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Degumming of Soybean Oil: Quantitative Analysis of Phospholipids in Crude and Degummed Oil[1,2]

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ABSTRACT

Phospholipids of crude and degummed soybean oils were isolated and separated by column chromatography and thin layer chromatography. Standards and specific spray reagents were used to identify the phospholipids. Phospholipids identified in the crude and degummed oils were phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid, lysophosphatidylcholine and lysophosphatidylethanolamine. Several unknown phosphorus-containing compounds were present. Samples of crude and degummed oil were collected from four soybean oil processors over four consecutive days. Total and individual phospholipids were quantitated by determining the phosphorus content. The total phosphorus content of crude oil varied among companies, with the average ranging from 453 to 676 ppm. The average total phosphorus content of the degummed oil of the four processors ranged from 12 to 84 ppm. Processors removed an average of 86-98% of the phosphorus present in the crude oil during the degumming process. There was also a daily variation in phosphorus removal within the individual companies. During the degumming process, the proportion of phosphatidylcholine decreased and the proportion of unknown, nonpolar phosphorus compounds increased in samples from all companies. Significantly higher proportions of phosphatidic acid and lyso compounds were found in the degummed oil of some but not all companies.

INTRODUCTION

Crude soybean oil must undergo several refining steps to produce a final product with bland flavor and odor. The first step is degumming with water (with or without a degumming agent such as phosphoric acid) to remove easily hydrated materials which are primarily phospholipids. Degumming removes 76-98% of the phosphorus-containing compounds (1) and residual phospholipids are removed during subsequent refining with caustic soda. High levels of residual phospholipids require greater amounts of caustic for refining, which increases the loss of neutral oil. The presence of excess phospholipids in the final oil causes darkening of the oil and poor flavor stability (2).

Chapman (3) found crude soybean oil to contain 39.0% phosphatidylcholine, 23.3% phosphatidylethanolamine, 20.3% phosphatidylinositol, 4.8% phosphatidic acid, and 12.5% unknown compounds. Hvolby (4) classifies phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol as hydratable phospholipids and phosphatidic acid and lysophosphatidic acid as nonhydratable. Nielsen (5) determined that the nonhydratable phosphorus compounds also include inorganic phosphate, inositolmonophosphate, and glycerophosphate. He attributes these phosphorus compounds to decomposed materials which are present in soybeans, with only small amounts formed during processing of the oil. Mounts et al. (6) attributes an increase in nonhydratables in exported beans to splitting and breakage of the seeds during transport which could activate enzymes that cause decomposition of phospholipids. Similar decomposition occurs during storage (7) and in beans that have been freeze-damaged (8).

The purpose of this investigation was to identify and quantitate the phospholipids not removed during the degumming of soybean oil. Crude and degummed soybean oil samples from four oil processors were analyzed for total phosphorus and individual phospholipids were quantitated.

MATERIALS AND METHODS

Samples

Crude and degummed soybean oils were collected on four consecutive days from four different soybean oil processing plants in Lincoln, NE, Decatur, IL, Fort Wayne, IN, and Stuttgart, AR. Samples were collected from one of the processors at two times during the year (November and February).

Separation Techniques

Crude and degummed oils were separated into neutral and polar lipids by a chromatographic system based on that of Hirsch and Ahrens (9). Chromatographic columns (10 X 100 mm) with a 200 mL solvent reservoir were packed with 5 g of 100-200 mesh Bio-Sil A (Bio-Rad

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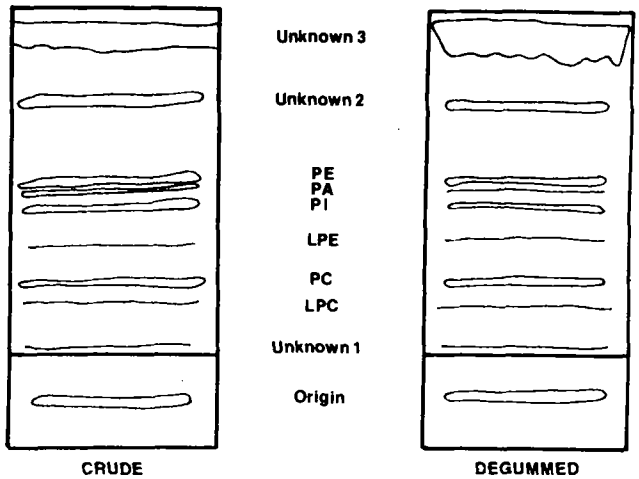

FIG. 1. Thin layer chromatograms of phospholipids in crude and degummed oil. See text for abbreviations and solvent system.

