A Kinetic Study of Phospholipid Extraction by Degumming Process in Sunflower Seed Oil

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ABSTRACT: During the refining process of vegetable oils (degumming), phospholipids are eliminated by thermal treatment with water (hydratable phospholipids, HP) and other degumming agents such as phosphoric acid, citric acid, or acid mixtures (nonhydratable phospholipids, NHP). Samples of pressed crude sunflower oils were degummed with water and acids, and the corresponding pellets (gums) and supernatant oils were obtained by centrifugation. During the water degumming process, a decrease of more than 98% in the phosphatidylcholine (PC) content was achieved in 5 min; phosphatidylethanolamine (PE) was the most difficult compound to be removed. Phosphatidylserine, phosphatidic acid, and phosphatidylinositol (PI) presented an intermediate behavior. The optimal contact time for quantitative extraction of the most important HP (PC, PI, and PE) in crude sunflower oils was 35 min. For acid treatments, a rapid elimination of the residual levels of PC was registered (5 min); the optimal contact times for the quantitative removal of the NHP were 35 min for phosphoric acid and acid mixture, and 25 min for citric acid. Taking into account that PE was the most difficult component to be removed, its level could be used as a monitor to evaluate the efficiency of the degumming process.

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KEY WORDS: Degumming process, HPLC–ELSD, phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phospholipids, sunflower oil.

Refining of oils removes constituents that could be harmful to health and increases functional characteristics like palatability and nutritional availability of desired fat components. For these purposes, it is essential to consider the refining process and the composition of the raw oil.

The stability and final quality of edible vegetable oils and their by-products are determined by the residual presence of certain minor compounds like phospholipids, and their composition provides good information about the proper oil processing and storage conditions (1). During the refining process (degumming), phospholipids are eliminated by thermal treatment with water (hydratable phospholipids, HP) and other degumming agents such as phosphoric acid, citric acid or acid mixtures (nonhydratable phospholipids, NHP). According to research performed on soybean oils, the presence of NHP is influenced by factors such as temperature and moisture during the storage of seeds, and cellular damage, which would facilitate the action of phospholipase D, yielding higher levels of phosphatidic acid (2).

In addition, different types of acid degumming processes can be carried out depending on the type and amount of phosphatides present in the raw oil (3).

Solid phase extraction (SPE) methodology is one of the most powerful tools for the separation and refining of lipids, allowing a better concentration of the different components (4,5).

For the determination of the phospholipid content in sunflower oils, isocratic high-performance liquid chromatography (HPLC) techniques with an ultraviolet detector are used (6). Good results have been reported with this method, although a better resolution of the different phospholipids is achieved with an elution gradient (7,8). For this purpose, the evaporative light-scattering detector (ELSD) appears to be the best choice (9,10); it has better sensibility, avoids solvent interference due to its evaporation, improves baseline stability, and is compatible with the use of an elution gradient (11). Nevertheless, as an additional analytical tool, the total phosphorus content, estimated by a spectrophotometric method, is usually applied.

The main aim of the present work was to study the kinetic extraction of the different phospholipids present in crude sunflower oils such as: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA), for monitoring the efficiency of the degumming process under conditions normally used by the oil industry.

EXPERIMENTAL PROCEDURES

Materials. (i) Solvents. Chloroform, *n*-hexane, acetone, methanol, *tert*-butyl methyl ether (*t*-BME) were HPLC grade (J.T. Baker Inc., Phillipsburg, NJ). Ammonium hydroxide was analytical reagent grade.

(ii) Phospholipid standards. PC, PE, PI, PS, and PA with purities greater than 98% were purchased from Sigma Chem-

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ical Co. (St. Louis, MO). To obtain the calibration curves, standards were dissolved in chloroform/methanol (2:1).

(iii) Samples. Pressed crude and refined sunflower seed oils were provided by Molinos Río de La Plata S.A. Water- and acid-degummed oils and their corresponding pellets were obtained by processing the crude oils at the laboratory.

Degumming process. Water degumming (WD) on crude sunflower oils: 2.5% water, $T = 40°C$, 5–55 min, moderate agitation. Acid degumming (AD) on water-degummed oils (t = 35 min): 2.5% phosphoric and citric acid solution (10%) and acid mixture (phosphoric acid/citric acid, 50:50), 70°C, 5–35 min, moderate agitation. In both cases, samples were then centrifuged at 8,540 × g, 5°C, for 30 min, yielding the corresponding pellets and supernatant oils.

