HPLC Analysis of Phospholipids in Crude Oil for Evaluation of Soybean Deterioration[1]

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Damage to soybeans due to pre-harvest stress, storage, and export shipment has been related to an increase in the nonhydratable phospholipid content of crude oil. Phospholipids in crude soybean oil extracted from such distressed soybeans have been analyzed by gradient high-performance liquid chromatography. Crude oil was fractionated by solid phase extraction using sequential elution for recovery of phosphatides. High-performance liquid chromatography of the concentrated phospholipids was accomplished on a Lichrosorb Si-60 10 μ column, 250 × 4.6 mm with ultraviolet detection at 206 nm. A 20-min solvent gradient of 2-propanol/hexane/water (42:56:2, 51:38:11) gave retention profiles of phospholipid distribution (major subclasses) that changed with impact of stress applied to plant or seed. Soybeans stored at high moisture levels (16% and 20% moisture) for up to 28 days yielded oils having phosphorus contents which decreased in direct relationship to days of storage. Retention profiles were unusable for fractions isolated from oils with phosphorus content below 100 ppm. Data show that during progressive damage, the content of phosphatidylcholine and phosphatidylinositol decreased while the phosphatidic acid content increased.

KEY WORDS: Chromatography, crude soybean oil, degumming, enzymes, high performance liquid chromatography, oil quality, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine.

Deterioration of soybeans can result from preharvest and postharvest events. In the field, damage can result from an early frost, causing plant death and immature soybeans, or from heavy rains delaying the harvest and generating high moisture levels in the beans (1). After harvest, improper handling and storage (2,3) and export shipment (4) cause damage to the bean. Such soybean stress results in activation of intrinsic enzymes, i.e., lipoxygenases, lipases, phospholipases, etc., which cause changes in the extracted crude oil decreasing its processibility, increasing refining losses, and reducing the stability of finished oils (5,6). Phospholipids, which normally are almost totally removed from crude oil by a simple water degumming process (7), become increasingly nonhydratable as bean deterioration proceeds and degummed oils are difficult to process into finished oils (8). Such damage to phospholipids has been attributed to the action of phospholipase-d (9) which results in an increase in the phosphatidic acid content correlated with an increase in the calcium and magnesium content of the oil (10). A recent study at this laboratory reported the quality of soybeans stored at different moisture levels, showing that, during storage, destruction of phospholipids was rapid and was accompanied by an increase in nonhydratable phospholipids (11). We now report an investigation of the distribution of phospholipids in crude oils extracted from stressed soybeans, as separated and quantitatively analyzed by solvent partition and high-performance liquid chromatography (HPLC).

EXPERIMENTAL PROCEDURES

Materials. All solvents used were reagent grade suitable for HPLC. Standards used for characterization were: phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS)—all obtained from Avanti Polar Lipids (Pelham, AL).

Test samples were crude oils: i) obtained during a previous study (11) from soybeans stored at different moisture levels and kept frozen until use; ii) hexane-extracted, according to laboratory procedures previously described (12), from a series of samples representative of soybeans damaged during a hurricane in the fall of 1985 and collected from farms in Georgia by David Wilson, Coastal Plains Station, Tifton, GA.

Oil characteristics. Oils were characterized according to AOCS standard methods (13) for: free fatty acid content (FFA) (Ca 5a-40), peroxide value (PV) (Cd 8-53), and phosphorus (P) (Ca 12-55).

High performance liquid chromatography. One-gram portions of crude oil were partitioned by solid phase extraction as described previously (12), to obtain the phospholipid fraction which was weighed and then dissolved in chloroform. HPLC analyses were performed with a Spectra-Physics SP 8700 system (Spectra-Physics, Inc., San Jose, CA), equipped for solvent mixing and flow programming. The sample was measured with a Hamilton syringe into a 100 μL injection loop and the elution was monitored with an LDC UV-VIS variable wavelength spectrometer (Milton-Roy, Inc., Riviera Beach, FL) set at 206 nm. All analyses were performed using a stainless-steel column (250 × 4.6 mm I.D.) packed with 10 μm Lichrosorb Si-60 (Alltech/Applied Science Labs, Deerfield, IL). Separations were performed at room temperature (ca. 20°C) and a flow-rate of 4 mL/min was maintained. The column was equilibrated with a ternary solvent system (2-propanol/hexane/water, 42:56:2). Sample elution was by a 20-min linear gradient to 51:38:11, and a 5-min return to the equilibration solvent. Previous investigators have reported the development of this separation system and its application to the analysis of complex phospholipids from animal, plant and biological lipids (14–19). The analog signal from the absorption detector was interfaced with a real-time computer (Mod-Comp Inc., Fort Lauderdale, FL) programmed to calculate peak areas and component relative percentage composition.

