Quantitative Determination of Phospholipids in Sunflower Oil

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ABSTRACT: Phospholipids from sunflower oil samples were enriched by using solid-phase extraction (SPE) cartridges and subsequently separated and analyzed by high-performance liquid chromatography (HPLC) with an ultraviolet detector. The recovery of individual phospholipids at different total concentrations in model oils and the repeatability of the method were investigated. The results demonstrated the utility of SPE–HPLC for quantitative analysis of phospholipids in sunflower oil and the effectiveness for concentrating, separating, identifying, and quantitating phospholipids in samples with phosphatide contents as low as 0.1%. Samples of sunflower oil at different stages of processing were analyzed, and phospholipid profiles in hexane-extracted oil, hot-pressed oil, and water-degummed oils were compared.

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KEY WORDS: High-performance liquid chromatography, phospholipids, solid-phase extraction, sunflower oil, water degumming.

The stability and quality of vegetable oils are influenced by the presence of minor constituents, such as phosphatides. Phospholipids may act as antioxidants, the antioxidative activity being attributed to their synergistic action, their metal scavenging activity, and their catalytic activity to decompose hydroperoxides (1,2). Measurement of phospholipid components also can be used to evaluate the quality of crude oil from oilseeds that have been damaged due to environmental conditions in the field and/or improper handling, storage, and transportation (3). The major phospholipids in sunflower seed oil are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidic acid (PA), with a total concentration lower than 1.2% (4). Most of these phospholipids are hydratable and can be removed from the crude oil by using a water-degumming process. Sunflower oils must also be considered as a source of lecithin, with potential uses as an additive for foods, feeds, and cosmetics (5).

Development of a methodology for quantitative analysis of phospolipids becomes important to handle the quality of nonrefined oils and to evaluate the efficiency of the degumming process. Although the phosphatide content in oils can be estimated from the total phosphorus percentage, there is a need for knowledge of the phospholipids profile. The more recent official methods for phospholipids, such as AOCS Ja7b-91 (6) and IUPAC 5.302 (7), use high-performance liquid chromatography (HPLC) and have been developed for quantitative analysis of vegetable lecithin concentrates. The low concentration of phospholipids in vegetable oils requires some concentrating method before HPLC analysis. This can be achieved by traditional solid–liquid column chromatography or by solid-phase extraction (SPE) prepacked cartridges. The principles of SPE and the methods for isolation and fractionation of lipids in biological, environmental, and food matrices have been extensively reviewed by Ebeler and Shibamoto (8) and by Christie (9). The SPE method offers several advantages, such as rapidity, high yield and low volume of eluting solvent, that avoid transfer steps, thereby reducing contamination and sample losses. However, development of any SPE method requires adequate selection of sorbent and elution solvents and optimization of all steps to ensure accurate and reproducible results (8). The analysis of phospholipids in vegetable oils is rather recent (3,10–12). Mounts and Nash (3) analyzed phospholipids in crude soybean oils by SPE–HPLC and reported relative area percentage and total phosphorus content. Retention profiles were unusable for fractions isolated from oils with a phosphorus content lower than 100 ppm. The authors referred to a previous paper (10) for SPE methodology, in which the phosphorus analysis suggested incomplete recuperation of phospholipids (10). Singleton (11) described a method to enrich phospholipids from crude peanut oil by using a silica precolumn and subsequently separating the individual phospholipids from the remaining neutral lipids within the chromatographic system. An improvement on the analysis system of this method, which requires appropriate software and incorporates additional hardware into the HPLC unit, has been published recently (12).

The main objective of this research was to develop an SPE–HPLC procedure to successfully recover, separate, and quantitate the phospholipid classes present in sunflower oil in a wide range of concentrations (0.1–1.2%), and to apply the method to study the effect of processing on phospholipid composition.

