A Conversion Factor to Determine Phospholipid Content in Soybean and Sunflower Crude Oils

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ABSTRACT

The major phospholipids (PL) from soybeans and sunflowers were separated by 2-dimensional thin layer chromatography (TLC) and the fatty acid composition of each PL was determined by gas liquid chromatography (GLC). PL from soybeans and sunflowers contained high percentages of linoleic and palmitic acids. Only phosphatidylinositol (PI) from both oilseeds were similar in fatty acid composition and the principal acid was palmitic acid. Sunflower phospholipids, except for PI, contained twice as much oleic acid as did those from soybeans. Sunflower PI contained very low but measurable quantities of heptadecanoic acid. The molecular weights (MW) of individual PL were based on their fatty acid composition. The MW found for soybeans and sunflower PL were quite similar even though their fatty acid compositions were different. The average MW of PL in crude soybean and sunflower oils was determined based on the MW of individual PL and their composition in the PL fraction. From that MW, a factor for converting phosphorous content in oil to its PL content was calculated. For both oils, the factor was 25.

INTRODUCTION

In several studies of developing and mature oilseeds, the polar lipid fraction or individual phospholipids (PL) were isolated and their fatty acid compositions were determined (1-8). The fatty acid data, however, were not used to determine the molecular weights (MW) of the individual PL. Such information, along with a knowledge of the PL composition of the oil, may enable a more realistic determination of PL contents of extracted crude oils on the basis of their elemental phosphorous content.

Factors are used to convert elemental phosphorous to PL content. The conversion factor for "acetone insolubles" in crude soybean oil is 30-31.7 (9,10) because it contains substantial amounts of nonphosphorous lipids, i.e., glycolipids and neutral lipids. However, the validity of this factor when used with degummed and refined oils has been questioned recently (11). On the other hand, the factor for other crude oils is given as 25.5 (see L.V. Cocks and C. Vanrede, A Laboratory Handbook for Fat and Oil Analysis, Academic Press, New York, 1966, pp. 137-147) and was calculated from a lecithin with an empirical formula $C_{44}H_{86}PO_9N$ (12).

The purpose of this study was to (a) determine more precise individual PL MW based on their fatty acid compositions, (b) use those results and the PL compositions of the oils to calculate their respective average PL MW and (c) use those weights to calculate conversion factors for soybean and sunflower crude oils.

MATERIALS AND METHODS

Crude oils were extracted from mature soybeans (mixed var.) and sunflower seed (var. Master Farmer) with chloroform/methanol (2:1) after they were ground in liquid nitrogen as previously described (13). The crude oil was centrifuged at 23,400 x g and the clear supernatant oil removed. The remaining pellet was rinsed several times with chloroform and dissolved in ca. 2 ml chloroform. Both the clear supernatant oil and dissolved pellet were used separately as PL sources for this study, the oil to determine its PL composition and the pellet to determine PL fatty acid composition.

Individual PL were separated by 2-dimensional thin layer chromatography (2D-TLC) on precoated Silica Gel 60 plates (14) and identified with specific spray reagents (15). Two samples (dissolved pellets from above) spotted separately (2.0 mg, 3 x 5 μl) on the lower left- and right-hand corners were developed on a single plate. Both samples were eluted with basic solvent (chloroform/methanol/7 N ammonium hydroxide, 65:30:4) for 10 cm; then, after drying with a hand-held dryer, the plate was rotated 90° and one of the samples was eluted with acidic solvent (chloroform/methanol/acetic acid/water, 170:25:25:4) for 9.0 cm. After drying, the plate was rotated 180° and the other sample eluted with the acidic solvent for 9.0 cm. Small amounts of neutral lipid and free fatty acids from the 2 samples overlapped; however, this did not affect the separation of PL (Fig. 1). Five to 6 such chromatograms yielded enough individual PL for fatty acid analysis. PL composition of crude soybean and sunflower oils were also obtained by a 3-solvent system, 2D-TLC, as previously described (13).

