Phosphorus-to-Phospholipid Conversion Factors for Crude and Degummed Sunflower Oils

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ABSTRACT: Phospholipid composition and phosphorus content of several crude and degummed sunflower oils were measured in order to compare theoretical and experimental factors used to convert phosphorus content to phospholipid content. Differences in phospholipid content between sunflower oils obtained by different extraction and degumming methods were also considered. From FA and phospholipid compositions, average theoretical conversion factors of 24.7 and 23.0 were found for crude and degummed sunflower oils, respectively. The experimental conversion factor for degummed oils was in good agreement with the theoretical value. In contrast, fitted experimental factors were significantly lower for crude oils. The differences could be attributed to phosphorus from sources other than phospholipids and to the presence of minor phospholipids not quantified by chromatographic analysis. The relative phospholipid concentrations of oils depended on the method of extraction and the type of degumming. Solvent-extracted oils had a higher total phospholipid content, being generally more concentrated in PC and PE. The content of nonhydratable phospholipids was relatively low; acid or enzymatic degumming removed 40 to 70% of these phospholipids.

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KEY WORDS: Acid degumming, conversion factor, enzymatic degumming, high-performance liquid chromatography, phospholipids, phosphorus, sunflower oil, water degumming.

Phospholipids are natural components of sunflower seed oils that can be analyzed quantitatively by chromatographic techniques (1). The phospholipid content in crude sunflower oil varies from 0.6 to 1.2%, and oils obtained by extraction usually have a higher phospholipid content than those obtained by pressing (2,3). The major phospholipids in sunflower seed oil are PC, PE, PI, and PA. Most of these phospholipids are hydratable and can be removed from the crude oil using a water-degumming process. Nonhydratable phospholipids, mainly calcium and magnesium salts of PA and lysoPA, glycerophosphates, and inorganic phosphates remain in the oil after water degumming (2,4). More efficient degumming can be obtained by acid treatment, where the hydratability of these compounds is increased by addition of either phosphoric or citric acid (5). Also, nonhydratable phospholipids can be removed by an enzymatic treatment that makes use of special biochemical reactions such as enzyme-catalyzed hydrolytic breakdown of the phospholipid molecule (6). In previous papers, it was observed that total content and composition of phospholipids in sunflower oil were strongly affected by the extraction and water-degumming processes (3,7). The knowledge of vegetable-oil phospholipid content is necessary to evaluate oil quality and the effectiveness of the degumming process. The total content of phosphatides in oils is commonly determined by ashing the sample and measuring the phosphorus spectrophotometrically (AOCS Method Ca 12-55: Ref. 8). To convert the percentage of total phosphorus to the equivalent phosphatide content, a multiplication factor of 30 is usually applied, although a value of 25 has been suggested for soybean and sunflower crude oils because of their phospholipid content and individual FA phospholipid composition (9). However, the spectrophotometric method has the disadvantage that only the total concentration of phosphorus is determined, including both inorganic phosphates and organic phosphatides. A number of methods are available to determine the phospholipid composition of an isolated phosphatide mixture (2,4). AOCS official method Ja 7-86 (8) estimates the phospholipid content of lecithin by TLC separation and phosphorus analysis using conversion factors in the range of 22.03–27.03 depending on the phospholipid being considered. AOCS method Ja 7b-91 is for the direct determination of single phospholipds in lecithin by HPLC, and it is not applicable to lysophospholipids. In contrast, the analysis of phospholipids in vegetable oils is rather recent, it being more common to report relative area percentages from the chromatographic analysis together with the total phosphorus content than the direct chromatographic quantification. Direct quantitative determination of individual phospholipids in sunflower oils can be performed by a solid-phase extraction (SPE)-HPLC method (1). From previous work that simultaneously quantified phospholipids from chromatographic analysis and reported total phosphorus content (3,7), a divergence between both methods of phospholipid content estimation in vegetable oils was suggested.

The aim of this work was to obtain theoretical and experimental factors to convert the phosphorus content of crude and degummed sunflower oils to phospholipid content.

