Fractionation of Soybean Phospholipids by High-Performance Liquid Chromatography with an Evaporative Light-Scattering Detector

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ABSTRACT: Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) from 23 soybean lines with a wide range of fatty acid compositions were resolved into seven fractions by high-performance liquid chromatography (HPLC). Fraction identities were assigned from fatty acid compositions determined by gas chromatography (GC). A mass detector, i.e., an evaporative light-scattering detector, was used for HPLC quantification. The detector response was a power function of PC and PE concentrations. Various correction methods were applied to the detector response to obtain the best agreement between phospholipid (PL) fatty acid compositions determined by GC and that calculated from the corrected HPLC fraction percentages. The corrected HPLC fraction composition also was compared with that calculated from stereospecific distribution data using a 1-random-2-random hypothesis. Correlation between PL-fatty acid and HPLC-fraction percentages showed that genetic modification of soybean oil composition caused changes in PL species, which alter physical properties and may alter the physiological functions of PL in biomembranes.

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Phospholipids (PL) are a major component of biological membranes. The chemical composition of the various PL classes determines their physical properties, which can affect the biological function of membranes (1,2). Biomembrane composition may be altered by nutritional and environmental factors (1,3–6). Genetic modification of soybean oil composition is accompanied by alteration of PL fatty acid composition (7,8), but little is known about how oil modification affects the molecular species composition (7).

The combinations of fatty acyl groups in PL molecular species can be partially resolved and their proportions determined. The most frequently used procedure depends on hydrolysis with phospholipase C and analysis of the resulting 1,2-diacyl-*sn*-glycerols (DG) by high-performance liquid chromatography (HPLC) (9–13) or gas chromatography (GC) (3,14–17) after making suitable derivatives. These multistep methods are time consuming and are subject to analytical bias. HPLC of unmodified PL avoids these problems, but these methods suffer from problems in quantification. Response to HPLC fractions using ultraviolet absorption is dependent on the number and configuration of the double bonds in the PL species. Refractive index response is sensitive to changes in temperature and solvent gradient. The evaporative light-scattering detector (ELSD) has many advantages, but its response to the amount of PL in the eluate is nonlinear (18–21). Methods based on mass spectroscopy also give biased results (22–25).

We wished to determine whether the distribution of acyl groups in the various PL molecular species could be predicted from the stereospecific distribution of the acyl groups, which has been reported (8). In soybean triglycerides, the percentages of the molecular species agree fairly well with that calculated by a 1-random-2-random-3-random distribution (26), but such a comparison has not been carried out for PL. In this paper, we report attempts to fit ELSD data with correction factors to make them agree with analyses of the acyl composition of the total phosphatides obtained by GC. The corrected ELSD data are compared with a 1-random-2-random fit of the stereospecific data.

MATERIALS AND METHODS

Materials. Twenty-three soybean lines with a wide range of fatty acid compositions were obtained from the Agronomy Department, Iowa State University (Ames, IA) and Pioneer Hi-Bred International Inc. (Des Moines, IA). These lines, classified into six categories based on their fatty acid compositions are shown in Table 1. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of these soybeans were isolated as described by Wang *et al*. (8) and stored at –20°C until analyzed. PC and PE standards from soybean were purchased from Sigma Chemical Co. (St. Louis, MO).

Chromatographic conditions. An HPLC system, including a Shimadzu (Columbia, MD) LC-600 solvent delivery sys-

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TABLE 1
Fatty Acid Composition of Soybean Selections (mol%)

Category	I.D.[a]	16:0	18:0	18:1	18:2	18:3
Typical	1	12.9	4.3	22.3	52.5	8.0
	2	10.1	4.3	24.3	52.9	8.4
	3	11.4	4.1	30.8	46.5	7.1
	4	11.6	4.2	28.8	48.3	7.1
High 16:0	11	33.3	7.3	11.7	35.3	12.4
	12	28.8	4.5	14.6	43.4	8.8
	A17	16.0	4.3	24.8	51.2	3.6
	A19	28.8	4.0	14.0	42.9	10.4
	A21	20.8	4.0	17.4	48.9	8.9
	A24	16.7	4.1	19.4	48.1	11.7
	spf358	28.4	3.9	11.3	40.1	16.3
High 18:0	5	12.0	15.1	16.4	47.6	9.0
	6	10.6	20.3	16.8	44.0	8.3
	7	10.7	23.9	16.5	41.1	7.8
	8	11.8	21.8	17.2	41.0	8.3
	stea	8.2	23.6	18.1	42.3	7.8
High 16:0 and 18:0	453	23.6	17.3	9.4	38.3	11.5
	561	25.6	20.1	7.8	36.7	9.8
Low 16:0	10	3.1	2.7	15.4	64.8	13.9
	spf457	3.4	2.8	21.0	68.3	4.5
	spe153	3.6	2.4	17.5	61.4	15.1
Low 18:0	A5	11.0	4.2	35.3	44.5	4.9
	spb201	10.2	4.6	26.9	55.6	2.7

[a]I.D., identification code.

tem; a Rheodyne (Cotati, CA) 7125 injector with a 20-µL sample loop; a Phenomenex (Torrance, CA) Luna C18(2), 5-µm particle size, 250 × 4.60-mm column, a Shimadzu CR501 Chromatopac integrator, and a Varex ELSD IIA (Burtonsville, MD) were used for PL separation and quantification. The mobile phase was methanol/chloroform/acetonitrile/water (87.5:5:3.75:3.75, by vol), which was a modification of the solvent of Demandre *et al.* (27). Elution was isocratic with a mobile phase flow rate of 1 mL/min. The nebulizing nitrogen gas flow rate and the temperature of the drift tube of the ELSD were 42 mm (equivalent to 2 L/min) and 125°C, respectively. Approximately 250 µg of PC and 150 µg of PE from all samples were injected in the system, and all analyses were duplicated.