After visualization with iodine, each PL spot was carefully scraped into a 15 x 150 mm culture tube. Individual PL were saponified with 2.0 ml 0.5N $NaOH/CH_3OH$ at 83 C for 6 min without removal of the silica gel. Fatty acids were esterified with 3.0 ml BCl_3/CH_3OH (10%) at 83 C for 5.5 min. Fatty acid methyl esters were extracted from the reaction mixture with hexane and the vol reduced to

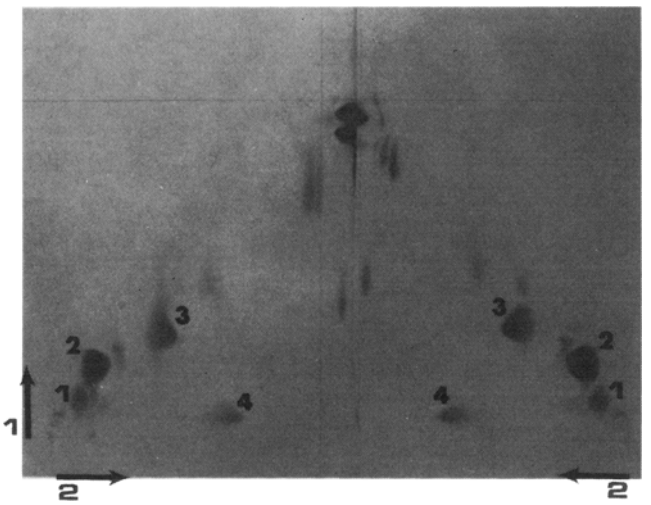

FIG. 1. 2D-TLC of 2 crude soybean lipid samples on one TLC plate. Origins are lower left- and right-hand corners. Basic solvent (#1), chloroform/methanol/7N NH_4OH, 65:30:4, from bottom to top. Acidic solvent (#2), chloroform/methanol/acetic acid/water, 170:25:25:4, from right to left and after drying, from left to right. Spots are identified as 1: phosphatidylinositol; 2: phosphatidylcholine; 3: phosphatidylethanolamine; 4: phosphatidic acid. Sunflower phospholipids migrated in the same way and plates were identical to the one shown.

TABLE I

Phospholipid Compositions of Soybean and Sunflower Crude Oils

Phospholipid	% Individual phospholipids in total phospholipids[a]				
	Soybeans			Sunflowers	
	Mixed var. (this study)	Harwood (1975)[b]	Privett (1973)[c]	Master Farmer var. (this study)	Borodulina (1974)
Phosphatidic acid	4.8	1.5	5.0	2.2	2.2
Phosphatidylinositol	20.3	17.5	14.0	27.9	24.0
Phosphatidylethanolamine	23.3	21.4	26.0	21.2	18.2
Phosphatidylcholine	39.0	43.6	45.0	48.7	55.4
UNK	12.5	4.3	6.3	-	-

[a]Based on P analyses of the individual and total phospholipids.

[b]Composition also included, cardiolipin-3.7%, phytoglycolipid-6.1%, *N*-acylphosphatidylethanolamine-1.1%.

[c]Composition also included, phosphatidyl glycerol-diphosphatidyl glycerol-3.3%.

TABLE II

Fatty Acid Composition of Major Phospholipids of Soybean and Sunflower[a]