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TABLE 1

Storage-Damaged Soybeans[a] (moisture content 16%)

Moisture content[b] (%)	Storage time (days)	FFA (%)	PV (meq/kg)	P (ppm)	Phospholipid composition[c]				
					PE	PI	PA	PS	PC
16	1	0.47	0	866	13	26	13	17	31
	3	0.55	0	801	17	22	14	20	27
	5	0.68	0	694	18	22	17	19	25
	7	0.76	0	694	19	23	15	19	24
	9	0.80	0.18	661	20	25	9	24	22
	13	0.95	0.18	426	23	18	18	24	17
	15	1.16	0.20	224	24	24	15	20	17
	17	1.27	0.42	253	22	16	22	23	16
	20	1.43	0.42	246	24	18	21	23	14
	22	1.53	0.69	228	23	19	21	23	14
	24	1.77	0.67	107	22	20	23	22	12
	27[d]	2.02	0.92	57	—	—	—	—	—

[a]All soybeans Century 1984.
[b]Temperature variation during storage 16% — 41 to 48°C.
[c]Relative area percent by HPLC.
[d]HPLC analysis is not possible when crude oil P < 100 ppm.

TABLE 2

Storage-Damaged Soybeans[a] (moisture content 20%)

Moisture content[b] (%)	Storage time (days)	FFA (%)	PV (meq/kg)	P (ppm)	Phospholipid composition[c]				
					PE	PI	PA	PS	PC
20	3	0.62	0	502	22	24	10	21	23
	5	0.88	0	449	26	12	22	20	20
	7	1.01	0.5	434	26	25	16	18	15
	10	1.32	8.3	289	27	17	22	25	9
	12	1.04	3.0	280	29	15	24	19	13
	14	1.06	3.5	196	28	16	25	17	14
	17	1.07	3.0	135	29	16	29	13	13
	19[d]	1.81	4.2	54	—	—	—	—	—
	21	1.82	4.9	46	—	—	—	—	—
	24	1.77	4.3	10	—	—	—	—	—
	26	2.04	5.7	6	—	—	—	—	—
	28	2.34	3.3	6	—	—	—	—	—

[a]All soybeans Century 1984.
[b]Temperature variation during storage 20% — 47 to 49°C.
[c]Relative area percent by HPLC.
[d]HPLC analysis is not possible when crude oil P < 100 ppm.

TABLE 3

Hurricane-Damaged Soybeans[a]

Sample[b]	Oil yield (%)	FFA (%)	PV (meq/kg)	P (ppm)	Phospholipid composition[c]				
					PE	PI	PA	PS	PC
Control 1	20.4	.45	2.2	988	17	29	9	16	29
2	20.1	.39	2.2	894	17	23	14	19	27
1D	18.5	5.9	6.2	499	18	48	15	10	10
2D	12.5	2.4	3.0	823	16	24	19	16	25
3D	17.8	1.4	2.0	899	12	24	22	16	28
4D	22.9	6.2	2.8	717	17	44	12	11	14
5D	19.9	1.0	1.4	947	13	16	24	19	30
6D	19.9	2.0	2.7	811	16	22	21	16	25
7D	21.2	1.1	4.0	870	11	23	20	18	27
8D	17.8	2.4	5.8	617	16	38	10	17	20
9D	26.7	6.4	6.8	517	17	49	13	12	9
10D	25.3	7.2	2.4	793	14	46	8	12	20
11D	20.8	2.2	7.3	529	19	40	11	12	19
12D	19.9	3.4	13.5	505	21	45	8	13	13

[a]Provided by D. Wilson, Coastal Plains Station, ARS, Tifton, Georgia, 1985.
[b]Control — Undamaged Century Soybeans; D — Damaged soybeans.
[c]Relative area percent by HPLC.

Procedure. Two Sep-Pak separations of 1-g of crude oil were performed for 12 samples of stored soybeans, each having 16% and 20% moisture and 12 samples of hurricane-damaged soybeans. Duplicate HPLC analyses were performed on the phospholipid fraction obtained from each Sep-Pak fractionation.

Data analysis. Component peak area and relative percent composition were correlated to experimental variables such as moisture, time of storage, oil yield, P, FFA and PV using an SAS statistical program for personal computers. The linear relationships developed were used to evaluate the impact of seed stress on phospholipid composition.
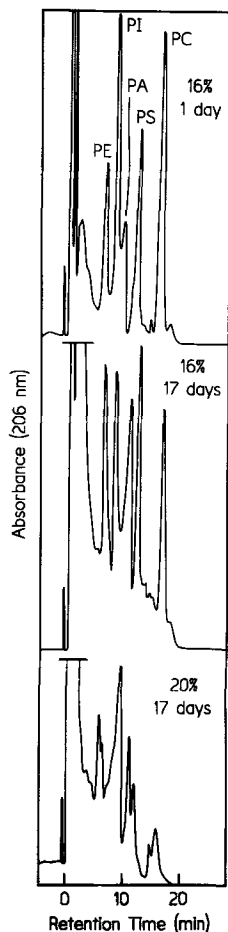

FIG. 1. Retention profile of phospholipids isolated from extracted crude oils of stored soybeans. Absorbance chromatogram obtained for samples injected with a ternary solvent system 2-propanol-hexane-water (42:56:2), then eluted with a 20-min linear gradient ending with 2-propanol-hexane-water (51:38:11). Column: 250 × 4.6 mm i.d. packed with Lichrosorb Si-60 10 μm. Flow-rate: 4 mL/min. PA: phosphatidic acid, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PI: phosphatidylinositol and PS: phosphatidylserine.