HPLC peak identification. Individual peaks were collected from the column outlet at the appropriate time while the column was disconnected from the detector. The solvent was evaporated, the PL converted to fatty acid methyl ester (FAME) with methanolic sodium methoxide, and components of the fractions were determined by their fatty acid compositions and elution order.

Optimization of correction factors. Pure soybean PC and PE (Sigma Chemical Co.) were diluted with the HPLC mobile phase to various concentrations. The concentration giving chromatographic peaks slightly larger than those from the sample analyses was assigned a value of 100, and the other concentrations were assigned proportionate values.

The response could be corrected by

$$C = A^{1/x} \quad [1]$$

where C is the corrected and A is the observed detector response and x is an arbitrary value. Several ways of evaluating x were compared using a mathematical application, unconditional minimization (UNCMIN) (28), which simultaneously optimizes several parameters for a target function among all 23 sets of data using a nonlinear least squares principle. The two target functions used were the determined GC fatty acid composition and the calculated molecular fraction percentages.

Calculation of molecular species composition from stereospecific distribution data. Previously reported (8) PL stereospecific distribution data for the 23 lines were used to calculate molecular species composition assuming the 1-random-2-random distribution. Theoretically, there are 25 molecular species with five possible fatty acids and two possible positions for esterification. An example of such a calculation is:

$$\%\ \text{molecular species A-B} = (\%\ \text{A at } sn\text{-}1 \times \%\ \text{B at } sn\text{-}2)/100 \quad [2]$$

In this situation, A-B represents the molecular species with A at the *sn*-1 position and B at the *sn*-2 position. A-B is different from B-A, in which B is at the *sn*-1 position and A is at the *sn*-2 position. When A/B is used in this paper, it represents the sum of A-B and B-A.

RESULTS AND DISCUSSION

HPLC separation and peak identification. Figure 1 shows an HPLC separation of a PC sample. A total of seven peaks were separated and identified as indicated on the chromatogram. Two peaks were mixtures of two molecular fractions with the same equivalent chain lengths (ECL; ECL were calculated by subtracting a value of 2 from the chain length for each double bond in calculating the chromatographic migration of a molecular fraction.): 16:0/18:3 + 18:2/18:2 and 16:0/18:2 + 18:1/18:2. For a PL with five possible acyl groups, there are 25 possible molecular species. But the *sn*-positional isomers, i.e., molecular species such as A-B and B-A, cannot be separated by the current HPLC separation conditions. By counting molecular species such as A-B and B-A as one molecular fraction, there are 15 possible fractions. (The term "fraction" will be used for a mixture of molecular species A-B and B-A that are *sn*-1-*sn*-2 positional isomers, and for a mixture of A-B, B-A, C-D, and D-C with equivalent carbon numbers. Such analyses are frequently referred to as "molecular species" analyses in the literature.). The seven observed peaks included 16 of the 25 possible molecular species and 9 of the 15 possible molecular fractions. The other six molecular fractions were expected to be present in very low concentrations (based on the stereospecific distribution calculation) and were not detected. In some instances, they may have coeluted and have been masked by peaks of greater concentration and the same ECL. The seven observed fractions accounted for about 95% of the expected molecular species mass calculated from the stereospecific distribution for both PC and PE.

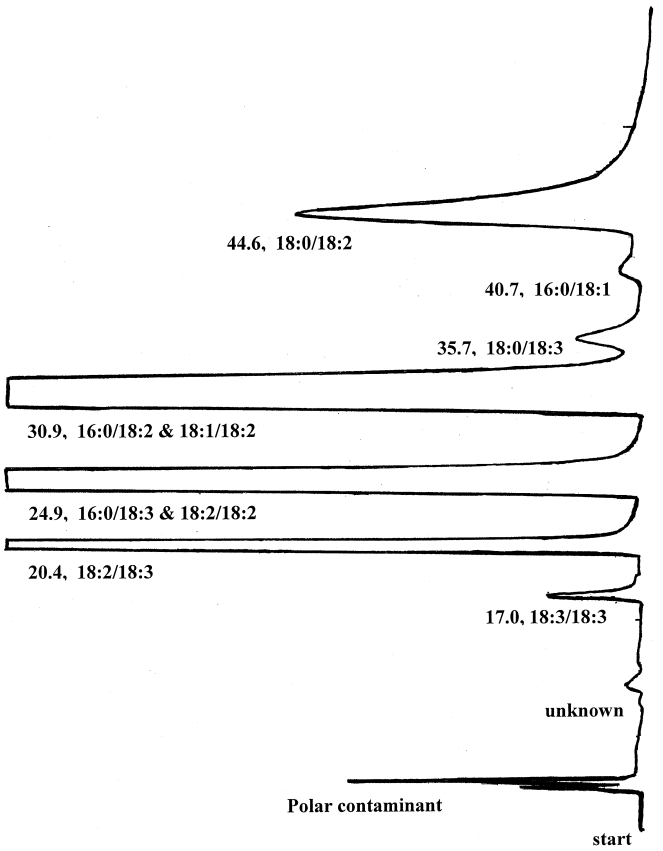

FIG. 1. High-performance liquid chromatographic separation of a typical phosphatidylcholine sample with peak retention times and identities. A/B represents the sum of A-B and B-A, where A-B represents the species with A at *sn*-1 and B at *sn*-2 and B-A represents the species with B at *sn*-1 and A at *sn*-2.