EXPERIMENTAL PROCEDURES

Materials. All reagents were of analytical reagent grade, except *n*-hexane and 2-propanol, which were of HPLC grade from J.T. Baker Inc. (Phillipsburg, NJ). Acetate buffer (pH 4.2) was prepared by mixing 26.5 mL of sodium acetate solution

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(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 (by vol). Standards of L-α-PE, L-α-PI, and L-α-PC from soybean and L-α-PA, sodium salt from egg yolk lecithin 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 the HPLC mobile phase to different concentrations. For the SPE step, 500-mg bonded oil SPE cartridges (J.T. Baker Inc.) were used. For comparative studies between methods, model oil samples were made by mixing refined sunflower oil with a powdered commercial soybean lecithin in a concentration that ranged from 0 to 1.75%. Crude industrial sunflower oils obtained by hot-pressing or hexane extraction, both with and without water degumming, and laboratory water-, acid-, and enzyme-degummed sunflower oils were also analyzed. Twice-distilled water, a 50 wt% solution of citric acid in water, 85% *o*-phosphoric acid ACS reagent grade, and Lecitase 10 L (a phospholipase A_2 from Novo Nordisk A/S, Bagsvaerd, Denmark) were used for oil degumming.

Oil degumming. Four different laboratory degumming processes were performed on 200-g samples of crude oil. The methods were: (i) water degumming at 80°C, with 2 wt% distilled water and mechanical stirring (500 rpm) for 5 min, (ii) citric acid degumming at 80°C with 0.3 wt% citric acid, mechanical stirring (500 rpm) for 5 min, followed by addition of 2 wt% distilled water; (iii) phosphoric acid degumming at 80°C with 0.15 wt% phosphoric acid, mechanical stirring (500 rpm) for 5 min, followed by addition of 2 wt% distilled water; (iv) enzyme-catalyzed degumming at 60°C with 0.15 wt% citric acid, NaOH (3 wt%) up to pH = 5, and 140 IU Lecitase 10 L in aqueous solution (0.2% vol/vol) with mechanic stirring (500 rpm) for 30 min. In all experiments, samples were then centrifuged at 1000 × *g*, for 5 min, yielding the respective degummed oils.

Sample preparation. A 2.5 g mass of oil was weighed into a 10-mL volumetric flask and filled to the mark with chloroform.

Phospholipid enrichment and separation. The oil samples were partitioned by SPE as described previously (1). Briefly, the SPE procedure consisted of: (i) sorbent conditioning with 2 mL methanol, 2 mL chloroform, and 4 mL hexane; (ii) sample loading: a micropipet was used to inject 50–150 mg of oil dissolved in chloroform (200–600 μL oil), using the highest mass when degummed oils were analyzed; (iii) TAG release from the sorbent bed: accomplished by passing 2.5 mL chloroform through; and (iv) phospholipid recovery by elution with 7 mL methanol containing 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 100 μL with mobile phase. The phospholipid fraction was quantified by HPLC analysis based on the IUPAC 5.302 standard method for soybean lecithin (10). A Waters HPLC system with a Waters 996 photodiode array detector set at 206 nm, a LiChrosorb Si-60 (250 × 4 mm, 5 μm particle size) column (Merck, Darmstadt, Germany) and a Millenium 2010 Chromatography Manager (Millipore Corporation, Milford, MA), were used. The phospholipid content, expressed as percentage in the oil, was obtained as:

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

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 in the SPE cartridge.

Total phosphorus content. Total phosphorus was determined by standard AOCS official method Ca 12-55 (8). The method determines phosphorus by ashing the sample in the presence of zinc oxide, followed by the spectrophotometric measurement of phosphorus as a blue phosphomolybdic acid complex.

FA. FA composition of oil and phospholipid samples was determined by GC analysis according to IUPAC 2.301-2.302 standard methods (10). The FAME were separated on a SP-2380 capillary column (30 m × 0.25 mm i.d., film thickness 0.2 μm; Supelco Co., Bellefonte, PA) maintained at 170°C for 15 min, then increased at 4°C/min to 210°C and held at 210°C for 10 min, using hydrogen as carrier gas. They were quantified by FID using an Agilent 4890 D gas chromatograph (Agilent Technologies Inc., Wilmington, DE).

Statistical analysis. All analyses were carried out in triplicate, and the mean values are reported. The precision of the average theoretical conversion factors was expressed as the confidence interval at a significance level of $\alpha = 0.05$. The goodness of fit was expressed by the square of the correlation coefficient (r^2) and the residual variation expressed as the root mean square error (RMSE).