Laboratories, Richmond, CA). Oil (0.25-0.50 g) was applied to the column in 5 mL of petroleum ether and duplicate columns were run for each sample. Neutral lipids were eluted with 100 mL of 4% ethyl ether in petroleum ether. Polar lipids were eluted with 10 mL of ethyl ether followed by 10 mL of methanol, and then 200 mL of 10% deionized distilled water in methanol. Solvents were evaporated on a rotary evaporator and the polar lipids were redissolved in 0.5 mL of chloroform/methanol (2:1, v/v). The polar lipids were further separated on LHP-K high performance thin layer chromatography (HPTLC) plates (Whatman Inc., Clifton, NJ). The solvent system used to separate the polar lipids into individual phospholipids was chloroform/ethanol/deionized distilled water/triethylamine (30:34:8:35, v/v/v/v) (10). Aliquots of the crude oil polar lipid fraction were chromatographed on duplicate plates, whereas the total degummed oil polar lipid fraction was chromatographed on one plate to ensure detectable amounts of phosphorus on the plate.

Identification

Identification of individual phospholipids was accomplished by the use of various spray reagents. Phospray (Supelco Inc., Bellefonte, PA) was used to determine phosphorus-containing compounds. Nitrogen-containing compounds were detected by fluorescamine (Supelco Inc., Bellefonte, PA) and ninhydrin. Dragendorff's reagent (Alkvis, Supelco Inc., Bellefonte, PA) was used to determine the presence of choline-containing compounds. Bial's orcinol reagent (Applied Science, State College, PA) was used to detect inositol compounds. Identity of the compounds was confirmed by the use of known standards (P-L Biochemicals Inc., Milwaukee, WI).

Phosphorus Determination

Total phosphorus was determined by first bringing the polar lipid fraction eluted from the column to 5 mL in a volumetric flask with chloroform/methanol (2:1, v/v). Duplicate 100 μL aliquots of the crude soybean oil polar fraction or 250 μL aliquots of the degummed polar fraction were then spotted onto a HPTLC plate. The plate was sprayed with 0.6% potassium dichromate in 55% (by wt) sulfuric acid and then charred at 140 C for 15 min. The spot and silica gel were scraped into a 30 mL micro-Kjeldahl flask, digested and analyzed for phosphorus according to the method of Rouser et al. (11) using half amounts of reagents. Phosphorus determination of individual compounds was accomplished by first separating the phospholipids by HPTLC. Then the plates were sprayed with 0.6% potassium dichromate in 55% (by wt) sulfuric acid and charred at 140 C for 15 min. The silica gel and compound to be analyzed were scraped into a 30 mL micro-Kjeldahl flask and phosphorus determined as before. The compounds at the origin and unknown 1 could not be analyzed for phosphorus using this method due to the high phosphorus content of the preadsorbent layer resulting in less than 100% recovery of phosphorus.

Statistical Analysis

Using F ratios from analyses of variance, significant differences were determined at the 1% level.

RESULTS

Total Phosphorus Analysis

The total phosphorus content of the 20 crude oil samples ranged from 402 to 709 ppm, with an average of 561 ppm. Average phosphorus content of crude oil from companies A, B, C and D were 461, 676, 617 and 570 ppm, respectively. The total phosphorus content of the 19 samples (one sample was broken during shipment) of degummed oil ranged from 9 to 163 ppm, with an average of 59 ppm. Average phosphorus content of degummed oil from companies A, B, C and D were 58, 12, 81 and 80 ppm, respectively. The percentage of phosphorus removed during the

TABLE I

Individual Phosphorus Compounds of Crude and Degummed Oil

Compound	Phosphorus content[a]			
	Crude		Degummed	
	ppm	% of total P	ppm	% of total P
LPC	10.5 ± 4.1	2.4	1.9 ± 1.2	4.4
PC	143.7 ± 11.4	32.7[d]	4.5 ± 2.6	10.5[d]
LPE	6.3 ± 2.4	1.4	2.2 ± 1.5[b]	5.1
PI	67.2 ± 15.3	14.3	4.5 ± 3.8[c]	10.5[c]
PA	73.7 ± 53.0	16.8	4.3 ± 3.0[c]	10.1[c]
PE	109.1 ± 25.3	24.8	8.5 ± 5.2[c]	19.9[c]
Unknown 2	6.0 ± 4.2	1.4[d]	8.9 ± 0.5	20.8[d]
Unknown 3	23.0 ± 10.7	5.2[d]	8.0 ± 5.2	18.7[d]

[a]Average of four companies unless otherwise noted.
[b]Average of three companies.
[c]Average of eight samples from company A.
[d]Significantly different at the 1% level.