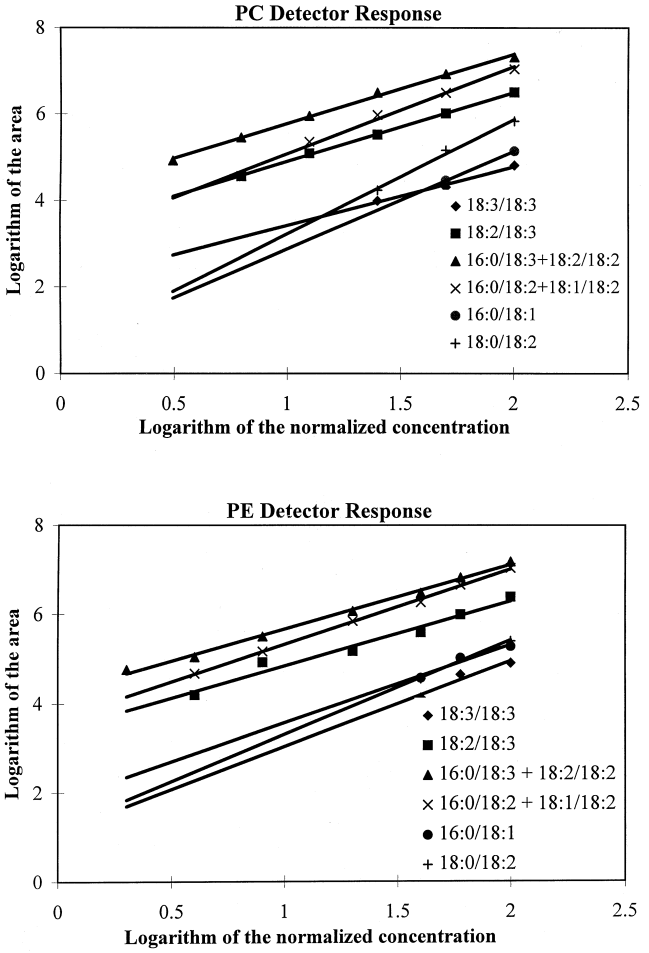

FIG. 2. Log-log plots of the evaporative light-scattering detector response for phosphatidylcholine (PC) and phosphatidylethanolamine (PE) standards. See legend of Figure 1 for meaning of PC, A/B, e.g., 18:2/18:3. The highest concentration used was assigned an arbitrary value of 100.

Nonlinear response of the light-scattering detector. The logarithm of the ELSD response vs. the logarithm of the normalized concentration of soybean PC and PE standard mixtures was linear (Fig. 2). The slopes and regression coefficients (R^2) of these linear plots are shown in Table 2. Because of its low concentration, a slope for the 18:0/18:3 fraction could not be obtained. An average slope was used for this fraction in subsequent calculations. Pure PC molecular species (18:2/18:2, 16:0/18:2, and 18:0/18:2) gave similar ELSD responses. Others have noted such responses, and slopes of 1.35 (18) and 1.81 (19) for general application, and 1.69 (29) and 1.634 (29) for specific compounds have been suggested. This nonlinear relationship has been attributed to variations in droplet size with solute concentration after evaporation of the solvent in the ELSD drift tube. The scattering of light by these droplets is a function of their diameter (18–20). The ELSD response also can decrease when the droplet size approaches the wavelength of light (18), but our samples were dilute enough to avoid this problem.

Some have attributed the differences in x values to varia-

TABLE 2
Slopes and Regression Coefficients of the Log-Log Plots of the Detector Response for Various Concentrations of the PC and PE Standards[a]

		18:3/18:3	18:2/18:3	16:0/18:3, 18:2/18:2	16:0/18:2, 18:1/18:2	16:0/18:1	18:0/18:2
PC	Slope	1.361	1.599	1.606	2.021	2.267	2.649
	R^2	0.984	0.999	0.997	0.992	1.000	0.992
PE	Slope	1.923	1.445	1.433	1.675	1.748	2.110
	R^2	0.935	0.970	0.996	0.998	0.955	0.988

[a]PC, phosphatidylcholine; PE, phosphatidylethanolamine.

tions in ELSD detector designs (18–20), but this obviously cannot account for the variation reported in Table 2 for the different molecular fractions. Possibly, the detector response is dependent on the refractive index and absorption coefficient of the PC species (18,19), but Stolyhwo *et al*. (19) reported that the *x* values for methyl esters that varied in chain length and saturation were almost identical, and even the detector response for a group of structurally distinctive compounds was very similar. The cause for various *x* values of the PL molecular fractions cannot readily be explained.

Data correction for the nonlinear response. The ELSD responses were corrected using Equation 1 and various values of *x*. These were $x = 1$; x = the average slope from plots of log A vs. log C for all HPLC peaks; x = the individual slopes giving the best fit for log-log plots of each HPLC fraction; x = the values for individual HPLC fractions that gave the best agreement between the fatty acid compositions calculated from the corrected HPLC fraction percentages and the fatty acid compositions for the unfractionated PL determined by GC; and x = the values of the individual HPLC peaks that gave the best agreement between the corrected HPLC fraction percentages and those that were calculated from a 1-random-2-random stereospecific distribution. Three PC samples, i.e., sample 1 with typical oil composition, sample 11 with an elevated palmitate percentage, and sample 7 with an elevated stearate percentage, were used to evaluate the effectiveness of the five correction methods. When a fatty acid was present in a peak representing two molecular fractions of similar ECL, the percentage of each molecular fraction was assumed to be equal to that calculated from the 1-random-2-random molecular species composition.

