SFF studies because it was readily available and in abundance.

Supercritical fluid fractionation studies were then scaled-up by a factor of four and performed utilizing the aforementioned home-built SFE/SFC system. Operational parameters from the analytical-scale SFF were translated to and optimized on the larger (semi-preparatory) extraction/chromatography system. In this case, the model system of soybean-based lecithin (2.0 g) was fractionated on neutral alumina (45 g). Adjustments were required for the modifier concentration and the fraction collection times, i.e., the modifier concentration was lowered to enhance the fractionation of the individual phospholipids, consequently, elution volumes were increased due to the lower modifier concentrations.

Supercritical fluid extraction/supercritical fluid chromatography

Before SFE/SFC trials using defatted soyflakes were undertaken, SFE experiments using the conditions reported by MONTANARI et al. (1996) for selective SFE of PLs were evaluated. In contrast to the results of that study, we found a larger volume of extraction fluid was needed for exhaustive extraction of the phospholipids. As shown in Fig. 3, the last frac-
tion was collected after 650 min of extraction time and contained only 0.015 g of material, but still had PLs present when analyzed via HPLC. In collecting the fractions, over 1100 mL of ethanol were used, which is considerably more than the 80 mL used by MONTANARI et al. Our time-consuming extraction of PLs from defatted soyflakes was probably still not exhaustive, but to optimize extraction time and mass, the SFE step of the SFE/SFC trials was run for 100 min. corresponding to the first fraction in Fig. 3. Thus, only this initial amount of soyflake extract was further fractionated by SFC.

SFE runs were performed on 60 g batches of defatted soyflakes (under the aforementioned conditions), and they yielded an average extract of 1.35 g (relative standard deviation of 9.9%). The individual phospholipid profile from one extraction was as follows: 16.1% PE, 9.2% PI, 2.8% PA and 15.6% PC. These PL percentages corresponded to fractions containing 190.8, 108.7, 33.4 and 185.1 mg, respectively. These results were then used as a baseline to establish enrichment factors for the SFE/SFC process.

The SFE/SFC experiments utilized defatted soyflakes. This extraction-chromatography was a consecutive two-step process and not a continuous, on-line process. The soyflakes were first extracted with SC-CO₂/ErOH and the resultant lecithin-like extract was deposited on the head of the chromatographic column. SC-CO₂ modified with EtOH:H₂O (9:1) was then used to elute/fractionate the individual phospholipids.

Fig. 4 is representative of a typical SFE/SFC fractionation profile. The extract corresponding to fraction 1 of Fig. 3 was further fractionated via SFC. As can be seen, the phospholipids do not elute until the middle or latter fractions. The SFC step not only enhances the fractionation of the individual PLs, but it also increases PL purity by removing excess oil. The fractionation profile (Fig. 4) exhibits an enrichment of PC in the middle fractions (6 & 7) and then its enrichment declines through fraction 10. PI and PE eluted in these intermediate fractions and show a general elution profile under conditions where the mobile phase is not saturated with PLs. PI elution starts in fraction 6, is at a maximum in fraction 7 and finishes eluting in fraction 9. PE elutes in fractions 7.

![Graph](image)
through 12, with maximum enrichment being achieved in fraction 9. In addition, PA is eluted with increasing percentages in fractions 10 through 12. Even though the SFC was not carried out to completion with respect to PA, the elution of PA exhibits a typical chromatographic peak profile for elution chromatography.

Table 1 summarizes the presence of the individual phospholipids after supercritical fluid extraction and the enrichment after the chromatography step relative to other eluting constituents. The SFE data represent average PL values, while the SFC data represent the maximum (individual fraction) enrichment of the specific PLs that were extracted. The SFC enrichment data can be visualized in Fig. 4. For example, PC is at maximum enrichment in fraction 6 and constitutes 76.8% of that fraction. However, this accounts for only 54.4% of the total amount of PC. When all fractions that contain PC are summed together, 90.7% of the PC is recovered. PE exhibits maximum enrichment in fraction 9 at 74.9%, but this one fraction only accounts for 21% of the total PE. When all PE-containing fractions are added together, 81.5% of the total PE is recouped. PI has its maximum in fraction 7 (20.8%), but only yields 15.4% of the total PI. Furthermore, combining all PI-containing fractions only yields 26.9% of the total PI. Fraction 12 shows for PA a maximum enrichment at 55.8%, which accounts for 31.2% of the available PA. When all fractions containing PA are totaled, the PA recovery is 129%. This recovery above 100% is not surprising due to the small amount of PA that is naturally present and extractable from the defatted soyflakes. With the small amount of PA

Table 1 - Percentage amounts of PLs in extracts derived from SFE and SFC steps*

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>SFE</th>
<th>SFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylethanolamine</td>
<td>16.1</td>
<td>74.9</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>9.2</td>
<td>20.8</td>
</tr>
<tr>
<td>Phosphatidic acid</td>
<td>2.8</td>
<td>55.8</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>15.6</td>
<td>76.8</td>
</tr>
</tbody>
</table>

* = relative to other eluting constituents (oil and unidentified peaks).
that is present, a difference of a few milligrams can have a dramatic effect on the total recovery data (or figures). The deviation in the amount of extract from the soyflakes during the SFE stage has to be considered also. With the on-line SFE/SFC process, the exact amount of extract deposited on the chromatographic column is difficult to determine. This is why SFE experiments were performed independently (prior to SFE/SFC), to establish an average amount of extract deposited on the column and thus an average PL baseline. All of the PLs demonstrated enrichment after SFC, showing increases between 2.3 and 19.9 times. This is encouraging considering the small dimensions of the chromatographic bed and could undoubtedly be improved by using a larger, more efficient column. The fractionation/purification of the individual phospholipids could also be improved with a more efficient chromatographic column.

CONCLUSIONS

In this study, we have successfully demonstrated a two-step process of supercritical fluid extraction and supercritical fluid chromatography on a semi-preparative scale, to enrich and fractionate phospholipids from defatted soyflakes. Even though soyflakes were de-oiled in this process, it should be noted that many commercially utilized PL concentrates contain soybean oil. By using the described system, one can (1) extract the oil from the soyflakes, (2) fractionate residual oil away from the PL concentrates, and (3) further enrich and fractionate the individual PLs. Thus, the complete three step process could be sequentially performed on the same system. This SFE/SFC system, which is comparable to that previously described by KING et al. (1996), has now been shown to be applicable for the enhanced recovery and enrichment of a second, high value natural product from an oilseed matrix. In addition, only environmentally-benign (carbon dioxide) and GRAS cosolvents (ethanol, water) have been used for the extraction and fractionation (chromatography) of these potential nutraceuticals and high value components. Using the described system for fractionating PLs avoids the use of non-GRAS organic solvent systems that have been reported for this purpose (VAN DER MEEREN et al., 1990; DE MEULENAER et al., 1995).

This research also demonstrates how a process may be scaled up from analytical-scale instrumentation, similar to that reported by LEMBKE (1998) for enrichment and fractionation of fish oil ethyl esters. Results from analytical pSFC were transferred to an analytical-scale extractor, where this extractor was utilized as a packed-column chromatograph, the extraction cell being filled with a sorbent. Results from this analytical-scale fractionation were then scaled-up to the described semi-preparative scale SFE/SFC systems. These results can now be translated to a potential plant-scale operation for making enriched-PL concentrates for food and industrial use.

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the products to the exclusion of others that may also be suitable.

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