

Studies on Phospholipids in Crude Oils from Fresh and Storage—Damaged Soybeans*

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Abstract The soybean phospholipids are chemically changed significantly when the soybean seeds are stored under high heat and humidity conditions. LC analyses of the phospholipids showed that the contents of unsaponifiables (the main component of these is sterol) and PA are increased significantly, on the other hand, the contents of main phospholipids, PE, PI and PC, are decreased significantly. The difficulty of degumming storage—damaged soybean crude oil and high phosphorous content of degummed oil are apparently related to chemical changes of the phospholipids compositions.

Key words Storage—damaged soybean; Phospholipids; HPLC analyses

Soybean phospholipids are reported to undergo significant changes when the seeds are stored under adverse conditions^[1]. The phospholipids from damaged seeds are poorly recovered by hydration in the degumming step. The resulting degummed oils are difficult to process into finished products because of their relatively high content of phosphorus—containing compounds, usually referred to as nonhydratable phosphatides^[2-4]. Phospholipids are also poorly recovered from oils extracted from soybeans exported to Europe^[5,6].

The nature of the nonhydratable phosphatides from damaged seeds has not been well characterized. In early studies of Nielsen^[2,7], the nonhydratable phosphatides from American soybeans were reported to contain 18% inorganic phosphate, 2% inositolmonophosphate, 21% glycerophosphate, 20% lysophosphatidic acid and 39% phosphatidic acid. These acids were chiefly present as the Ca—Mg salts. A small amount of nitrogen was present in the form of serine and ethanolamine bound as salt or ethers with inositol or glycerolphosphoric acid. Hvolby^[8] reported a relation between the presence of Ca and Mg in soybean oil and the amount of nonhydratable phosphatides. Another study reported a loss of 72% of total phospholipids extracted in the oil with a significant decrease in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) and an increase in phosphatidic acid (PA) and lysophosphatidylcholine (LPC)^[1]. However, the analyses of these phosphatides were based on quantitative TLC, which may be questioned.

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This report deals with an investigation of the polar lipids in oils from fresh and storage-damaged soybean seeds. Solvent partition and high — performance liquid chromatographic (HPLC) systems were used to separate and quantitatively analyze soybean phospholipids.

1 Materials and Methods

1.1 Materials

Crude oils were prepared from fresh seeds and from seeds stored in constant humidity chambers over saturated dibasic sodium phosphate solution (75% relative humidity). By adiabatic heating in a forced — draft oven the seeds reached a temperature of 58 °C after 16 days and 64 °C after 18 days. The seeds were dried, flaked and extracted as described previously^[9]. The crude oil from soybean seeds stored 18 days had a free fatty acid content of 1.56% and peroxide value of 0.77.

1.2 Concentration of Polar Lipids

Different methods explored included silica column chromatography^[10], silica cartridge (Sep — Pak, Waters) and solvent partition^[11] with one, three and six separatory funnels. The procedure adopted consisted of partitioning 10g crude oil between petroleum ether equilibrated with 95% methanol (50ml each) in 6 separatory funnels. TLC examination of the lower layers (developing solvent, CHCl₃/MeOH/water; 65/25/4) indicated that phospholipids were most concentrated in the first two fractions. Fractions 3 to 6 contained polar triglycerides and unsaponifiables. Weight percent distributions of fractions from the different crude oils are summarized in Table 1.

Table 1 Weight percent distribution of lower layers from
solvent — partition between petroleum ether and 95% methanol

Crude oils	Fractions 1—2	Fractions 3—6	% Polar lipids ^a
Fresh	70.8%	29.1%	3.1
16—days storage	46.8	53.2	2.4
18—days storage	26.8	73.1	2.4

^aFractions 1—6.

1.3 HPLC Analyses

A large number of solvent systems were explored with both analytical and preparative microporous silica columns. Solvent programming was tried with different mixtures of hexane : isopropanol : water, usually 6 volumes of hexane, 8 volumes of isopropanol and varying water from 0.3 to 1.4 volumes^[12,13]. Adding 0.02 to 0.1 volume concentrated sulfuric acid^[14] sharpened up the peaks but the resolution of phospholipids was not as good as with the neutral solvent systems. A ternary mixture of acetonitrile : methanol : 85% phosphoric acid (130 : 5 : 1.5, v/v/v)^[15] was also tried, but the separation was poor. The system adopt-

ed consisted of sequential elution with three ternary solvent mixtures containing 6 parts of hexane, 8 parts of isopropyl alcohol and three levels of water: 0.30 (I), 0.75 (II) and 1.4 (III) parts. A Whatman Partisil M-9 column 10/50 was used on either a preparative scale (50mg sample) or analytical scale (2mg sample) with a UV detector set at 206nm. At solvent flow rate of 3ml per minutes the time-solvent sequence adopted was: 10min with solvent I, 50min with solvent II and 60min with solvent III. Analytical HPLC runs were integrated with the computer.