Table 3 compares the percentage of each HPLC fraction obtained using the five correction methods with the amounts expected from the 1-random-2-random calculation for PC using stereospecific data (8). Table 4 compares the fatty acid composition calculated from the HPLC fractions using the five correction methods with the composition obtained by GC on the unfractionated PC. Table 5 presents the values of the power factor *x* in Equation 1 for the various methods of correction for both PC and PE. The uncorrected ELSD data (assumption 1) and the data corrected with individual slopes from the standard curves (assumption 3) differed greatly from both the molecular fraction composition and the fatty acid composition. When the ELSD data were corrected with the average slope (assumption 2), the calculated fatty acid compositions fit the GC results fairly well, but fits for 18:1 and 18:3 were generally poorer than for other acyl groups. The poor fit for 18:1 may be because it accounted for a large proportion of the HPLC fractions that were small and undetected. When the ELSD data were corrected to optimize fit with the GC composition (assumption 4), they gave a good fit with fatty acid composition, but the fit with the 1-random-2-random data was not

TABLE 3
Comparison of HPLC Peaks (mol%) with Those Calculated by the 1-Random-2-random Hypothesis Using Various Correction Methods for PC[a]

Sample	Correction method[b]		18:3/18:3	18:2/18:3	16:0/18:3, 18:2/18:2	16:0/18:2, 18:1/18:2	18:0/18:3	16:0/18:1	18:0/18:2
#1		Calc.[c]	0.6	9.5	40.0	38.7	0.7	4.1	6.3
	1		0.2	7.1	54.2	35.0	—	1.1	2.4
	2		2.0	13.7	39.9	31.7	—	5.3	7.5
	3		5.9	19.4	66.7	7.0	—	0.7	0.4
	4		2.6	7.0	33.7	46.5	—	1.5	8.7
	5		0.2	7.1	39.7	41.7	—	3.9	7.5
#11		Calc.	0.8	9.3	32.9	46.7	0.9	4.8	4.8
	1		0.4	8.0	39.6	48.7	0.1	1.7	1.5
	2		2.8	14.2	32.9	36.6	1.2	6.4	5.9
	3		11.4	21.9	57.4	7.9	0.4	0.8	0.3
	4		3.9	6.8	26.7	54.1	0.2	1.6	6.7
	5		0.2	7.1	32.5	49.4	0.3	4.7	5.9
#7		Calc.	0.9	12.3	42.2	24.6	2.6	1.6	15.8
	1		0.3	9.4	57.4	21.1	1.1	0.3	10.3
	2		2.4	14.6	37.9	22.5	4.8	2.5	15.3
	3		7.6	21.1	63.3	5.2	1.7	0.4	0.7
	4		3.4	7.9	33.7	33.3	0.7	0.8	20.1
	5		0.2	8.2	40.6	30.9	0.9	2.0	17.1

[a]Samples are (#1) typical oil composition; (#11) elevated 16:0; (#7) elevated 18:0. HPLC, high-performance liquid chromatography; GC, gas chromatography. See Table 2 for other abbreviation.
[b]Correction methods used to correct original data: (1) No correction factor applied; (2) an average slope from the standard curve; (3) individual slopes from the standard curve; (4) optimized factors (*x* in Eq. 1) for best GC composition fit; (5) optimized factors (*x* in Eq. 1) for best HPLC fraction percentage fit.
[c]Calculated from a 1-random-2-random stereospecific distribution.

TABLE 4
Comparison of Fatty Acid Composition (mol%) Determined by GC with Those Calculated from the HPLC-Determined Molecular Fraction Percentage Using Various Correction Methods for PC[a]

Sample	Corr. method[a]		16:0	18:0	18:1	18:2	18:3
#1		GC determination	15.1	4.5	11.5	61.3	7.6
		Calculate by stereo-distribution	15.9	3.5	9.1	64.2	7.3
	1		13.8	1.2	6.9	72.3	5.8
	2		14.2	3.7	8.4	63.3	10.4
	3		5.1	0.2	1.6	75.0	18.1
	4		16.8	4.4	9.2	62.2	7.4
	5		16.7	3.7	9.5	64.8	5.2
#11		GC determination	25.4	3.4	8.1	53.4	9.8
		Calcuate by stereo-distribution	24.5	2.8	7.0	56.4	9.3
	1		24.5	0.8	5.7	60.5	8.5
	2		21.3	3.6	6.8	54.3	14.0
	3		9.5	0.4	1.2	60.5	28.5
	4		25.3	3.4	6.2	54.9	10.2
	5		25.6	3.1	7.3	56.9	7.2
#7		GC determination	12.3	11.6	8.5	59.3	8.4
		Calcuate by stereo-distribution	10.0	9.2	5.2	65.9	9.7
	1		8.8	5.7	3.9	74.3	7.3
	2		9.7	10.1	5.2	61.7	13.3
	3		3.8	1.2	1.1	72.9	21.0
	4		12.2	10.4	6.3	62.3	8.7
	5		12.2	9.0	6.5	66.2	6.0

[a]See Table 3.

as good as that with assumption 2. When the ELSD data were corrected to optimize fit for the 1-random-2-random data (assumption 5), the calculated fatty acid composition fit the fatty acid composition determined by GC fairly well. The two optimization methods (4 and 5) gave sets of correction factors that were generally similar except for the 18:3/18:3 fraction, and the averages of these values were close to the average value obtained from the best linear fits of the ELSD data used in assumption 2. Application of the five correction methods to PE gave results similar to those of PC. These results suggest that the combination of the fatty acyl groups in the molecular species of PC and PE can agree fairly well with the 1-random-2-random distribution theory. The unusually large correction factors for the 18:3/18:3 fraction of both PC and PE (Table 5), obtained when the optimization best fit with the 1-random-2-random calculation, suggest that there is something unusual about this fraction and probably its proportions are underestimated by the 1-random-2-random distribution.