TABLE II

Individual Phosphorus Compounds in Crude and Degummed Soybean Oil from Company A Collected in November and February

Compound	Phosphorus content[a]							
	Crude				Degummed			
	November		February		November		February	
	ppm	% of total P	ppm	% of total P	ppm	% of total P	ppm	% of total P
LPC	20.7 ± 15.8	5.3	8.6 ± 8.1	2.7	2.1 ± 2.4	5.6	2.1 ± 2.3	5.2
PC	147.0 ± 18.9	37.5	127.5 ± 19.8	39.8	7.8 ± 3.5	20.7	8.7 ± 5.8	21.6
LPE	7.5 ± 4.1	1.9	6.1 ± 3.3	1.9	2.0 ± 2.0	5.4	1.8 ± 2.4	4.5
PI	58.8 ± 8.8	15.0	40.9 ± 14.8	12.8	3.7 ± 2.6	9.9	5.2 ± 4.8	12.9
PA	24.6 ± 7.8	6.3	15.8 ± 8.1	4.9	5.8 ± 3.7	15.3[b]	4.0 ± 3.1	9.9[b]
PE	107.2 ± 36.5	27.4	82.4 ± 15.1	25.7	7.7 ± 4.7	20.4	9.0 ± 5.7	22.5
Unknown 2	10.3 ± 5.6	2.6	14.5 ± 12.3	4.5	4.5 ± 5.3	11.9	4.1 ± 4.3	10.2
Unknown 3	15.7 ± 6.6	4.0	24.6 ± 13.8	7.7	4.1 ± 1.9	10.8	5.4 ± 5.3	13.4

[a]Average of four samples
[b]Significantly different at the 1% level.

degumming process ranged from 64 to 99%, with the average percentage of phosphorus removed from the 19 samples being 89%. Average percentage of phosphorus removed by companies A, B, C and D were 87, 99, 86 and 86% respectively. Samples were collected from company A in November and February to determine if degumming efficiency was affected by storage of the soybeans. Although the average phosphorus removal in February (82%) was less than that in November (93%), the difference was not significant due to the degree of variation in the data.

Individual Compound Analysis

Thin layer chromatograms of phospholipids from crude and degummed soybean oil are shown in Figure 1. Compounds identified were phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylinositol (PI), lysophosphatidylethanolamine (LPE), phosphatidylcholine (PC), and lysophosphatidylcholine (LPC). There were also three bands of unknown compounds present. The analysis of the individual phosphorus compounds of crude and degummed soybean oil from the four companies is shown in Table I. The amounts of each compound are shown as well as the percentages of the total phosphorus for each compound. The percentage of a compound must be considered as a percentage of the total phosphorus recovered from that HPTLC plate and not as the absolute increase or decrease of a compound, since a decrease in the proportion of one compound will increase the proportion of all the other compounds. Statistical analysis showed that there were no significant differences in the daily variation of the individual phospholipids.

The analysis of the degummed oils proved to be difficult due to the larger amount of neutral lipid at the solvent front which distorted the phospholipids in the middle of the HPTLC plate. This neutral material contained high levels of phosphorus and could not be discarded. Therefore, for company B, LPE, PI, PA, and PE were analyzed as one band. For companies C and D, LPE could be separated as a single band, but PI, PA and PE were analyzed as one band. Since the neutral lipid did not present complications in the analysis of these compounds for company A, analysis of each compound was performed.

PC was found in the greatest percentage in crude oil, followed by PE, PI, PA, LPC, and LPE. A difference was found in the ranking of PI and PA by the individual companies. Companies A and B had higher percentages of PI than PA, whereas the opposite was true of companies C and D. Due to the difficulty in the analysis of LPE, PI, PA, and PE in the degummed oil, ranking of the phospholipids in degummed oil was not done. However, on the basis of the degummed oil samples from company A, PC was found in the greatest amount, followed by PE, PA, PI, LPC, and LPE.

The amount of PC, expressed as a percentage of the total phosphorus content, decreased after degumming in all samples, while the percentage of unknowns 2 and 3 increased (Table I). After degumming, there were increased percentages of LPC and LPE in the samples from companies C and D but no significant differences in samples from companies A and B. Since PI, PA and PE were not resolved in the degummed oil samples of companies B, C and D, one cannot make conclusions about how these phospholipids are changed by degumming. However, decreased percentages of PI and PE and increased percentages of PA were found in the degummed oil from company A.