Phospholipid	Fatty acid					
	16:0	17:0	18:0	18:1	18:2	18:3
Phosphatidylcholine						
Soybean	20.5 ± 0.5	-	5.5 ± 0.1	10.5 ± 0.3	58.8 ± 0.0	4.6 ± 0.3
Sunflower	18.2 ± 1.1	-	4.7 ± 0.1	23.5 ± 0.2	53.5 ± 1.3	-
Phosphatidylethanolamine						
Soybean	31.6 ± 1.6	-	3.2 ± 0.3	8.7 ± 0.9	53.2 ± 0.1	3.2 ± 0.5
Sunflower	29.6 ± 0.3	-	5.7 ± 0.2	17.4 ± 0.2	47.2 ± 0.3	-
Phosphatidylinositol						
Soybean	47.7 ± 1.5	-	8.2 ± 0.3	4.9 ± 0.5	36.2 ± 0.6	2.8 ± 0.6
Sunflower	45.4 ± 0.9	0.4 ± 0.0	10.5 ± 0.0	5.8 ± 0.1	37.8 ± 1.0	
Phosphatidic acid						
Soybean	34.0 ± 2.9	-	8.1 ± 0.5	11.9 ± 0.7	44.7 ± 1.4	1.3
Sunflower	34.2 ± 2.5	-	10.6 ± 0.5	22.4 ± 0.9	32.8 ± 3.3	-

[a]Area % ± SEM.

ca. 0.2 ml with a stream of dry nitrogen. Methyl esters were analyzed by gas liquid chromatography (GLC) using conditions previously described (16).

RESULTS AND DISCUSSION

Soybeans contained 7 PL as determined by 2D-TLC and molybdate spray reagent (Table I). Three PL were unidentified and accounted for 12.5% of the total. The data on soybean PL agreed well with Harwood results (17) and with those of Privett et al. (4). Only the 4 major PL identified in soybeans were used for fatty acid analysis. Only 4 PL were detected in sunflowers; their identities and relative distribution compared favorably with the findings of Borodulina et al. (18). Small amounts of phosphatidyl serine (PS) have, however, been reported in crude sunflower lecithin (3). PL from crude sunflower oil contained higher percentages of phosphatidylinositol (PI) and phosphatidylcholine (PC) than crude oil from soybeans, whereas the soybean crude oil contained higher percentages of phosphatidic acid, phosphatidylethanolamine (PE) and unknown PL.

The fatty acid compositions of the individual PL (soybean and sunflower) that remained in the supernatant oils and those PL that centrifuged down with the pellets were the same.

Linoleic acid was the major fatty acid in most of the PL analyzed (32-58%, Table II). A rather high percentage of palmitic acid was found in all soybean and sunflower PL (18-47%). Because palmitic and linoleic were the 2 major fatty acids in these oilseed PL, 1 mol of the most abundant PL molecular species would likely contain 1 mol each of palmitic and linoleic acids. Individual sunflower PL separated by LH-20 Sephadex chromatography were also found to contain high percentages of linoleic and palmitic acids (3). Generally, the percentage of palmitic acid is rather low in crude oils extracted from mature soybeans and sunflower seeds—11% and 5%, respectively (19,20). This is most likely because although PL contain high percentages of palmitic acid, they are generally found in crude oils at low levels (0.2-3.0%) and thus contribute very little to the total crude oil fatty acid profiles.

The fatty acid compositions of PI were quite similar between the 2 oilseeds whereas the fatty acid composition of the other PL differed (Table II). PC, PE and phosphatidic acid from sunflowers contained ca. twice as much oleic acid as did those from soybeans.

The molecular weights of individual PL from soybeans and sunflowers were computed based on their fatty acid compositions (Table III). PC from soybeans will serve to illustrate how these values were computed; data for the computation are presented in Table IV.

Average MW of one fatty acid/mol PC = Σ (molecular weight contribution all fatty acids). Av. MW of one fatty acid/mol PC = 274.5. MW of phosphocholine glycerol minus 2 hydroxyls = 223.2; then, molecular weight of PC = $2 \times 274.5 + 223.2 = 772.2$.

The av. MW of all PL in soybean and sunflower crude oils could then be determined based on the molecular weights of the individual PL and their relative composition of total PL (Table I): mol wt of all PL = Σ (decimal fract. of individual PL $\times$ MW of individual PL). In calculating the

TABLE III

Molecular Weights of Individual Phospholipids Based on Fatty Acid Composition[a]

Phospholipid	Soybean	Sunflower
Phosphatidic acid	681.6	681.2
Phosphatidylinositol	837.0	837.2
Phosphatidylethanolamine	724.4	725.8
Phosphatidylcholine	772.0	774.6

[a]Average of 2 determinations.

average MW of soybean PL, the unknown PL (12.5%) were assumed to be phosphatidylglycerol, since it has been identified as a component of soybean PL at mature stages of development (4,8). Furthermore, it was assumed that its fatty acid composition was similar to that of the other soybean PL fatty acids and its MW was thus computed 772.3. Its contribution to the av. PL MW was 96.5 MW units. The av. MW of soybean PL was computed to be 769.7, and that of sunflower PL, 779.5.

