Computation of Conversion Factors to Determine the Phospholipid Content in Peanut Oils[1]

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ABSTRACT

Peanut oil was separated into the various lipid classes by column chromatography. The polar lipid fraction which contained phospholipids was separated into individual major components by 2-dimensional thin layer chromatography. Conversion factors for calculating the concentration of total phospholipid in peanut oil from percent elemental phosphorus were determined by estimation of molecular weights for the respective components. A conversion factor of 23.6, 24.8, 26.6, 22.2, and 24.4 was found for phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, phosphatidic acid, and total phospholipid, respectively. These factors also were used to convert μg of phosphorus into μg of phospholipid.

The Official AOCS method has recommended the factor of 30 be used to convert elemental phosphorus (P) to phosphatides in soybean oil (1). While this factor may be used for the "acetone insoluble" fraction, its use for degummed and refined oils has been questioned recently (2,3). Also the "theoretical" conversion factor of 25.5 employed by Jamieson and McKenny (4) and recommended for use in "A Laboratory Handbook for Fats and Oils" may be too high since it was calculated from a lecithin with the empirical formula $C_{44}H_{86}PO_8N$ (5).

The fatty acid composition of phospholipid (PL) fractions from different oils varies. Therefore, on a weight percentage basis, conversion factors for different oils would be different. In this study, the average molecular weight (AMW) of the individual PL components and the total PL fraction were determined experimentally from the fatty acid composition as analyzed by gas liquid chromatography (GLC) to give a more precise conversion factor to determine percentage PL content in peanut oil from the elemental P assay.

MATERIALS AND METHODS

Peanut oil was extracted from 75 g (DWT) of mature seed by homogenizing the tissue 3 times in 250 mL chloroform/methanol (2:1, v/v) for 1 min followed by filtration. Nonlipid contaminants were removed by extracting with a modified Folch method using 0.7% NaCl water solution (6). A rotary evaporator was used to reduce the volume of the chloroform fraction to 250 mL giving a concentration of 130 mg/mL as determined by gravimetric analysis.

Crude lipid was separated into various lipid classes by silicic acid column chromatography as described by Pattee et al. (7). Approximately 300 mg (2.5 mL) of the crude lipid was applied to a 20-g silicic acid column. Solvents, elution volumes, fractions, and major components are as listed (Table I).

The acetone and methanol fractions were combined and reduced in volume to 1 mL under vacuum and under a stream of nitrogen. Only the polar lipid fraction was used in this study.

Phospholipids were separated by 2-dimensional TLC on silica gel G (500 μm, 20 × 20 cm) plates. Plates were developed in a chamber lined with filter paper using a basic solvent which consisted of C/M/7N NH_4OH (65:30:4, v/v/v). After development, plates were dried in a vacuum oven for 30 min. Plates were then developed in a second solvent with C/M/HOAC/H_2O (170:25:25:6, v/v/v/v) at a right angle to the first development. Individual PL were detected on the plate by spraying with Rhodamine 6G spray and viewing under a UV light. Phosphorus-containing spots (as determined on other plates using a phosphorus-specific spray) were scored and removed by a glass recovery tube (Kontes). Appropriate standards were chromatographed and three replicates were run per sample. Six plates were required to obtain enough material for fatty acid analysis by GLC.

Phospholipids were methylated by placing the absorbent containing lipids into a large test tube, adding 4.5 mL of 2.5% methanolic-HCl and refluxing for 2 hr at 63 C. After cooling, 0.5 mL of H_2O was added to each tube and the methyl esters were extracted with petroleum ether (bp 30-60 C). Fatty acid composition was determined by GLC on a 1/8 in. × 12 ft ESGG-X column (10%) using a flame ionization detector. Elemental P was determined spectrometrically at 830 nm using the aqueous phase (8).

RESULTS AND DISCUSSION

The 4 major phospholipids in peanut oil have been analyzed for their fatty acid compositions (Table II). These 4 compo-

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TABLE I

Lipid Class Separation

Elution vol and solvent	Main components	Fraction	Final vol. (mL)
75 ml 7% ethyl ether in hexane	Triglycerides	I	5
100 ml 7% ethyl ether in hexane	Diglycerides	II	25
425 ml chloroform	Diglycerides & free sterols	II	
400 ml chloroform/acetone (1:1)	Steryl glucoside & esterified steryl glucosides	III	1
100 ml acetone	Other glucosides & phospholipids	IV	1
300 methanol	Phospholipids		

TABLE II

Fatty Acid Composition of the Major Phospholipids and Total Phospholipid Fraction in Peanut Oil[a]

Component/fraction	Fatty acid				
	16:0	16:1	18:0	18:1	18:2
Total phospholipids	25.4	2.5	3.9	33.4	34.7
Phosphatidylethanolamine	24.2	3.0	5.9	40.8	26.4
Phosphatidylcholine	31.5	4.2	12.2	34.1	18.0
Phosphatidic acid	28.3	3.6	17.0	34.9	16.2
Phosphatidylinositol	31.8	11.3	9.9	26.6	20.3

[a]Area percent.

nents constitute 87.1% of the total polar fraction. Predominant fatty acids found in the phospholipids of peanut oil are palmitic, palmitoleic, stearic, oleic and linoleic. Traces of behenic and lignorceric were found; however, the concentration was less than 1%. Composition of the PL fraction, both qualitatively and quantitatively, is in agreement with that found by Sanders (9). Fatty acid composition of the PL fraction in other oilseeds is similar to the composition in peanuts qualitatively, but differs quantitatively. Therefore, there is a need to determine experimentally specific conversion factors for individual PL components as well as the total PL fraction for different oils. Conversion factors were determined for each component and the total PL fraction from the composition data presented in Table II.