2 Results

The polar lipids isolated in the first two lower layers from partition of crude oils in six separatory funnels with petroleum ether and 95% methanol, were further separated by HPLC into 16 fractions. HPLC chromatographies of analytical runs are compared in Figures 1 to 3 on samples from fresh and stored seeds. Main components were identified by comparing chromatographic behavior (TLC and HPLC) with those of authentic standards. Peak 1 corresponds to a mixture of unsaponifiable materials (pigments, tocopherol, sterols) and par-



Fig. 1 HPLC chromatography of phospholipids in crude oils from fresh soybean seeds

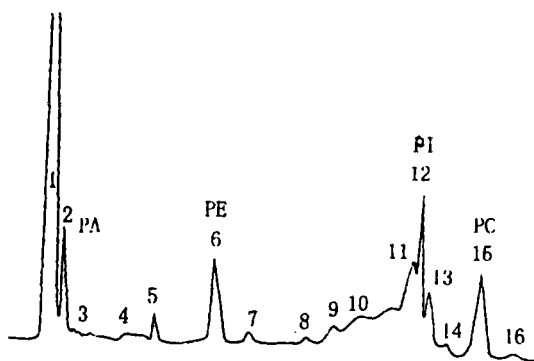


Fig. 2 HPLC chromatography of phospholipids in crude oils from soybean seeds storage-damaged 16 days

tial glycerides(mono—/diglycerides). Sterols appear to be the main component of this fraction. Peaks 2,6,12 and 15 correspond to PA,PE,PI and PC, respectively.

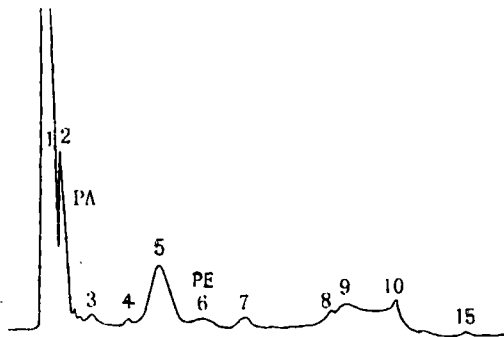


Fig. 3 HPLC chromatography of phospholipids in crude oils
from soybean seeds storage—damaged 18 days

Table 2 Relative area percent distribution of analytical HPLC fractions
from polar lipids of fresh and damaged seeds

PeakNo. ^a	Fresh seeds			Seeds stored 16 days			Seeds stored 18 days			Tentative indentifi- cation ^c
	Fr. 1—2 ^b	Fr. 3—6 ^b	Total	Fr. 1—2 ^b	Fr. 3—6 ^b	Total	Fr. 1—2 ^b	Fr. —6 ^b	Total	
1	27.7	85.3	44.5	59.1	64.3	61.9	82.9	62.0	67.6	Unsap.
2	2.1	14.2	5.6	2.7	35.7	20.2	2.6	37.0	27.8	PA
3	0.8		0.6	0.7		0.3	1.1		0.3	
4	2.0		1.4	1.2		0.6	0.6		0.2	
5	2.2		1.6	1.5		0.7	3.9		1.0	
6	15.5		11.0	6.4		3.0	3.0		0.8	PE
7	0.6		0.5	0.7		0.3	1.8		0.5	
8	1.5		1.1	1.1		0.5	1.1		0.3	
9	2.5		1.7	2.1		1.0	1.8		0.5	
10	6.2		4.4	3.8		1.9	1.1		0.3	
11	5.5		3.9	6.3		2.9				
12	12.2		8.1	4.1		1.9				PI
13	0.6		0.4	2.4		1.1				
14	0.4		0.3	0.4		0.2				
15	18.2		12.9	4.4		2.0	0.1		0.03	PC
16	0.3		0.2	0.1		0.4				
Percent	70.8	29.2	100.0	46.8	53.2	100.0	26.8	73.1	100.0	

^aSee Figures 1—3.
^bLower layers from solvent partition separation with 6 separatory funnels.
^cBased on TLC and HPLC comparison with reference compounds.

