Characterization of Phospholipids from Glyphosate-Tolerant Soybeans

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ABSTRACT: The phospholipids from three control and two glyphosate-tolerant soybean cultivars were isolated by extraction of soy flakes with hexane and characterized after separation by high-pressure liquid chromatography. In addition, several lots of commercial fluid lecithin were analyzed and the results were compared to values published in the literature. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid were identified as major components in these cultivars and in the commercial lecithin samples. The results showed that glyphosate-tolerant soybeans yield lecithin comparable and equivalent to conventional soybean cultivars.

Paper no. J8895 in *JAOCS 76*, 57–60 (January 1999).

KEY WORDS: Analysis of variance, glyphosate-tolerant, high-pressure chromatography, lecithin, phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phospholipids, soybeans.

A recent report (1) stated that the expression of the gene encoding glyphosate tolerance in soybean did not alter total protein, ash, fat, fiber, carbohydrate, amino acid, enzyme, or isoflavone content of the seed. Characterization of defatted meal and oil from glyphosate-tolerant soybeans also demonstrated equivalency to the parental control. Limited data suggested that the lecithin fraction from glyphosate-tolerant soybeans was equivalent as well. This study was undertaken to provide a more detailed characterization of the phospholipid composition from glyphosate-tolerant soybeans.

EXPERIMENTAL PROCEDURES

Five lots of soybeans (three control and two glyphosate-tolerant) were cracked, dehulled, and flaked in a pilot plant at NCAUR. The cracked beans were given a 30-s steam treatment prior to passage through the flaking rolls. This treatment produced a plastic flake 0.012–0.015 in thick that would not break during handling and extraction. The crude oil was obtained by extraction of flakes (1000 g) with hexane (1.5 L) in an all-glass Soxhlet extractor. After 5 h, the miscella was filtered through paper and the solvent was removed under a vacuum on a rotating evaporator. Crude oil extractions were performed in duplicate. Phospholipids (PL) were isolated from crude oil as follows. Duplicate samples of approximately 5 g oil were fractionated on a 10-g column of silica gel (60–200 mesh) with sequential elution by 200 mL chloroform, 100 mL acetone, 100 mL methanol, and 100 mL 0.1% phosphoric acid in methanol. The methanol and phosphoric acid/methanol fractions were combined for recovery of total PL. Solvent was removed by rotary evaporation at room temperature, and the residue was redissolved in chloroform. Duplicate samples were pooled and washed three times with 1 mL of saturated NaCl solution, followed by addition of sodium bicarbonate until neutral. The sample was dried with sodium or magnesium sulfate, filtered, dried under N_2, and frozen at 0°C until analyzed.

PL. Analytical high-performance liquid chromatography (HPLC) separation of PL was performed on a Thermo-Separation Products (Fremont, CA) Model SP 8800 ternary solvent delivery system with an SP 8500 dynamic mixer and a Rheodyne 7125 injector, equipped with 10–100 µL sample loops. Effluent detection was achieved with a Varex evaporative light-scattering detector (ELSD) (Model ELSD II, Alltech Associates, Inc., Deerfield, IL). The samples were eluted on a Metachem Inertsil silica column 5 µm, 250 × 3 mm i.d. for analytical separations. The solvent system was a linear gradient elution from (A) chloroform/tertiary-butyl-methyl ether (750:150, vol/vol) to (B) methanol/ammonium hydroxide/chloroform (920:70:10, vol/vol/vol) at 0.5 mL/min for 30 min and held at (B) for 10 min. This was followed by a reverse linear gradient to the starting solvent at 0.5 mL/min for 10 min. The analog signal from the ELSD was interfaced with the PC 1000 computer system *via* the SP 4500 data interface module, which was programmed to calculate the peak areas and relative percentage composition of the eluted components. Samples of commercial fluid lecithin were obtained from Lucas Meyer Co. (Decatur, IL) and Quincy Soybean (Quincy, IL).

Statistical analysis. Data were analyzed using a nested analysis of variance (ANOVA) procedure which compared tested class (control vs. glyphosate-tolerant) differences against the random effect of sample within class. Variance among samples was compared to variance between extracts

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of the same sample. Variance between the duplicate extracts was tested against the overall residual variation.