Individual components of oil samples collected from company A during November and February are shown in Table II. No significant differences were noted in the percentages of each compound in the crude oils ($P < 0.01$). However, the degummed oil showed a significantly higher percentage of PA in November when compared to the oil collected in February ($P < 0.01$) even though the mean phosphorus content in November was lower than in February (33 and 84 ppm phosphorus, respectively).

DISCUSSION

The total phosphorus content of the oils from the four processors is similar to that described by List et al. (1). They found the phosphorus content of the crude oil from five different processors ranged from 580 to 867 ppm, whereas the phosphorus content of degummed oil ranged from 12 to 167 ppm. Their samples were collected two weeks apart and showed a variation among the processors in the phosphorus content in both the crude and degummed oils but no variation in phosphorus removal within each processing plant. The average efficiency of phosphorus removal during the degumming process of their five processors was 87%. Privett et al. (12) and Chapman (3) found PC to be the major phospholipid present in crude soybean oil, followed by PE, PI, and PA. These results were confirmed in this study. LPE and LPC were not detected in either of the former studies, which could be due to the insufficient resolution of these minor components from other bands by those authors.

Phosphatidic acid generally has been considered to be the primary nonhydratable phospholipid (4). All of the phospholipids identified in crude oil were found to be present in degummed oil, albeit at much lower levels. Almost 40% of the phosphorus in degummed oil was found to be in two bands of unknown identity. Although the identity of the components of unknowns 2 and 3 has not

been determined, one can speculate that since these unknowns appear with nonpolar compounds on TLC chromatograms it is likely that they would not hydrate on contact with water and would not readily be removed during degumming. The proportion of these compounds would therefore increase with respect to the other compounds analyzed.

Kanamoto et al. (13) found that the removal of PA and PE added to completely degummed soybean oil was improved by the presence of PC. In the present study, higher levels of PC did not improve the removal of PA + PE + PI. Company B which had 131.7 ppm PC in its crude oil removed 98.9% of PA + PE + PI (185.8 to 2.0 ppm) whereas company C, which had 157.0 ppm PC in its crude oil, removed only 88.9% of PA + PE + PI (318.4 to 44.1 ppm). It is likely that degumming efficiency is affected by associations that phospholipids might have with calcium and magnesium ions, other phospholipids and other components of crude soybean oil.

Nielsen (5) suggested that the phosphorus compounds in degummed soybean oil are present in crude oil as decomposed phospholipids. Nakayama et al. (7) found phospholipase D to be present in a buffer extract of soybeans and felt that this enzyme was responsible for an increase in PA upon high moisture storage. Others have found that heat treating soybeans prior to oil extraction yielded degummed oil with low phosphorus content (14,15).

SUMMARY AND CONCLUSIONS

The phospholipids of crude and degummed soybean oil have been found to include phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylinositol (PI), lysophosphatidylethanolamine (LPE), phosphatidylcholine (PC), lysophosphatidylcholine (LPC), and three groups of unknown compounds. The total phosphorus content of crude and degummed soybean oil varied among four companies and varied from day to day within the respective companies. The efficiency of degumming varied among companies, with a low of 81% and a high of 98%.

Crude oil contained high percentages of PC, followed by PE, PI, PA, LPC, and LPE. There were differences in the ranking of the percentages of PI and PA among companies. Crude oil samples from companies A and B had greater percentages of PI than PA, whereas the reverse was found in the samples from companies C and D. Significantly increased percentages of unknowns 2 and 3 were found in the degummed oil samples. The degumming process significantly decreased the proportion of PC. Changes in the other phospholipids varied from company to company.

Oil collected from company A during November and February showed a significant difference in the amount of PA in the degummed oils with a significantly higher proportion of PA found in the degummed oil collected in November.

A trend of decreased proportions of PE and PC with increased proportions of their respective lyso compounds was noted after degumming. This could indicate that fatty esters of PC or PE were hydrolyzed to produce the increase in lyso compounds. There was also an increase in the proportion of PA, which could indicate the loss of the functional groups of some of the phospholipids to form additional PA. It is more likely that PC, PE and PI are more easily hydrated and removed than other phosphorus compounds during the degumming process.

Our studies indicated that the degumming process removed each of the phospholipids to some degree, but that some phospholipids were more easily removed than others. Therefore, the term nonhydratable phospholipids is a misnomer, the 'nonhydratable' phospholipids being hydrated to some extent. Further study is necessary to identify the unknown phosphorus-containing compounds in degummed oil and to determine how the condition of crude oil and degumming parameters affect hydratability.

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