Comparison of determined and calculated molecular fraction and fatty acid percentages of PL. Assumption 4 was used to calculate the molecular fraction percentages from the ELSD data. Tables 6 and 7 compare these results with the 1-

TABLE 5
Correction Factors (Eq. 1) for Various Ways of Data Correction for PC and PE

	Correction method[a]	18:3/18:3	18:2/18:3	16:0/18:3, 18:2/18:2	16:0/18:2, 18:1/18:2	18:0/18:3	16:0/18:1	18:0/18:2
PC	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	1.917	1.917	1.917	1.917	1.917	1.917	1.917
	3	1.361	1.599	1.606	2.021	1.917	2.267	2.649
	4	1.688	1.975	1.852	1.735	2.533	2.191	1.754
	5	3.202	1.999	1.838	1.777	2.473	1.897	1.818
PE	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	1.722	1.722	1.722	1.722	1.722	1.722	1.722
	3	1.923	1.445	1.433	1.675	1.722	1.748	2.110
	4	1.928	1.737	1.731	1.640	2.055	1.659	1.655
	5	10.526	2.176	1.930	1.839	2.441	2.288	1.927

[a]See Table 3 for explanation and Table 2 for abbreviations.

TABLE 6
Comparison of Fatty Acid Composition (mol%) and the Mole Percentages of the HPLC Peaks for PC[a]

Category	I.D.[b]		16:0	18:0	18:1	18:2	18:3		18:3/18:3	18:2/18:3	16:0/18:3 + 18:2/18:2	16:0/18:2 + 18:1/18:2	18:0/18:3	16:0/18:1	18:0/18:2
Typical	1	D	15.1	4.5	11.5	61.3	7.6	C	0.6	9.5	40.0	38.7	0.7	4.1	6.3
		C	16.8	4.4	9.2	62.2	7.4	D	2.6	7.0	33.7	46.5	—	1.5	8.7
	2	D	13.8	4.8	13.8	59.8	7.8	C	0.6	9.2	38.3	39.0	0.9	4.7	7.3
		C	15.1	4.4	11.2	60.7	8.6	D	3.7	7.4	31.7	46.6	0.1	1.8	8.6
	3	D	14.4	3.7	8.8	65.8	7.5	C	0.5	9.8	44.1	35.6	0.7	2.9	6.3
		C	17.0	3.9	7.6	63.4	8.0	D	3.1	7.6	35.7	44.8	0.2	1.2	7.5
	4	D	15.0	3.7	8.3	64.2	8.9	C	0.8	11.5	44.3	34.3	0.7	2.5	5.9
		C	16.2	2.8	7.4	64.7	8.8	D	3.3	8.4	38.7	43.0	—	0.8	5.6
	Avg. diff.		–1.7	0.3	1.7	0.0	–0.3		–2.5	2.4	6.7	–8.3	0.7	2.2	–1.2
	SD		0.7	0.4	0.9	1.6	0.5		0.5	0.5	1.2	0.8	0.1	0.6	1.1
High 16:0	11	D	25.4	3.4	8.1	53.4	9.8	C	0.8	9.3	32.9	46.7	0.9	4.8	4.8
		C	25.3	3.4	6.2	54.9	10.2	D	3.9	6.8	26.7	54.1	0.2	1.6	6.7
	12	D	21.4	2.8	6.9	61.0	7.9	C	0.7	11.2	44.6	37.3	0.4	2.3	3.5
		C	21.3	3.0	7.2	60.2	8.2	D	3.5	6.9	30.3	52.1	—	1.1	6.1
	A17	D	17.5	2.7	6.2	69.1	4.5	C	0.1	5.3	47.7	39.2	0.3	2.4	5.0
		C	20.1	2.9	6.3	66.9	3.7	D	0.5	4.8	38.1	50.0	—	0.7	5.8
	A19	D	21.0	2.9	6.4	60.0	9.9	C	0.9	10.3	37.5	42.3	0.9	3.1	5.0
		C	23.3	2.5	5.5	58.7	10.0	D	3.8	7.8	31.5	51.1	—	0.9	5.0
	A21	D	20.2	3.3	9.2	60.2	7.1	C	0.6	9.5	39.7	41.0	0.6	3.7	4.9
		C	20.7	2.5	7.5	60.9	8.4	D	3.3	7.2	32.4	51.0	—	1.1	5.0