SPE methodology. Sep-Pak Vac Diol Cartridges (1 g) were obtained from Waters (Milford, MA). The cartridges were conditioned with 4 mL methanol, 4 mL chloroform, and 8 mL *n*-hexane. Samples (≈400 mg) were previously dissolved in 0.5 mL chloroform, eluted with 8 mL chloroform, 2 mL acetone, and 14 mL methanol/ammonium hydroxide (0.5 mL/100 mL of a 25% ammonium hydroxide solution), according to methodology of Carelli *et al*. (6) modified by us. The sequential elution was carried out using a vacuum chamber. The methanolic fractions were collected in conical vials and evaporated to dryness under high-purity nitrogen.

HPLC analysis. Phospholipids were analyzed in a Waters HPLC system consisting of a Waters 600 Controller solvent delivery, a Waters 717 plus Autosampler injector, Millenium Software 2010 v. 2.10; and an Evaporative Light Scattering MK III Detector (Varex, Rockville, MD).

The column used was a Lichrospher Si-60/II (3 µm, 150 × 4.6 mm i.d.). The mobile phase consisted of solvent A (chloroform/*t*-BME, 75:25) and solvent B (chloroform/methanol/ammonium hydroxide, (1:97:2) [Abidi *et al*. (8) modified by us]. It was pumped at 0.5 mL/min.

The HPLC–ELSD determinations were carried out with an elution gradient and the ELSD parameters were $T = 55°C$, gas flow (high-purity nitrogen) 1.8 standard liters per minute.

The elution cycle was initiated with a linear gradient from 100 to 0% solvent A in 30 min, then held at 100% solvent B for 10 min and ended with a linear gradient from 0 to 100% solvent A in 10 min.

Phosphorus determination. Residual phosphorus content was determined in samples of supernatant oils degummed with water and citric acid, according to the Norma Instituto Argentino de Racionalización de Materiales 5597/70 (12).

Statistical analysis. Analysis of variance was applied to the data using a SYSTAT statistical package (13).

RESULTS AND DISCUSSION

A typical chromatogram of a sample of crude sunflower oil is shown in Figure 1. A good peak resolution after the purification step by SPE is observed. The main compounds present in this sample are PC (43.5 ± 3.4%), PE (23.1 ± 1.0%), and PI (20.9 ± 0.5%). A chromatogram corresponding to refined oil is superimposed on Figure 1, evidencing that the phospholipid content of this sample is undetectable. The methodology of SPE was appropriate for purifying and concentrating the phospholipid fractions corresponding to different stages of the degumming process.

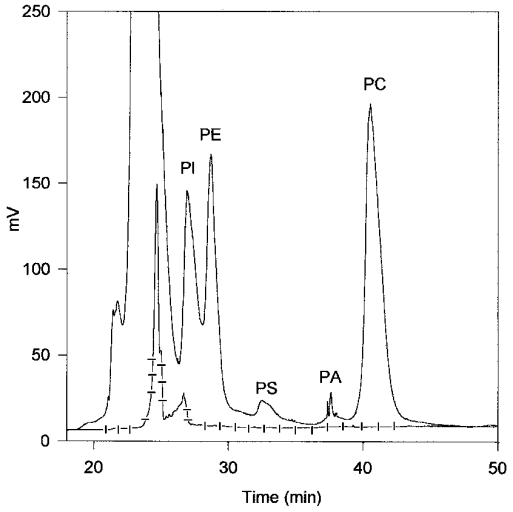

FIG. 1. High-performance liquid chromatogram of crude sunflower oil (—) with refined oil (ш) superimposed. Peaks: PI, phosphatidylinositol; PE, phosphatidylethanolamine; PS, phosphatidylserine; PA, phosphatidic acid; PC, phosphatidylcholine.

The kinetic evolution of each phospholipid in water-degummed oils is shown in Figure 2A. A differential behavior of these compounds has been registered: the content of PC drastically decreased after 5 min of WD (≈99%), PE was removed with the most difficulty ($P < 0.05$). PS, PA, and PI showed moderate removal results. At 35 min of treatment, a significant decrease of the total phospholipid content was achieved ($P < 0.05$). These results are in agreement with the evolution registered for the residual phosphorus content in supernatant oils (Fig. 3A).

The information obtained for this process is in accordance with available data on hydratability rates mentioned by other authors (3), showing that PC exhibits the highest value, followed by PI, PA, PS, and PE.

It is important to consider that the hydratability condition of each compound is related to the chemical structure, swelling, capacity to form ordered structures, and ability to generate vesicles of mixed materials (mainly PC, PI, and PE) due to the intersolubility that these compounds could exhibit (14).

Based on this information, the supernatant oil corresponding to 35 min of WD was chosen as the initial sample for the subsequent step of AD.

The HPLC–ELSD profiles corresponding to the AD process with phosphoric acid can be observed in Figure 2B. Rapid elimination of the remnant content of PC and PA (5 min) was registered. PE appears to be the most difficult compound to remove.