The av. MW of PL could be used to compute a factor for converting the percentage phosphorus (% P) in crude soybean and sunflower oils to the percentage PL. The source of all elemental phosphorous in crude oils is dissolved PL and since there is 1 mol P/mol PL, the relationship between % PL and % P in oils can be expressed by the following:

$$\% \text{ PL} = \% \text{ P} \times \frac{1}{\text{wt fract. P}} \qquad [1]$$

Calculating the wt fraction P in soybean oil from its average PL MW as just found:

$$\text{Wt fract. P} = \frac{\text{mol wt P}}{\text{mol wt PL}} = \frac{30.97}{769.7} = .0402$$

$$\text{And, } \frac{1}{\text{Wt fract. P}} = 24.8$$

For Sunflowers:

$$\text{Wt fract. P} = \frac{\text{mol wt P}}{\text{mol wt PL}} = \frac{30.97}{779.5} = .0397$$

$$\text{And, } \frac{1}{\text{Wt fract. P}} = 25.2$$

These values are fairly close and average 25.0; therefore, one equation, % PL = % P × 25, could be used to calculate the percentage of PL in both soybean and sunflower crude oils.

The % PL in crude, uncentrifuged, soybean oil was computed from the MW of its PL and compared to the value obtained using the equation with actual raw data.

Raw data:

Absorbance (A) of total-lipid phosphorous from sample at 820 nm = 2.351
Weight of total lipid sample applied to TLC plate = 13.5 mg
Based on standard curve with the phosphorus procedure (21), μmol P = A/2.75 and μg P = 11.26 × A
Av. MW of soybean PL = 769.7

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

$$= \frac{\text{A}/2.75 \times 769.7 \text{ μg/μmol}}{13.5 \text{ mg}} \times 100$$

$$= 4.87$$

Using % PL = % P × 25:

$$\% \text{ P} = \frac{\text{mg P}}{\text{mg total lipid}} \times 100$$

$$= \frac{11.26 \times \text{A}}{13.5 \text{ mg}} \times 100$$

$$= 0.196$$

Then: % PL = % P × 25 = 0.196 × 25 = 4.90.

For comparison, if we multiply by 30, then % PL = 5.88, a 20% higher value.

The 2 values (4.87 and 4.90) are in good agreement and indicate total % PL in crude oils can be determined by % P × 25, as well as from av. PL MW.

Since PL fatty acid composition and the composition of PL in crude oils were quite different between soybeans and sunflowers (Tables I and II), it is surprising that the av. PL MW were so similar (Table III) and yielded almost identical conversion factors. It is not known whether 25 or some other number would be obtained as the factor for other oilseeds, if the approach just described is used; however, the results of this study indicate that % P × 25 would give more realistic values of PL content in crude soybean and sunflower oils than the factor 30.

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TABLE IV

Data for Computing the Molecular Weight of Soybean Phosphatidylcholine (PC)

Fatty acid	FA composition of PC (as decimal)	FA MW (dissociated)	MW contribution of each FA[a]
16:0	0.205	255.4	52.4
18:0	0.055	283.5	15.6
18:1	0.105	281.4	29.5
18:2	0.588	279.4	164.3
18:3	0.046	277.4	12.8

[a]Weight contribution of each fatty acid = decimal fraction × MW fatty acid.