Quantitative analyses based on integration of the relative peak areas showed that peak 1 increased from 44.5% in the polar fraction from fresh seeds to 61.9% and 67.6% in the polar fraction from seeds stored 16 and 18 days (Table 2). Peak 2 due to PA increased, respectively, from 5.6 to 20.2 and 27.8%. On the other hand, the main phospholipids PE, PI and PC showed a significant decrease from 8–12% each in fresh seeds to 0–0.8% in damaged seeds. The remaining unidentified components decreased from 16% to 3%.

Preparative HPLC runs were made on the same polar fractions and the fractions corresponding to the analytical runs (Figures 1–3) were separated and weighed. The fractional weight distribution showed the same trends in phospholipid composition as the analytical HPLC analyses (Table 3). Fractions 1, 2, 6, 9 and 11 attributed, respectively, to sterols (and other unsaponifiables), PA, PE, PI and PC were tentatively identified by mixed chromatography (TLC and HPLC) with authentic compounds. TLC showed three unidentified components between PA and PE, two between PE and PI, one between PI and PC and one after PC (corresponding perhaps to sphingomyelin and lyso PC).

Table 3 Relative weight percent distribution of preparative HPLC fractions from polar lipids of fresh and damaged seeds*

Peak No.	Fresh seeds			Seeds stored 16 days			Seeds stored 18 days		
	Fr. 1–2	Fr. 3–6	Total	Fr. 1–2	Fr. 3–6	Total	Fr. 1–2	Fr. 3–6	Total
1(unsap.)	9.4	95.1	34.5	35.2	81.7	58.5	94.2	75.8	80.4
2(PA)	3.7	4.9	4.0	4.5	18.3	11.4	1.4	24.1	18.5
3	1.9		1.3	1.9		0.9			
4	2.3		1.6	2.5		1.3	1.2		0.3
5	3.2		2.3						
6(PE)	19.4		13.7	7.8	3.9		0.6		0.1
7	1.2		0.8	3.6		1.8			
8	6.5		4.6	18.8		9.4	2.5		0.6
9(PI)	18.3		13.0	15.3		7.6			
10	2.4		1.7	2.7		1.4			
11(PC)	26.7		18.8	9.3		4.6	0.1		0.02
12	5.1		3.6	3.4		1.7			
Percent	70.8	29.2	100.0	50.1	49.9	100.0	25.0	75.0	100.0

*See Footnotes in Table 2.

Further fractionation was carried out on the less polar lipids in lower layers 3 to 6 from solvent partition of crude oils. Three fractions were collected by HPLC with one solvent system (hexane : isopropanol : water, 6 : 8 : 0.05) (Table 4). Fraction 3 had the same relative retention as PA and increased from 4.8% in the polar lipids from fresh seeds to 18.3 and 24.2% in the corresponding lipids from seeds stored 16 and 18 days, respectively.

Table 4 HPLC fractionation of lower layer partition fractions 3—6*

Fractions	Fresh seeds	Stored seeds	
		16 Days	18 Days
1	51.1%	40.3%	22.0%
2	44.0	41.4	53.6
3	4.8	18.3	24.2

* One solvent system = hexane:isopropanol:water, 6:8:0.05.

3 Summary

Solvent partition and HPLC systems were developed to separate and analyze quantitatively soybean phospholipids. After 16 and 18 days of storage at 75% relative humidity, the polar lipid fractions in soybeans seeds showed significant changes in unsaponifiable and phospholipid composition. The unsaponifiable components, represented mainly by sterols, and PA increased significantly in the polar lipid fraction of aged soybean seeds. The phospholipids PE, PI and PC, on the other hand, showed a significant decrease in the lipids from aged seeds. These chemical changes are apparently related to the difficulty in degumming and further processing of crude soybean oil.

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新大豆及储存受害大豆的毛油中磷脂成分研究

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摘要 大豆在储运中因湿度和温度过高而受损害后,磷脂组份发生较大的化学变化,色谱分析表明,不可皂化部分(主要是甾醇)和 PA 含量增加,而主要磷脂成份 PE、PI、PC 含量减少。受损害大豆毛油水化脱胶困难及脱胶后油中磷含量偏高均与磷脂组份发生化学变化有关。

关键词 储运受害大豆;磷脂;高效液相色谱分析

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