EXPERIMENTAL PROCEDURES

Materials. Solvents *n*-hexane and 2-propanol were HPLC-grade from J.T. Baker Inc. (Phillipsburg, NJ). All other chem-

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icals were analytical-reagent grade. Acetate buffer (pH 4.2) was prepared by mixing 26.5 mL of sodium acetate solution (0.2 M) and 73.5 mL of acetic acid solution (0.2 M), both made with twice-distilled water. The HPLC mobile phase was *n*-hexane, 2-propanol, and acetate buffer in the proportion 8:8:1 vol/vol/vol. Standards of PE, PA, PI and PC, from soybean with purities greater than 98%, were obtained from Sigma Chemical Co. (St. Louis, MO). To obtain calibration curves, standard solutions were prepared by dissolving the phospholipids in HPLC mobile phase to different concentrations. A complete mixture was reached with sonication. For the SPE step, 500-mg bonded diol SPE cartridges from J.T. Baker Inc. were used. For recovery studies, model oils that simulated the phospholipid composition of crude sunflower oils (4) were made by mixing triolein (Sigma Chemical Co.) and refined sunflower oil with phospholipid standards. Two samples of crude and water-degummed sunflower oil were employed for repeatability studies. Four oil samples from the same sunflower seed lot at different stages of the industrial process were provided by a local factory. Crude oils obtained by either hot-pressing or hexane extraction, both with and without water degumming, were used.

SPE methodology. Before extraction, the diol phase cartridge was conditioned with 2 mL methanol, 2 mL chloroform, and 4 mL hexane. A micropipet was used to inject 50–150 mg of oil dissolved in chloroform. A large percentage of the triacylglycerols was released from the sorbent bed by passing 2.5 mL chloroform through. None of the phospholipids were eluted in this fraction as demonstrated by recovery studies. Phospholipids were recovered by elution with 7 mL methanol that contained 0.5 mL/100 mL of a 25% ammonia solution. The eluate was collected into a conical vial, evaporated to dryness under nitrogen, and made up to a given volume (100 µL) with mobile phase. This phospholipid fraction was analyzed in detail by HPLC. The cartridges may be reused after suitable washing in reverse elution order.

HPLC analysis. HPLC analysis of phospholipids was based on the IUPAC standard method for soybean lecithin (7). The HPLC system consisted of a Varian Vista Chromatograph with an injection valve of a 10-µL sample loop, an ultraviolet (UV) Varian detector set at 206 nm, and a Varian 4400 Integrator (Varian Associates Inc., Palo Alto, CA), Two different HPLC columns were used: a 300 × 4 mm Micropak Si-10 (10 µm) column (Varian Associates Inc.) and a 250 × 4 mm LiChrosorb Si-60 (5 µm) column (Merck, Darmstadt, Germany). Separations were performed at room temperature by isocratic elution with hexane, 2-propanol, and acetate buffer in proportion 8:8:1 vol/vol/vol. The stabilization of the column was achieved by passing the mobile phase at a flow rate of 0.2 mL/min until a stable baseline was obtained. Then, the flow rate was increased to 2 mL/min. To enable quantitative determinations of the separated phospholipids, calibration curves were used. The phospholipid content, expressed as percentage in the oil, was obtained as:

$$\%\text{PL} = 100\ C_{\text{PL}}\ V/M \qquad [1]$$

where C_{PL} represents the phospholipid concentration obtained from the calibration curve in mg/mL, V is the volume in mL of phospholipid concentrate that constitutes the sample to be injected to the HPLC system, and M is the weight in mg of oil loaded into the SPE cartridge.

RESULTS AND DISCUSSION

Typical HPLC chromatograms of phospholipids are presented in Figure 1, where an adequate resolution can be observed. Although both HPLC columns were suitable for phospholipid analysis, a slightly better performance was obtained with the Lichosorb Si-60 column. Retention times of crude sunflower oil phospholipids were close to those obtained for standard soybean phospholipids. Figure 2 shows the calibration curves obtained to quantitate the amount of individual phospholipids. Standard solutions were prepared by dissolving the standard phospholipids in HPLC mobile phase to concentrations between 0.2 and 4.5 mg/mL. Calibration curves exhibited excellent linearity over the entire concentration range with regression coefficients in the order of 0.995–0.999.