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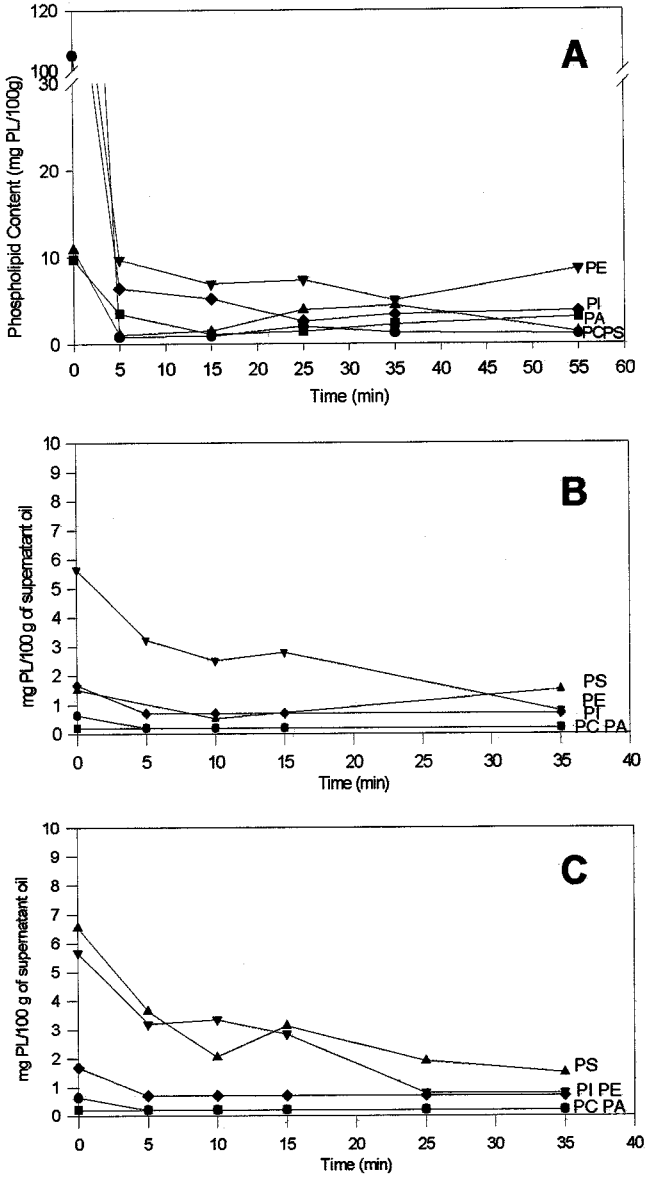

FIG. 2. Kinetic evolution of the phospholipid (PL) content. (A) Water-degummed oils, T = 40°C, 2.5% of water; (B) acid-degummed oils, T = 70°C, 2.5% phosphoric acid; (C) acid-degummed oils, T = 70°C, 2.5% citric acid. ●, PC; ■, PA; ▲, PS; ▼, PE; ◆, PI. Plotted values are the mean of three determinations. See Figure 1 for abbreviations.

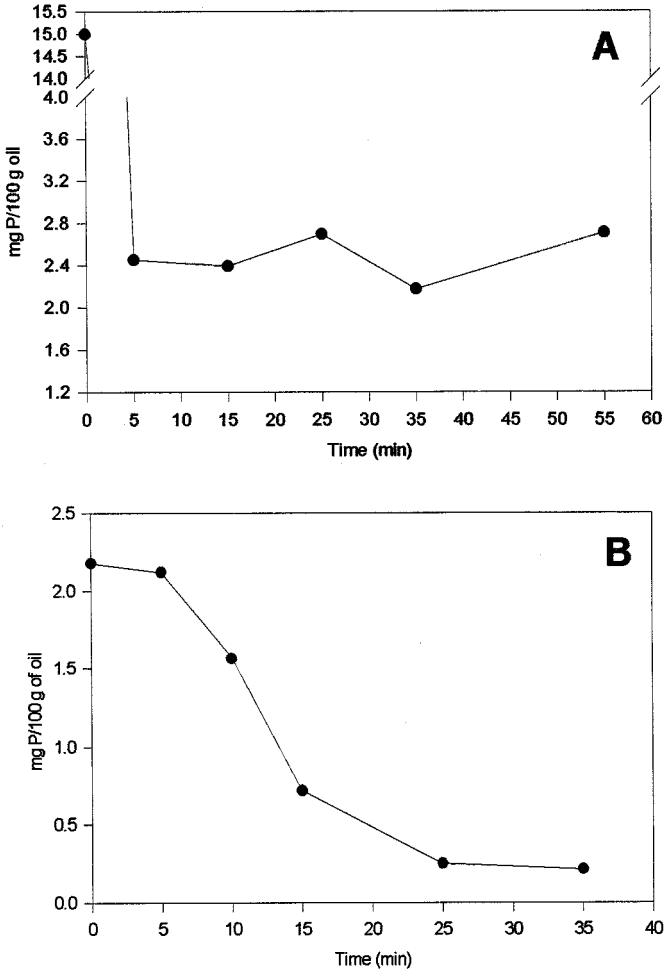

FIG. 3. Residual phosphorus content in supernatant oils during (A) water degumming, T = 40°C, 2.5% of water; (B) acid degumming, T = 70°C, 2.5% citric acid. Plotted values are the mean of three determinations.