	A24	D	16.5	2.3	5.5	65.3	10.6	C	0.7	10.2	40.9	39.2	0.7	2.9	5.4
		C	20.2	3.3	6.4	61.4	8.7	D	3.2	7.7	33.7	47.9	—	1.0	6.6
	spf358	D	22.4	3.2	7.1	56.4	11.1	C	1.3	12.2	36.6	41.9	0.7	3.2	4.1
		C	24.0	2.3	5.6	57.0	11.1	D	4.5	7.7	30.4	51.7	—	1.1	4.6
	Avg. diff.		–1.5	0.1	0.7	0.7	0.1		–2.5	2.7	8.1	–10.0	0.6	2.1	–1.0
	SD		1.5	0.7	1.1	1.9	1.0		1.0	1.4	3.0	2.4	0.2	0.6	0.9
High 18:0	5	D	11.7	8.6	6.6	63.2	10.1	C	1.0	12.9	43.5	28.0	1.7	1.9	11.1
		C	13.9	8.3	5.9	62.0	10.0	D	4.3	8.6	33.9	36.0	0.5	0.7	16.0
	6	D	11.5	11.2	11.5	57.8	8.2	C	0.9	10.8	38.3	30.7	2.0	3.2	14.1
		C	11.9	9.9	8.8	61.3	8.1	D	3.1	7.4	31.4	37.0	0.6	1.2	19.2
	7	D	12.3	11.6	8.5	59.3	8.4	C	0.9	12.3	42.2	24.6	2.6	1.6	15.8
		C	12.2	10.4	6.3	62.3	8.7	D	3.4	7.9	33.7	33.3	0.7	0.8	20.1
	8	D	11.2	11.9	8.6	58.6	9.9	C	0.8	9.4	33.0	31.7	2.9	3.2	19.1
		C	14.1	10.5	5.9	59.9	9.6	D	3.8	7.6	31.9	34.6	0.8	1.1	20.2
	stea	D	9.6	13.9	9.6	59.8	7.3	C	0.6	8.4	36.1	26.9	2.8	2.5	22.7
		C	11.0	13.0	7.7	60.9	7.3	D	2.9	6.6	29.7	33.8	0.7	1.1	25.4
	Avg. diff.		–1.4	1.0	2.0	–1.5	0.0		–2.7	3.1	6.5	–6.6	1.8	1.5	–3.6
	SD		1.2	0.4	0.8	1.9	0.2		0.5	1.3	3.3	2.2	0.5	0.6	1.7
High 16:0 & 18:0	453	D	16.5	11.7	7.3	53.3	11.5	C	1.3	11.9	32.7	33.0	3.2	3.1	14.9
		C	18.3	9.8	5.2	55.5	11.2	D	5.0	7.6	26.4	40.5	0.7	1.0	18.9
	561	D	16.0	10.8	3.4	58.3	11.6	C	1.4	12.6	35.3	29.7	3.7	1.2	16.2
		C	19.1	9.7	2.9	56.2	12.1	D	5.4	8.2	28.2	38.3	0.6	0.6	18.7
	Avg. diff.		–2.5	1.5	1.3	–0.1	–0.1		–3.9	4.4	6.7	–8.1	2.8	1.3	–3.2
	SD		1.0	0.6	1.1	3.0	0.5		0.2	0.0	0.7	0.8	0.4	1.1	1.1
Low 16:0	10	D	8.6	6.8	16.2	60.3	8.1	C	0.6	9.3	39.0	35.1	1.4	3.6	11.0
		C	9.3	6.5	12.9	63.6	7.7	D	3.0	7.6	34.7	40.9	0.4	0.9	12.5
	spf457	D	7.0	6.0	10.1	73.0	3.9	C	0.2	6.1	51.0	29.3	0.5	2.0	10.8
		C	8.5	5.6	10.5	70.7	4.7	D	1.8	5.2	44.4	37.5	—	—	11.1
	spe153	D	9.1	5.6	19.9	56.7	8.7	C	0.7	9.7	35.3	39.0	1.4	5.1	8.8
		C	9.7	5.5	14.8	61.8	8.2	D	3.3	7.6	32.3	44.5	0.3	1.3	10.8
	Avg. diff.		–0.9	0.3	2.7	–2.0	0.0		–2.2	1.5	4.7	–6.5	0.9	2.9	–1.3
	SD		0.5	0.2	2.8	3.9	0.7		0.6	0.6	1.8	1.4	0.3	0.9	0.9
Low 18:3	A5	D	13.3	3.8	12.0	66.6	4.1	C	0.2	5.5	44.8	38.6	0.4	4.2	6.3
		C	15.0	4.3	10.7	65.8	4.2	D	1.2	4.9	36.2	47.9	—	1.2	8.6
	spb201	D	13.3	4.2	15.3	64.3	3.0	C	0.1	3.4	40.6	42.7	0.3	5.4	7.4
		C	14.1	4.4	12.5	64.4	4.7	D	2.5	3.5	34.2	49.6	—	1.4	8.8
	Avg. diff.		–1.3	–0.3	2.1	0.4	–0.9		–1.7	0.2	7.5	–8.1	0.4	3.5	–1.8
	SD		0.6	0.2	1.1	0.6	1.1		1.0	0.5	1.5	1.6	0.1	0.8	0.7