Recovery studies were conducted on triolein and sunflower oils with a known phospholipid composition, similar to the crude sunflower oil profile. This was attained by mixing triolein and refined sunflower oil, without a detectable phosphorus content, with phospholipid standards at concentrations

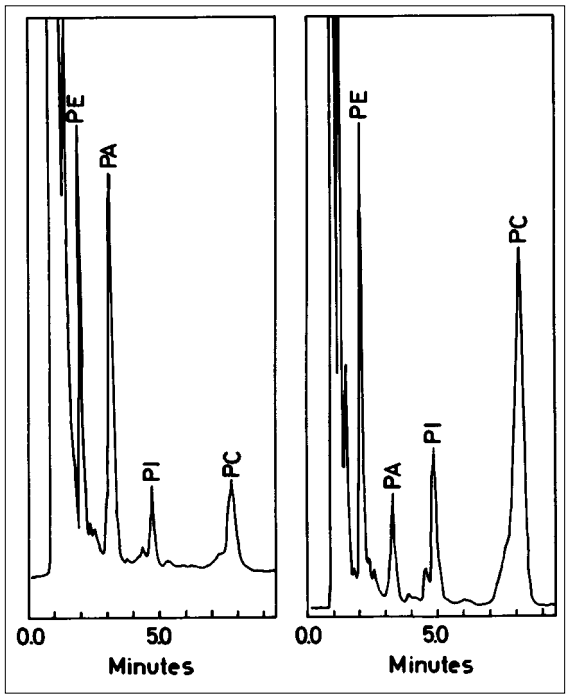

FIG. 1. High-performance liquid chromatography of water-degummed and crude sunflower oil phospholipids: PE: phosphatidylethanolamine; PA: phosphatidic acid; PI: phosphatidylinositol; PC: phosphatidylcholine. Column: 250 × 4 mm Lichrosorb Si-60 (Merck, Darmstadt, Germany); detection: ultraviolet 206 nm, flow rate: 2 mL/min.

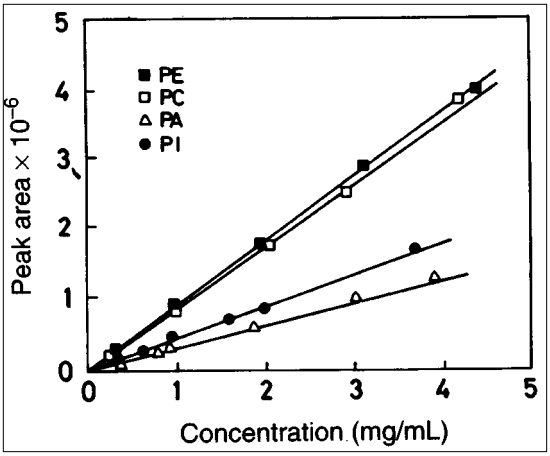

FIG. 2. Calibration curves for different phopholipids. See Figure 1 for abbreviations.

between 0.1 and 1%. As shown in Table 1, the recoveries were high for all phospholipids in both triolein and sunflower oil for different total phospholipid concentrations. This study also showed good precision for the method, with coefficients of variation for independent tests ranging from 1 to 9%. The repeatability of the method was also studied with two different samples, crude and water-degummed sunflower oil, which represent the higher and lower concentrations. The results of analyses for four independent tests are reported in Table 2. The precision of the measurements was good for the total content [coefficient of variation (Cv) of 3.9 and 8.8% for crude and degummed sunflower oil, respectively] and for individual phospholipids (Cv values less than 10%). The analysis of data showed the utility of the SPE–HPLC technique as a suitable analytical tool for sunflower oils; it is able to concentrate, separate, and quantitate phospholipids in concentrations as low as 0.1%. Moreover, SPE cartridges can be regenerated, and its use for concentrating is more economical and rapid than other alternative procedures, such as preparative- scale thin-layer chromatography (TLC), HPLC, or gradient elution chromatography from open columns.