phospholipids shows that PC and PA are present in traces after 5 min of treatment. The content of PI diminishes more quickly than PE and PS ($P < 0.05$). After 15 min of processing, the level of PE decreased rapidly down to traces, while PS exhibited a smoother evolution (Fig. 2C). In this case, the evolution of the residual phosphorus content exhibited a decrease along the degumming process, reaching the lowest level by 25 min (Fig. 3B).

The results obtained for AD with acid mixture show the presence of PC and PA at trace levels after 5 min of treatment, as observed for both acids used separately. In this case, PE is present at higher levels than PI, PC, and PA in the supernatant oils ($P < 0.05$). PI content diminishes after 15 min, while PS levels remain relatively constant during the whole process (Fig. 4A).

The evolution of the total phospholipid content relative to the AD process can be observed in Figure 4B. For phosphoric acid and acid mixture, a minimal quantity is reached at 35 min of treatment. Citric acid exhibits a notorious superiority as a degumming agent when compared with the other agents assayed, attaining the lowest residual phospholipid content by 25 min ($P < 0.05$). Also, it can be observed that after 15 min of treatment, the presence of citric acid in the acid mixture facilitates the removal of the remnant phospholipids present in the degummed oils.

Taking into account the results presented above, a comparative analysis of the kinetic extraction of phospholipids by both degumming processes (WD and AD) can be performed. Approximately 35 min is required for removing the main HP with WD. For AD with citric acid, 25 min is the optimal contact time to allow the elimination of the remnant NHP. In the case of phosphoric acid and acid mixtures treatments, 35 min

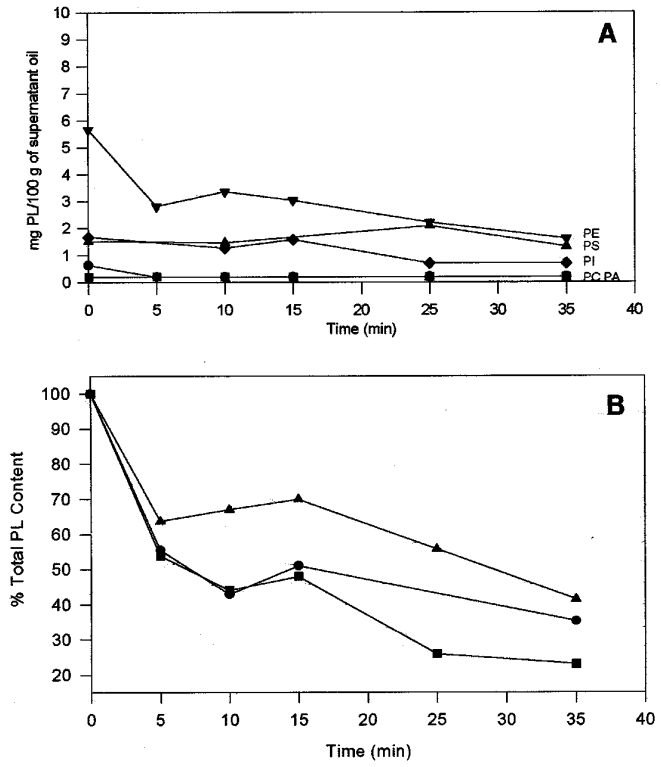

FIG. 4. (A) Kinetic evolution of the phospholipid content in acid-degummed oils, T = 70°C, 2.5% acid mixture (phosphoric acid/citric acid, 50:50). ●, PC; ■, PA; ▲, PS; ▼, PE; ◆, PI. Plotted values are the mean of three determinations. (B) kinetic evolution of the total phospholipid content in acid-degummed oils. T = 70°C, 2.5% degumming agent. ●, PA; ■, citric acid; ▲ = acid mixture (phosphoric acid/citric acid, 50:50). % Total PL content = total phospholipid content referred to T = 0 min. See Figure 1 for abbreviations.

is the time chosen for this purpose. PC is easily removed by both water and AD agents. PE could be used as a marker to evaluate the efficiency of the degumming process.

This information is a good technological tool to obtain the best yields during the vegetable oil refining process, operating under the conditions previously mentioned.

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