RESULTS AND DISCUSSION

Each of the experimental samples of soybeans was processed into crude oil. PL isolated from the neutral lipids (hereafter referred to as extracts) were separated and quantified by HPLC. Typical HPLC chromatograms are shown in Figure 1. The mean compositions of the control and glyphosate-tolerant PL are shown in Table 1. From ANOVA, the significance of variation between duplicate extractions suggested that samples were not homogenous, but no sample could be shown to be statistically ($P < 0.05$) different from any other sample. There were differences between extracts from common samples. ANOVA (Table 2) showed that differences between extracts were considerably greater than experimental error. The mean compositions of the control and glyphosate-tolerant lecithins are shown in Table 3 along with similar data for several commercial fluid lecithins and data taken from the literature (2,3). Data from the present study shows that PL from glyphosate-tolerant soybeans were equivalent to unmodified cultivars, and their compositions fell into the ranges reported in the literature.

Soybean lecithin as reviewed by Scholfield (4) is a complex mixture of PL including phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) as major components, with phosphatidic acid (PA) generally reported as a minor constituent. Published values (5–11) indicated a rather wide range of values for the individual PL, which may be in part due to differences in isolation and extraction methods. Interpretation of published values is complicated further when analyses were performed on preparations containing appreciable amounts of triacylglycerides. Other workers (3) have extracted soybean PL with chloroform/methanol. This solvent system apparently has the greatest effect on PC, since PE and PI show relatively good agreement regardless of which solvent was used. PA has been re-

TABLE 1
Mean Composition of Control and Glyphosate-Tolerant Phospholipids

			Phospholipid (%)			
Sample	Extract	Replicates	PE[b]	PC	PI	PA
1. C[a]	A	3	26.6	36.4	21.4	15.7
	B	2	24.4	30.0	17.3	28.3
2. C	A	3	26.1	34.9	18.3	20.7
	B	3	29.6	34.3	17.5	18.3
3. GT	A	3	28.9	33.2	17.7	20.0
	B	3	25.6	31.7	18.5	24.2
4. C	A	4	31.9	30.9	17.3	20.0
	B	3	32.6	30.2	17.3	20.1
5. GT	A	6	30.6	31.7	17.5	20.2
	B	3	33.2	33.2	17.8	17.8
Pooled S.D.			0.93	0.76	0.69	0.74

[a]Control; GT, glyphosate-tolerant; S.D., standard deviation.
[b]PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PA, phosphatidic acid.