RESULTS AND DISCUSSION

The FA analysis of two crude sunflower oils obtained by hexane extraction and pressing from the same lot of seeds, their phospholipid fractions, and a commercial soybean lecithin were done. The following results were obtained: extracted sunflower oil ($C_{16:0}$ = 6.0%, $C_{18:0}$ = 3.8%, $C_{18:1}$ = 24.4%, $C_{18:2}$ = 65.8%); pressed sunflower oil ($C_{16:0}$ = 5.9%, $C_{18:0}$ = 3.8%, $C_{18:1}$ = 24.6%, $C_{18:2}$ = 65.7%); extracted-oil phospholipid fraction ($C_{16:0}$ = 11.6%, $C_{18:0}$ = 3.8%, $C_{18:1}$ = 16.1%, $C_{18:2}$ = 68.5%); pressed-oil phospholipid fraction ($C_{16:0}$ = 12.2%, $C_{18:0}$ = 3.0%, $C_{18:1}$ = 15.7%, $C_{18:2}$ = 69.1%); soybean lecithin ($C_{16:0}$ = 24.5%, $C_{18:0}$ = 5.4%, $C_{18:1}$ = 7.8%, $C_{18:2}$ = 56.5%, $C_{18:3}$ = 5.8%). Only small differences in the content of individual FA were observed between pressed and extracted oils or between their phospholipid fractions. However, the phospholipid FA composition differed from its oil composition, showing a higher concentration of palmitic acid. This difference has already been reported by other researchers (9,11). Studies in genetically modified soybeans demonstrated that phospholipid FA composition changed with oil FA modification (11).

The average phospholipid M.W. (M_{PL}) can be estimated from the average FA M.W. (M_{FA}) and the phospholipid composition of the oil according to the following equations:

TABLE 1
Range of Phosphorus Content and Phospholipid Composition in Crude and Degummed Sunflower Oils in Comparison with a Soybean Lecithin Sample

Oil samples	Phosphorus (ppm)	CPL (wt%)	Phospholipid distribution (%) PE	PA	PI	PC
Pressed [a]	320–953	0.29–0.74	15.0–23.4	14.8–36.7	15.8–27.2	19.0–50.1
Extracted [b]	342–657	0.59–1.20	17.0–25.6	10.7–22.0	15.5–30.5	28.9–52.0
Degummed[c]	15–251	0.02–0.58	2.1–36.3	18.0–89.0	2.3–30.2	2.5–42.0
Soybean lecithin	30, 100	69.41	26.0	23.0	21.0	30.0

[a] $n = 11$.
[b] $n = 10$.
[c] $n = 21$. CPL, chromatographic phospholipid content in wt% on oil.

$$M_{PL} = \left[\frac{W_{PC}}{221.2 + M_{FA}} + \frac{W_{PI}}{217.2 + M_{FA}} + \frac{W_{PE}}{179.1 + M_{FA}} + \frac{W_{PA}}{136.0 + M_{FA}} \right]^{-1} \quad [2]$$

with $M_{FA} = [\sum W_i / M_i]$, where W_i and M_i represent the mass fraction and the M.W. of the ith FA. Since variation in FA compositions of individual phospholipids has low influence on their M.W., this calculation was performed taking into account the whole-phospholipid FA composition.

From the M_{PL}, a theoretical factor (K_{th}) for converting phosphorus content in oil (P) to its total phospholipid content (TPL_{th}) can be calculated.

$$K_{th} = M_{PL}/30.97 \quad [3]$$

$$TPL_{th}\ (wt\%) = K_{th}\ 10^{-4}\ P\ (ppm) \quad [4]$$

Such information may enable a more realistic estimation of phospholipid contents in crude and degummed oils on the basis of their elemental phosphorus content.

Table 1 shows the variability of phospholipid composition in crude and degummed sunflower oils. In addition, Figures 1 and 2 present the effect of different degumming processes on phospholipid content in extracted and pressed sunflower oil, respectively. Relative phospholipid concentrations depended on the extraction method and type of degumming. In agreement with previous studies (1,3) the total content of phospholipids depends on the method of extraction, being higher and generally more concentrated in PC and PE in solvent-extracted oils. The content of nonhydratable phospholipids was relatively low. The total removal of PI, PE, and PC was more than 95%, while PA was partially removed during water degumming. PA in pressed oil was significantly more hydratable than in extracted oil, indicating that most PA is complexed with calcium or magnesium in the oil obtained by solvent extraction (7). Acid and enzymatic degumming treatments removed 40 to 70% of the nonhydratable phospholipids. PA is the main constituent of residual phospholipids, particularly in the enzymatic degummed oil.