The AMW for one fatty acid/mol PL is equal to the sum of the contributions of all the fatty acids. Weight contribution of each fatty acid is derived by the following equation:

$$\text{AMW} = \Sigma\ [\text{decimal fraction} \times \text{MW of the fatty acid}]^{-}$$

Therefore, the AMW of one fatty acid/mol phosphatidylethanolamine (PE) was 274.9. The MW of phosphoglycerol ethanolamine moiety was 181. Hence, the MW of PE was:

$$\text{MW of PE} = 2 \times 274.9 + 181.1 = 730.9$$

Atomic weight of elemental P is 30.97. Thus, the weight fraction P in PE was:

$$\frac{\text{Atomic wt P}}{\text{MW PE}} = \frac{30.97}{730.9} = 0.0424$$

Because there was one mol P/mol PE the conversion factor for PE in peanut oil is:

$$\frac{1}{0.0424} = 23.6$$

Conversion factors for phosphatidylcholine, phosphatidylinositol and phosphatidic acid were calculated in a similar manner and found to be, respectively, 24.8, 26.6, and 22.2.

To determine the AMW of the total PL fraction, the MW contribution of each PL must be calculated using the percentage composition data presented in Table III. The unidentified PL (12.9%) was assumed to be phosphatidylglycerol since it has been previously identified in mature seeds. The calculated MW of phosphatidylglycerol was 744.9 and its contribution was 96.1 MW units. Hence the computed AMW for the total PL fraction in peanut oil was 756.8. The wt fraction of P in the total PL is:

$$\text{Wt fraction P} = \frac{756.8}{756.8} = 0.0409$$

$$\text{and } \frac{1}{0.0409} = 24.4$$

The conversion factor for the total PL calculated from the percentage concentration of the individual PL was 24.4, as compared to 24.5 from the fatty acid composition of the total PL.

TABLE III

Phospholipid Composition of Peanut Oil and Molecular Weight Data

Phospholipid	% PL	AMW
Phosphatidylethanolamine	39.44	730.9
Phosphatidylcholine	18.82	768.2
Phosphatidic acid	6.62	686.4
Phosphatidylinositol	22.22	824.2
Unidentified phospholipid	12.90	744.9

Conversion of Percentage Phosphorus to Percentage Phospholipid

Using the conversion factor (24.4) to determine % PL from the original data, the raw data and equations are as follows.

Absorbance (A) at 830 nm of total P from a sample (400.17 mg) applied to a silica column was 2.757. The amount of total PL applied to a thin layer chromatography plate was 60 μL from a total volume of 1 mL. Using the y-intercept (0.689) and the slope (0.127) from the standard P assay the following equations can be written.

$$\mu\text{g PL} = \frac{\left(\frac{\text{A-0.689}}{0.127}/30.97\right)(756.8\ \mu\text{g}/\mu\text{mol})}{60\ \mu\text{L}} \times 10^3$$

and

$$\mu\text{g P} = \frac{\text{A-0.689}/0.127}{60\ \mu\text{L}} \times 10^3$$

$$\%\ \text{PL} = \frac{\mu\text{g PL}}{\mu\text{g total lipid}} \times 100$$

then

$$\%\ \text{PL} \frac{\left(\text{A-0.689}/0.127\ /30.97\right)(756.8\ \mu\text{g}/\mu\text{mol}) \times 10^3}{60\ \mu\text{L}} / 4.00170 \times 10^5 \times 100$$

$$= 1.66$$

Using % PL = % P × 24.4

$$\text{and } \%\ \text{P} = \frac{\mu\text{g P}}{\mu\text{g total lipid}} \times 100,$$

then

$$\%\ \text{P} = \frac{\text{A-0.689}/0.127 \times 10^3}{60} / 4.00170 \times 10^5 \times 100 = 0.0678$$

$$= 0.0678$$

and % PL = 0.0678 × 24.4 = 1.65

The values calculated for % PL in the total PL fraction from the raw data and from the conversion factor were in agreement and demonstrated that % PL in peanut oil could be determined by multiplying the experimentally derived conversion factor aby the % P as assayed by the phosphorus procedure. From the results of this study, it is recommended that a conversion factor of 24.4 be used to convert elemental P into % PL or μg PL for the total PL fraction in peanut oil.

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ERRATUM

Please note the following corrections to the article "Heterogeneity within Commercial Contract Analysis Samples of Shea-Nut Kernels," by S.J. Kershaw and E. Hardwick, which appeared in the June issue of *JAOCS* (58:706, 1981). In the legends to Figs. 2-4, the values given as "coefficient of variation" are actually "variance," and the legends should read: (Fig. 2) coefficient of variation, 26.4; (Fig. 3) coefficient of variation, 18.8; and (Fig. 4) coefficient of variation, 111.3.