The SPE–HPLC procedure was applied to the quantitative analysis of sunflower oil samples with different types of processing but obtained from the same seed lot. The weigh percentage and relative percentage of each phospholipid in the oils, as averages of two independent determinations, are summarized in Table 3. The samples contained significantly higher amounts of PA when compared with literature data (4). The phosphatide content of crude oils depends on the sunflower seed and on the method of extraction. The oil obtained by solvent extraction has a higher concentration than that obtained by pressing. However, no significant differences in the phospholipid profiles were observed. Table 3 shows that the average efficiency of the water-degumming process was approximately 83%, practically independent of the amount of phospholipids and the method of oil extraction. Degummed oils contained higher percentages of PA and lower percentages of PC than original crude oils. This is consistent with the fact that PA is the less hydratable and PC is the most hydrophylic of the phosphatides occurring in sunflower oil.

In conclusion, a sure, reproducible, and simple SPE–HPLC method has been developed to concentrate sunflower phospholipids from the bulk neutral fraction and to separate and quantitate the individual phospholipids. It can be used to

TABLE 1
Recovery of Standard Phospholipids

	Total amount of phospholipids added					
	0.1% in oil[a]		0.6% in triolein[b]		1% in oil[b]	
Phospholipid	Recovery (%)	Cv (%)	Recovery (%)	Cv (%)	Recovery (%)	Cv (%)
PE	94.9	4.9	101.6	5.2	94.3	3.9
PA	98.3	6.2	100.2	7.1	100.3	7.4
PI	98.3	8.7	99.3	1.2	100.8	6.5
PC	104.8	7.6	103.3	6.5	107.4	4.1

[a]$n = 4$.
[b]$n = 3$; Cv = coefficient of variation; PE, phosphatidylethanolamine; PA, phosphatidic acid; PI, phosphatidylinositol; PC, phosphatidylcholine.

TABLE 2
Precision Studies of SPE–HPLC Method in Sunflower Oils

	Crude sunflower oil[a]		Degummed sunflower oil[a]	
Phospholipid	wt (%)	Cv (%)	wt (%)	Cv (%)
PE	0.193	1.2	0.021	2.4
PA	0.168	9.2	0.065	9.2
PI	0.202	7.7	0.007	5.8
PC	0.476	2.7	0.009	9.9
Total	1.039	3.9	0.102	8.8

[a]$n = 4$; Cv = coefficient of variation; SPE, solid-phase extraction; HPLC, high-performance liquid chromatography. See Table 1 for other abbreviations.

TABLE 3
Quantitative Analysis of Phospholipids in Sunflower Oil with Different Processing[a]

Oil samples	Phospholipids				
	PE	PA	PI	PC	Total
Pressed	0.150	0.109	0.151	0.327	0.737
	(20.3%)	(14.8%)	(20.5%)	(44.4%)	
Pressed–degummed	0.048	0.034	0.017	0.033	0.132
	(36.3%)	(25.9%)	(13.0%)	(24.8%)	
Extracted	0.250	0.141	0.235	0.569	1.195
	(20.9%)	(11.8%)	(19.7%)	(47.6%)	
Extracted–degummed	0.040	0.037	0.043	0.087	0.207
	(19.3%)	(18.0%)	(20.7%)	(42.0%)	

[a]See Table 1 for abbreviations.

monitor the quality of crude sunflower oil, which can be affected by preharvest events, postharvest conditions, and type of processing. Besides, the technique should be applicable to other vegetable oils with phospholipid contents as low as 0.1%.

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