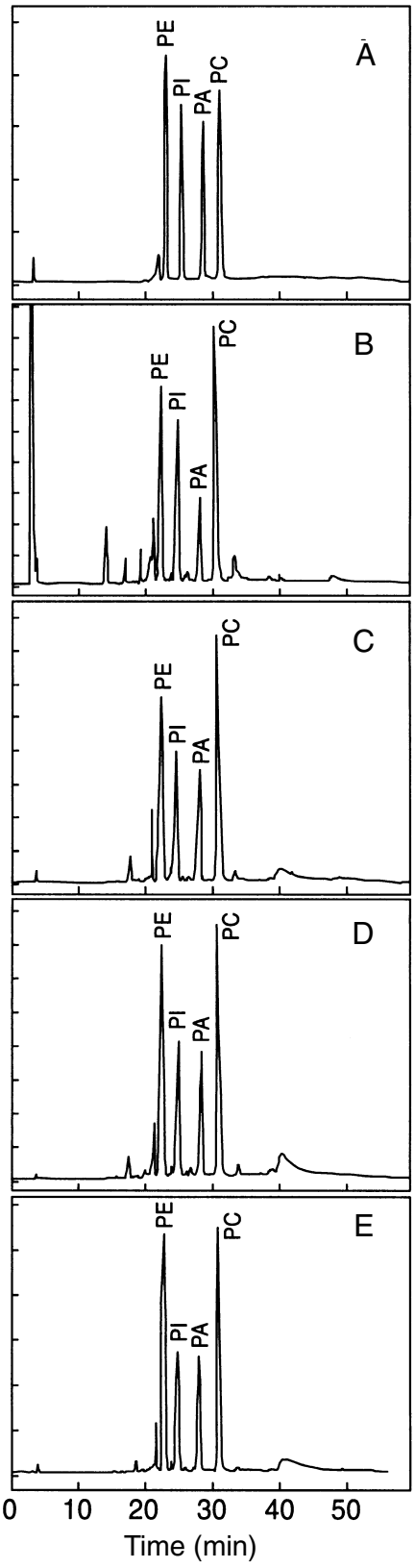

FIG. 1. High-performance liquid chromatograms of phospholipids A, standards; B, commercial fluid lecithin; C, control cultivars; D, E, glyphosate-tolerant cultivars. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PA, phosphatidic acid.

TABLE 2
Analysis of Variance for Sample, Extraction, and Duplicate Analysis

		Mean squares[a]			
Source	dF	PE[b]	PC	PI	PA
Class	1	8.24	0.84	4.12	0.02
Sample (class)	3	70.79[c]	18.40	5.58	17.20
Extract (sample class)	5	11.18[d]	11.65[d]	4.61[d]	47.35[d]
Residual	X[e]	0.91	0.55	0.50	0.56

[a]Residual mean squares (MS) was the test for extract; MS, extract, MS was the test for sample MS; and sample MS was the test for class MS.
[b]See Table 1 for abbreviations.
[c]$P < 0.05$.
[d]$P < 0.01$.
[e]X—Residual dF was 24 for PE and PC, 19 for PI, and 22 for PA.

ported as a component of granulated or acetone-precipitated soybean lecithin between 7 and 14%, yet PA appears to be absent in chloroform/methanol-extracted preparations.

The presence of PA in soybean PL is indicative of enzymatic-induced deterioration within the intact seed or during processing into oil and meal. Soybeans stored at high moisture levels (16–20%) yield crude oil with decreasing phosphatide levels and increased PA content. Data presented by Mounts and Nash (12) suggest that susceptibility of PL to attack by phospholipase-D is PC > PI > PE with PA as the final product. Other factors promoting the degradation of soybean PL include heat, moisture, and cellular disruption during normal extraction of soy flakes with hexane (13,14). Thus, the presence of PA in commercial soybean lecithin is not surprising. Mounts *et al.* (15) recently reported the effects of genetic modification on the content and composition of bioactive constituents of soybean oil. The PL content of 12 soybean genotypes showed a wide range of values for PE, PI, PC, and PA. Nine of the 12 oils showed PA levels ranging from 2.1 to 6.1%, while three samples had PA contents of 15.2, 27.7, and 35.0%. It was concluded that elevated PA levels were not related to genetic modification, but to preharvest and postharvest damage to the soybeans.

The presence of PA in levels somewhat higher than the literature prompted some additional studies in which composition of the column-fractionated PL was compared to that obtained by direct precipitation with acetone. The agreement between the two methods generally was quite satisfactory, which indicated the PL are not degraded by the column fractionation procedure. Another possibility to account for the presence of PA was the conditioning step in which the cracked beans were steamed for a very short time to aid in the flaking step. Comparison of the conditioned vs. nonconditioned treatment showed little, if any, difference in the PA content. In fact, the nonconditioned samples usually were higher in PA than the conditioned samples. These results indicate that PA was present in the beans prior to extraction and did not arise during the processing steps used to isolate the PL from the beans. The results presented here represent only two lines of glyphosate-tolerant soybeans and further work is required to fully characterize other lines.

ACKNOWLEDGMENTS

Ray Holloway and Kathy Rennick assisted with the experimental work.

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TABLE 3
Mean Phospholipid Composition of Soybeans and Commercial Lecithin

	Phospholipid (%)			
Sample	PE[a]	PC	PI	PA
Control beans	28.9	32.8	18.1	20.0
Glyphosate-tolerant	29.8	32.3	17.4	20.5
Commercial fluid lecithin[b]	25.4	40.9	22.3	11.4
Commercial fluid lecithin[b]	27.1	37.6	24.5	10.8
Literature values[c]	29–34	33–41	15.1–21.0	7–14

[a]See Table 1 for abbreviations.
[b]Present study.
[c]See References 2 and 3.

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[Received June 2, 1998; accepted October 5, 1998]