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Utilization of Membrane-produced Oilseed Isolates in Soft-serve Frozen Desserts

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ABSTRACT

Consumption of frozen desserts in the United States has increased steadily in recent years. However, rising costs of milk solids-not-fat (MSNF) used in dessert formulas may cause manufacturers to consider less-expensive nondairy protein sources as an alternative with the resulting products labeled "nondairy". Use of soy protein isolates and concentrates as food ingredients is rapidly gaining acceptance in the United States. Glandless cottonseed and peanut protein isolates are expected to become available in the next few years. A membrane isolation process which employs ultrafiltration membranes to produce protein isolates directly from oilseed flour extracts has now been developed. Performance of these isolates in frozen desserts was assessed. Taste panel scores of dessert samples for color, odor, textures, flavor and overall acceptability were statistically analyzed. Results showed MIP soy isolate could replace MSNF (a) at the 80% level without flavor or texture loss, (b) at the 60% level without loss in overall acceptability and (c) at the 40% level without quality loss in color and odor. MIP peanut isolate replaced MSNF (a) at the 80% level without textural change, (b) at the 60% level without loss in overall acceptability or desirable flavor and odor and (c) at the 40% level without color loss. MIP cottonseed SP isolate was used to replace MSNF (a) at the 60% level without flavor loss, (b) at the 40% level with no textural changes and (c) at the 20% level without loss in overall acceptability. Based on these results, MIP oilseed isolates (especially soy and peanut) are a possible alternate source of protein for use in soft-serve frozen desserts to the replacement levels stipulated.

INTRODUCTION

Consumption of frozen desserts in the United States has increased steadily in recent years. This trend is expected to continue. However, the spiraling costs of milk solids-not-fat (MSNF) used in dessert formulas may cause manufacturers to consider less-expensive nondairy protein sources as an alternative. Products in which nondairy proteins are incorporated would have to be labeled "nondairy" since Federal standards permit only dairy proteins in ice cream, ice milk or mellorine.

Futch (1979), in a survey of frozen dessert manufacturers found that 94% of those responding considered price an important factor in maintaining frozen dessert sales (1). An analysis reported by Boehm (1976) showed household comsumption of frozen desserts to be responsive to changes in retail prices, especially in the short term (2).

The alternative to MSNF most frequently employed in frozen desserts to date has been whey solids. A number of investigations have been made to determine the effects of whey solids on ice cream and other dairy products (3-7).

In general, the investigators agreed that whey solids, especially solids from sweet wheys, could satisfactorily replace MSNF to the limits allowed by present Federal standards of identity and perhaps beyond. However, some reduction in quality was reported from loss of firmness and smoothness and from the appearance of a pinkish color when the colorant, annatto, was used in the cheese process generating the whey.

Grey (1979) cites a trend toward the use of nondairy products in the dairy industry (8). Garland et al. (1979) reported research in which defatted, glandless cottonseed flour, glandless cottonseed storage protein isolate, deglanded cottonseed flour, soy flour, soy protein concentrate and soy protein isolate were substituted for various levels of MSNF in a frozen dessert formula (9).

In the work to be described here, oilseed protein isolates produced from defatted soy, glandless cottonseed and peanut flour by industrial ultrafiltration (UF) and reverse osmosis (RO) membranes were evaluated as replacements for MSNF at levels of 20, 40, 60 and 80% in a soft-serve frozen dessert formula. A control in which none of the MSNF were replaced was also included in statistically designed experiments.

The oilseed isolates evaluated were produced by a membrane isolation process (MIP) developed by investigators at Texas A&M University's Food Protein Research and Development Center (FPRDC) (10-13). Using the MIP, protein is extracted from oilseed flours following conventional procedures. However, protein is ultrafiltered directly from the liquid extract instead of being removed by isoelectric precipitation as is conventionally done. MIP isolates possess functional and nutritional properties that differ from those of conventional isolates. Thus, the performance of MIP isolates in soft-serve frozen dessert was assessed using a sensory test panel and analytical and color measurements.

EXPERIMENTAL PROCEDURES

Preparation of Oilseed Isolates

Soy and peanut MIP isolates were prepared following the