[a]Comparison of fatty acid composition (mol%) determined (D) by GC and calculated (C) from HPLC molecular fraction percentages, and a comparison of mole percentages of HPLC peaks (D) and those predicted (C) by 1-random-2-random hypothesis. Factors optimized for the best GC fatty acid composition (Assumption 4) were used to correct the areas. See Table 3 for abbreviations. PC, phosphatidylcholine.
[b]See Table 1.

TABLE 7
Comparison of Fatty Acid Composition (mol%) for Phosphatidylethanolamine[a]

Category	I.D.[b]		16:0	18:0	18:1	18:2	18:3		18:3/18:3	18:2/18:3	16:0/18:3 + 18:2/18:2	16:0/18:2 + 18:1/18:2	18:0/18:3	16:0/18:1	18:0/18:2
Typical	1	D	22.1	3.4	9.4	56.8	8.6	C	0.5	7.6	34.6	46.6	0.6	5.3	4.8
		C	24.5	2.6	8.5	58.1	6.4	D	0.4	8.4	30.2	49.3	—	6.6	5.1
	2	D	20.2	3.6	11.4	57.2	7.5	C	0.5	8.2	35.1	44.1	0.7	5.8	5.6
		C	22.4	2.9	10.7	56.7	7.3	D	0.9	9.7	28.8	46.7	—	8.1	5.8
	3	D	20.6	2.9	9.6	60.1	6.8	C	0.6	9.6	40.5	41.6	0.4	3.6	3.6
		C	22.8	2.7	7.2	60.5	6.8	D	0.8	9.2	32.7	46.9	—	5.1	5.3
	4	D	20.1	2.8	7.9	61.5	7.8	C	0.7	10.2	38.9	42.5	0.5	3.3	3.9
		C	23.7	2.8	6.5	60.0	7.0	D	0.9	8.9	32.1	48.1	—	4.4	5.6
	Avg. diff.		–2.6	0.4	1.4	0.1	0.8		–0.1	–0.1	6.3	–4.0	0.6	–1.6	–1.0
	SD		0.7	0.4	0.8	1.2	1.0		0.2	1.2	1.4	1.6	0.1	0.6	0.8
High 16:0	11	D	26.4	1.8	7.2	55.2	9.5	C	0.6	8.5	32.7	46.7	1.1	4.5	5.9
		C	27.8	2.2	7.1	54.6	8.3	D	0.8	9.2	27.1	52.5	0.4	6.0	3.9
	12	D	26.6	1.6	5.0	59.6	7.3	C	0.5	8.0	37.1	48.4	0.3	2.9	2.9
		C	28.0	1.8	5.3	57.7	7.2	D	0.7	8.5	29.1	53.7	—	4.2	3.7
	A17	D	23.0	1.5	4.3	66.9	4.4	C	0.1	5.0	44.5	45.1	0.2	2.2	2.9
		C	24.5	2.0	4.9	64.7	3.9	D	0.0	5.7	36.0	51.5	—	2.7	4.0
	A19	D	26.8	2.1	5.3	56.9	9.0	C	0.8	9.1	35.2	48.1	0.5	3.3	3.0
		C	28.1	1.6	4.6	57.0	8.7	D	0.9	10.0	29.4	53.2	—	3.3	3.2
	A21	D	23.2	1.8	6.4	60.9	7.8	C	0.5	8.2	35.1	48.3	0.4	3.8	3.6
		C	25.9	1.7	6.0	57.9	8.5	D	1.2	10.0	30.4	50.8	0.1	4.3	3.3
	A24	D	22.5	1.4	4.8	62.8	8.6	C	0.5	8.5	37.1	46.2	0.5	3.3	3.9
		C	25.8	2.3	5.4	58.8	7.7	D	0.8	9.5	30.8	50.5	—	3.8	4.7
	spf358	D	26.9	2.0	5.7	55.2	10.1	C	1.0	10.5	34.9	47.0	0.4	3.5	2.6
		C	28.5	1.5	5.4	55.6	9.0	D	1.0	9.8	28.8	53.3	—	4.0	3.1
	Avg. diff.		–1.9	–0.1	0.0	1.6	0.5		–0.2	–0.7	6.4	–5.1	0.4	–0.7	–0.1
	SD		0.8	0.5	0.5	1.6	0.6		0.2	0.7	1.3	1.4	0.2	0.5	1.1
High 18:0	5	D	18.2	5.9	5.3	61.2	9.5	C	0.8	10.0	37.3	36.3	1.6	2.6	11.5
		C	19.6	5.7	5.0	60.6	9.1	D	0.9	11.8	33.6	39.1	0.9	3.1	10.6
	6	D	15.2	7.6	9.8	58.9	8.5	C	0.7	10.8	40.6	34.5	1.1	3.7	8.6
		C	16.1	6.5	8.3	61.3	7.7	D	0.8	10.5	33.4	38.1	0.8	4.2	12.2
	7	D	17.5	8.3	6.4	60.2	7.6	C	0.7	10.1	40.6	31.6	1.8	2.3	12.9
		C	16.6	7.6	6.0	61.6	8.1	D	0.9	10.7	34.1	35.5	1.0	3.6	14.2
	8	D	16.0	8.3	6.8	57.3	11.7	C	0.9	10.8	36.9	31.7	2.3	2.9	14.4
		C	17.0	7.6	6.5	60.1	8.8	D	1.1	11.1	33.4	35.0	1.1	4.2	14.1
	stea	D	14.3	9.7	8.7	60.3	7.0	C	0.5	8.4	37.0	33.1	1.9	3.3	15.8
		C	15.8	9.1	7.6	60.2	7.2	D	1.1	11.0	40.8	27.8	2.5	3.2	13.7
	Avg. diff.		–0.8	0.7	0.7	–1.2	0.7		–0.2	–1.0	3.4	–1.7	0.5	–0.7	–0.3
	SD		1.0	0.3	0.5	1.5	1.3		0.2	1.2	4.3	3.9	0.7	0.6	2.2
High 16:0 and 18:0	453	D	21.9	6.8	5.5	55.4	10.5	C	1.3	12.1	36.0	38.1	1.5	3.1	7.8
		C	23.0	4.8	4.8	56.5	10.9	D	0.8	9.6	31.4	35.7	1.0	4.5	17.2
	561	D	21.3	6.0	2.7	59.2	10.8	C	1.2	12.6	39.3	35.8	1.6	1.5	8.1
		C	23.1	5.4	2.8	58.4	10.3	D	1.2	12.2	31.3	42.2	0.9	2.3	9.9
	Avg. diff.		–1.4	1.3	0.3	–0.1	0.1		0.3	1.5	6.3	–2.0	0.6	–1.1	–5.6
	SD		0.5	1.0	0.6	1.4	0.6		0.4	1.5	2.3	6.2	0.1	0.3	5.3
Low 16:0	10	D	11.9	7.3	14.8	58.6	7.5	C	0.5	9.0	38.2	34.7	1.4	3.9	12.3
		C	12.4	5.6	12.2	61.5	8.4	D	1.2	11.3	34.7	35.9	1.0	5.6	10.2
	spf457	D	13.0	5.6	9.2	68.9	3.5	C	0.1	4.8	45.6	36.3	0.5	3.1	9.5
		C	13.7	4.6	9.0	68.6	4.1	D	0.7	5.8	42.6	38.9	—	2.7	9.2
	spe153	D	12.9	4.6	18.3	56.3	8.0	C	0.7	9.3	35.4	40.7	0.9	6.3	6.7
		C	14.5	3.8	13.8	60.4	7.5	D	1.0	10.4	33.3	41.4	—	6.3	7.6
	Avg. diff.		–0.9	1.2	2.4	–2.2	–0.3		–0.5	–1.5	2.9	–1.5	0.6	–0.4	0.5
	SD		0.6	0.5	2.2	2.3	0.7		0.2	0.8	0.7	1.0	0.2	1.1	1.5
Low 18:3	A5	D	19.7	2.6	10.9	63.2	3.6	C	0.1	4.3	40.9	44.8	0.2	5.5	4.1
		C	20.2	2.9	10.0	63.5	3.4	D	0.0	5.3	35.5	48.0	—	5.4	5.7
	spb201	D	19.4	2.9	12.6	62.4	2.6	C	0.1	3.3	37.4	47.2	0.2	6.7	5.1
		C	21.3	2.7	12.2	60.5	3.3	D	0.6	4.3	32.1	49.4	—	8.2	5.4
	Avg. diff.		–1.2	0.0	0.6	0.8	–0.3		–0.2	–1.0	5.3	–2.6	0.2	–0.7	–1.0
	SD		1.0	0.3	0.3	1.5	0.6		0.4	0.0	0.1	0.7	0.0	1.1	0.9