RESULTS AND DISCUSSION

Characterization of hexane-extracted crude oils and the phospholipid fraction isolated from each are presented in Tables 1, 2 and 3. Increasing levels of soybean damage are indicated by higher FFA content and a lower P con-

tent. HPLC analysis of the phospholipid fraction from storage-damaged beans was not possible when the phosphorus content of the crude oil was less than 100 ppm (27 days — 16% moisture; 19 days — 20% moisture). Destruction of phospholipids was extensive and the resultant chromatograms were difficult to interpret.
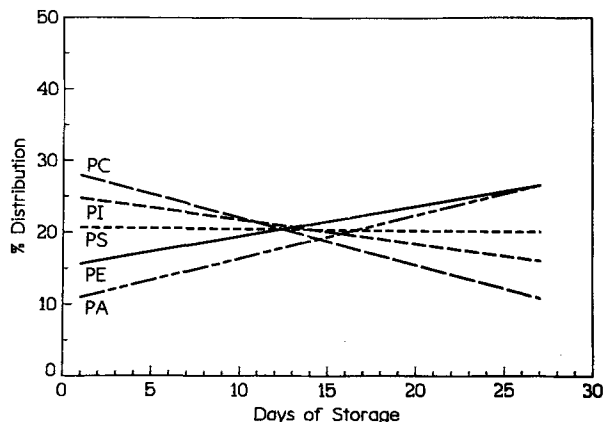
FIG. 2. Linear regression plot of relative phospholipid composition distribution versus time of storage for crude oils extracted from soybeans stored at 16% moisture. Linear equations: PC: $-0.751X + 29.336$ $r^2 = 0.86$, PI: $-0.287X + 24.648$ $r^2 = 0.13$, PS: $-0.229X + 18.552$, $r^2 = 0.19$, PE: $0.389X + 15.491$ $r^2 = 0.46$, PA: $0.419X + 11.974$ $r^2 = 0.34$.

The major components of soybean phospholipids are PC, PE and PI (20). Typical HPLC chromatograms are presented in Figure 1 and graphically illustrate the impact of storage deterioration on the phospholipid pattern. Peaks were identified by relative elution volumes of pure samples of the individual phospholipids. The linear relationship of the relative percentage distribution of the phospholipid classes to time of storage is presented in Figures 2 and 3. As the time of storage increases, there is progressive destruction of the phospholipids, i.e., loss of P content. The linear relationships show that the relative content of PC and PI decreases significantly, while that of PE and PA increases. Relative content of PS remains about the same. The rate of increase in the relative content of PA is greater with the soybeans stored at 20% moisture which is consistent with the faster deterioration of soybeans observed at this moisture level.

These findings are interpreted to indicate that the susceptibility of PLs to attack by the phospholipase-d enzyme is PC > PI > PS > PE and PA as the product of the enzymatic reaction.

Results of analyses obtained on the crude oil and its phospholipid fraction extracted from 12 samples of hurricane-damaged soybeans are presented in Table 3. There is little formation of phosphatidic acid in these samples and the phosphorus content remains high, while the FFA and PV increase. This finding indicates that under the conditions of soybean deterioration related to hurricane damage, the hydrolytic and oxidative enzymes play a greater role in oil compositional changes than does the phospholipase-d enzyme. The results presented here show that the HPLC of phospholipids of crude oil is a good tool for the evaluation of deterioration of soybeans.
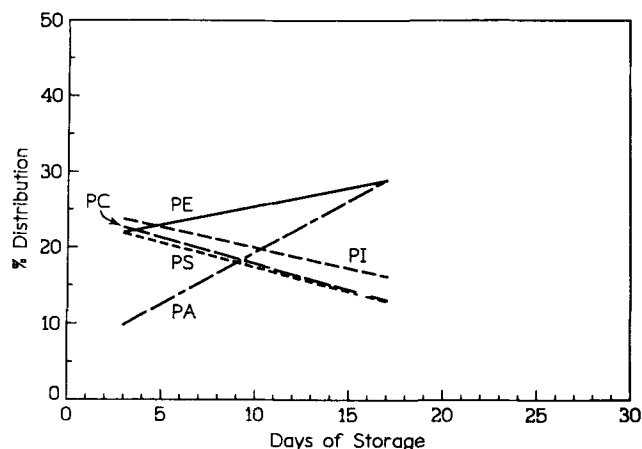
FIG. 3. Linear regression plot of relative phospholipid composition distribution versus time of storage for crude oils extracted from soybeans stored at 20% moisture. Linear equations: PE: 0.399X + 22.799 r^2 = 0.33, PS: -0.491X + 24.030 r^2 = 0.22, PI: -0.366X + 21.215, r^2 = 0.09, PC: -0.637X + 21.550 r^2 = 0.34, PA: 1.097X + 10.405 r^2 = 0.64.

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