Table 1 also shows the range of phosphorus content and phospholipid compositions found in different samples. From individual calculations the following average theoretical conversion factors were obtained $K_{th} = 24.7 \pm 0.2$ for crude industrial sunflower oil ($n = 21$, significance level $\alpha = 5\%$); $K_{th} = 23.0 \pm 0.6$ for degummed sunflower oil with phospholipid content lower than 0.1% ($n = 21$, significance level $\alpha = 5\%$); and $K_{th} = 24.25$ for soybean lecithin. These factors are similar to those recommended for soybean lecithin in AOCS official method Ja 7-86 (8). The lowest value for degummed oils is due to their higher percentage of PA. This phospholipid has the lowest M.W. and preferentially remains in the oil after degumming. The total phospholipid contents (TPL_{th}) calculated from the phosphorus content given in Table 1 by using the theoretical factors were significantly higher than the experimental chromatographic phospholipid content (CPL), especially for crude pressed sunflower oils. These discrepancies are discussed below.

The method of applying a conversion factor to calculate the phospholipid oil content from its phosphorus content was validated by using refined oil/lecithin mixtures. Model oil samples with a lecithin concentration in the range of 0 to 1.75% were analyzed by spectrophotometric and chromatographic methods. A correlation between the total phospholipid content measured by HPLC (CPL) and the phospholipid content calculated from phosphorus determination using the theoretical conversion factor found for soybean lecithin (TPL_{th}) gave $TPL_{th} = 1.054$ CPL ($r^2 = 0.999$, RMSE = 0.025).

Figure 3 shows the correlation between phosphorus and phospholipid contents in pressed crude oils, hexane-extracted crude oils, and degummed oils. Experimental data for degummed oils can be represented as CPL (wt %) = 0.002083 P (ppm) ($r^2 = 0.951$, RMSE = 0.058), or CPL (wt %) = 0.00244 P (ppm) − 0.053 ($r^2 = 0.912$, RMSE = 0.050), in reasonable

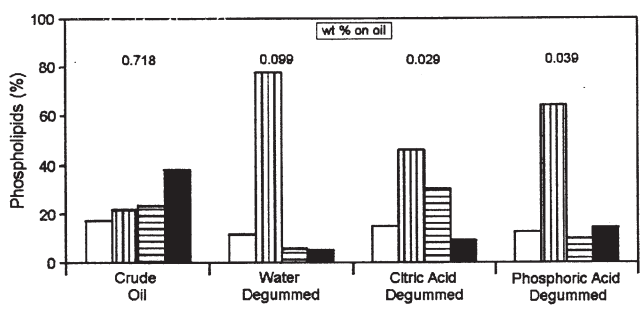

FIG. 1. Effect of degumming on phospholipid content in extracted sunflower oil. Open box, PE; vertically lined box, PA; horizontally lined box, PI; solid box, PC.

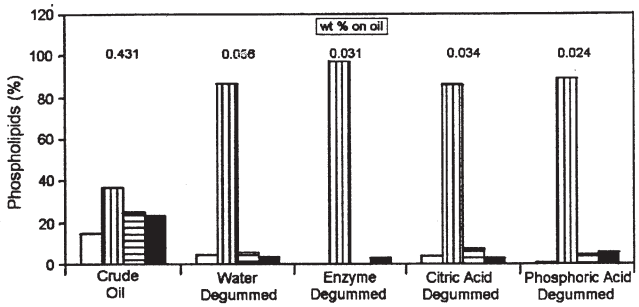

FIG. 2. Effect of degumming on phospholipid content in pressed sunflower oil. Open box, PE; vertically lined box, PA; horizontally lined box, PI; solid box, PC.