[a]See Footnote *a* of Table 6.
[b]See Table 1.

TABLE 8
Correlations Among Phospholipid Fatty Acid Percentages

		16:0	18:0	18:1	18:2	18:3
16:0	PC	1.000	–0.514[a]	–0.526[a]	–0.339	0.330
	PE	1.000	–0.736[a]	–0.621[a]	–0.251	0.219
18:0	PC	—	1.000	–0.004	–0.363	0.270
	PE	—	1.000	0.149	–0.105	0.240
18:1	PC	—	—	1.000	–0.039	–0.429[a]
	PE	—	—	1.000	–0.097	–0.376
18:2	PC	—	—	—	1.000	–0.690[a]
	PE	—	—	—	1.000	–0.736[a]
18:3	PC	—	—	—	—	1.000
	PE	—	—	—	—	1.000

[a]Values represent significant correlation coefficients at 5% level. For abbreivations see Table 2.

random-2-random molecular fraction percentages and the observed and calculated fatty acid percentages for PC and PE from 23 soybean lines. For both PC and PE, the determined percentages for 16:0/18:2 + 18:1/18:2 and 16:0/18:3 + 18:2/18:2 were considerably greater and less, respectively, than percentages calculated from the 1-random-2-random theory. For PC, the determined percentages of 18:3/18:3 also were considerably greater than the theoretical calculations. These observations apply to all categories of the soybean samples and suggest that aside from the biased placement of acyl groups on the *sn*-1 and *sn*-2 positions, the combination of two positions also may be biased. These results may be partially explained by the noncoincidence of the peak biosynthesis of different fatty acids during maturation of the beans. Thus, at different seed developmental stages, the relative availability of various fatty acyl groups for PL synthesis may be different (30,31). Such a hypothesis was invoked to account for discrepancies between the determined and the calculated triglyceride molecular fractions (26).

In all PC and PE samples, 16:0/18:3 + 18:2/18:2, 16:0/18:2 + 18:1/18:2, 18:2/18:3, and 18:0/18:2 were the major molecular fractions. 16:0/18:1 was present in higher concentrations in PE than in PC. Nishihara and Kito (14) reported that 16:0/18:2, 18:0/18:2, 18:1/18:2, and 18:2/18:2 were the major fractions of PC and PE in soybean hypocotyl and cotyledon.

Correlation of soybean oil fatty acid and PL molecular species compositions. When soybean oil composition is modified, the fatty acid composition of PL changes in a fashion similar, but to a lesser extent, to the triglyceride (8). Table 8 shows the correlation among various fatty acid percentages for PC and PE. The negative correlations between 16:0 and 18:0, 16:0 and 18:1, 18:1 and 18:3, and 18:2 and 18:3 seem to reflect accepted precursor–product relations. Table 9 shows how the seven HPLC fraction percentages correlate with the PL fatty acid percentage. Naturally, when the percentage of an acyl group increased, the percentage of PL fractions containing it tended to increase. But, superimposed on these trends were the acyl group precursor–product correlations noted in Table 8, which sometimes countered the expected trend. Frequently, the correlations found for PC and PE agreed in sign and significance, suggesting that the placement of acyl groups on these PL follows similar rules.

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TABLE 9
Correlation Among Phospholipid Fatty Acid Composition and Its Molecular Fraction Percentage

		18:3/18:3	18:2/18:3	16:0/18:3 + 18:2/18:2	16:0/18:2 + 18:1/18:2	18:0/18:3	16:0/18:1	18:0/18:2
16:0	PC	0.282	0.094	–0.471[a]	0.757[a]	–0.407	0.287	–0.565[a]
	PE	–0.214	–0.137	–0.730[a]	0.803[a]	–0.450[a]	–0.036	–0.617[a]
18:0	PC	0.345	0.261	–0.313	–0.882[a]	0.948[a]	–0.191	0.988[a]
	PE	0.419[a]	0.492[a]	0.536[a]	–0.972	0.853[a]	–0.225	0.934[a]
18:1	PC	–0.309	–0.280	0.172	–0.007	–0.062	0.408	–0.013
	PE	0.013	–0.156	0.213	–0.213	–0.094	0.679[a]	0.019
18:2	PC	–0.731[a]	–0.504[a]	0.899[a]	0.002	–0.489[a]	–0.492[a]	–0.297
	PE	–0.452[a]	–0.580[a]	0.665[a]	0.003	–0.168	–0.350	–0.105
18:3	PC	0.867[a]	0.868[a]	–0.647[a]	–0.137	0.422	0.044	0.209
	PE	0.600[a]	0.830[a]	–0.502[a]	–0.152	0.319	–0.223	0.286

[a]Values represent significant correlation coefficients at 5% level. For abbreviations see Table 2.

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