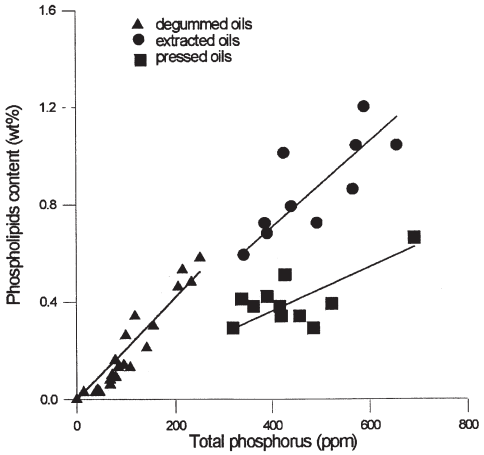

FIG. 3. Correlation between phosphorus and phospholipid content in sunflower oils.

agreement with theoretical calculations. Fitted experimental factors were significantly lower for crude oils, especially for pressed oils ($K_{exp} = 9.1$, $r^2 = 0.962$, RMSE = 0.081) than for extracted oils ($K_{exp} = 17.6$, $r^2 = 0.980$, RMSE = 0.123).

Differences between experimental and theoretical conversion factors can be attributed to the following: (i) The presence of meal particles and phosphorus content from sources other than phospholipids, which is also determined by the spectrophotometric method. We observed that crude oils that were free of solid particles (obtained by chloroform dilution, filtration, and solvent removal) presented the same phospholipid content but lower phosphorus content, giving a conversion factor very close to that of degummed oils. (ii) The presence of other minor phospholipids (i.e., lysophospholipids) not quantified by the chromatographic method. However, the four major phospholipids were determined, and the remaining fraction was relatively low in sunflower oils (1,2). (iii) Experimental errors in the determination of the total phosphorus content in crude sunflower oils by the AOCS method Ca 12-55 (8).

In conclusion, experimental factors for sunflower oils were obtained in order to estimate the total phospholipid content from the phosphorus content. These factors are significantly lower than theoretical values, particularly for crude oils. Although the chromatographic method has the advantage of identifying and quantifying phospholipids separately, the proposed factors allow a fairly good estimation of the total phospholipid content in crude and degummed sunflower oils.

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REFERENCES

1. Carelli, A.A., M.I.V. Brevedan, and G.H. Crapiste, Quantitative Determination of Phospholipids in Sunflower Oil, *J. Am. Oil Chem. Soc. 74*:511–514 (1997).
2. Padley, F.B., F.D. Gunstone, and J.L. Harwood, Occurrence and Characteristics of Oils and Fats, in *The Lipid Handbook*, 2nd edn., edited by F.D. Gunstone, J.L. Harwood, and F.B. Padley, Chapman & Hall, London, 1994, pp. 47–223.
3. Brevedan, M.I.V., A.A. Carelli, and G.H. Crapiste, Changes in Composition and Quality of Sunflower Oils During Extraction and Degumming, *Grasas Aceites 51*:417–423 (2000).
4. Dijkstra, A., Degumming, Refining, Washing and Drying of Fats and Oils, in *Proceedings of the AOCS World Conference and Exhibition on Oilseed Technology and Utilization*, Budapest, Hungary, American Oil Chemists' Society, Champaign, 1992, pp. 138–151.
5. Dimic, E., D.J. Karlovic, and J. Turkulov, Pretreatment Efficiency for Physical Refining of Sunflowerseed Oil, *J. Am. Oil Chem. Soc. 71*:1357–1361 (1994).
6. Buchold, H., Enzymatische Phosphatidentfernung aus Pflanzenölen, *Fat Sci. Technol. 95*:300–304 (1993).
7. Crapiste, G.H., M.I.V. Brevedan, and A.A. Carelli, Water Degumming of Sunflower Oil, in *Advances in Oils and Fats, Antioxidants, and Oilseed By-Products, Volume II the Proceedings of the World Conference on Oilseed and Edible Oils Processing*, edited by S.S. Koseoglu, K.C. Rhee, and R.F. Wilson, AOCS Press, Champaign, 1998, pp. 32–35.
8. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, AOCS Press, Champaign, 1993.
9. Chapman, G.W., A Conversion Factor to Determine Phospholipid Content in Soybean and Sunflower Crude Oils, *J. Am. Oil Chem. Soc. 57*:299–302 (1980).
10. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th edn., edited by C. Paquot and A. Hautfenne, International Union of Pure and Applied Chemistry, Blackwell Scientific Publications, Oxford, 1992.
11. Wang T., E.G. Hammond, and W.R. Fehr, Phospholipid Fatty Acid Composition and Stereospecific Distribution of Soybeans with a Wide Range of Fatty Acid Composition, *J. Am. Oil Chem. Soc. 74*:1587–1594 (1997).

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