Evaluation of Self Degumming Properties of Phospholipids in Soybean Oil Using HPLC[1]

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ABSTRACT

Freshly extracted crude soybean oil was obtained from a commercial soybean oil refinery, divided into aliquots, stored at 24 C and 5 C for varying lengths of time up to two weeks, and analyzed for total phosphorus. The concentrations of phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylinositol (PI) and phosphatidylcholine (PC) were determined by phosphorus analysis following separation by HPLC. Portions of the aliquots were degummed, and total phosphorus and individual phospholipids were determined as described above. Total phosphorus decreased 41% in crude oil stored at 24 C and 34% in crude oil stored at 5 C over two weeks. PE and PC precipitated more readily than PI and PA. Total phosphorus remaining in degummed oil was least after 12 hr storage of the crude oil and increased as the storage period became longer. The PE, PI and PA concentrations showed similar trends in degummed oil; however, the PC concentration was least at 0 hr and increased thereafter. These results are discussed in terms of phospholipid-phospholipid interactions and possible interactions between phospholipids and other compounds present in the oil.

INTRODUCTION

The occurrence of phospholipids in soybean oil is important because of the detrimental effects these compounds may exert on oil stability and quality. Several studies indicate that phospholipids chelate with trace metals such as iron and carry them through the refining process. This subsequently contributes to oil instability due to the autoxidation of unsaturated fatty acids (1-3). Other studies, however, have shown phospholipids act as synergists for tocopherols and inhibit autoxidation of polyunsaturated fatty acids in model systems based on soybean oil (4).

Phospholipids remaining in refined oil may also contribute to oil discoloration when heated (1). Burkhardt and Fuller (5) found that phospholipids caused discoloration in safflower oil when heated above 100 C prior to degumming. Garibay (6) reported that if phospholipids are not adequately removed by the degumming and refining operations, they will precipitate at hydrogenation temperatures, resulting in a dark-colored oil.

Phospholipids behave as amphipathic substances in which the polar regions of the molecule migrate toward water and the nonpolar regions migrate away from water. Due to this behavior, phospholipids will form a monolayer between oil and water surfaces, allowing the phospholipids to become hydrated from the oil when water is added (7). Problems arise, however, with phospholipids which cannot be completely removed by the addition of water: the so-called nonhydratable phospholipids. Phosphatidic acid, phosphatidylglycerol and other phospholipid decomposition products generally are classified as nonhydratable (8). All phospholipids found in crude oil (PC, PE, PA, PI) remain in degummed oil, but in different ratios (9). It has been reported that the bulk of the nonhydratable phospholipids exist as calcium and magnesium salts (8,10,11), and these show little or no affinity for water. Phospholipids also may become nonhydratable by forming complexes with native protein in the oil. Kito et al. (12) proposed that phospholipids and protein interact to form an inverted micelle whereby the polar regions of phospholipids align around a protein core. Rushing (13) detected amino acid residues in a degummed oil extract, which suggests some interactions between nonhydratable phospholipids and protein may occur.

Arutyunyan (10) studied sunflower oil and found some of the phospholipids in crude oil precipitate if the oil is stored for a short period of time. He termed this phenomenon self degumming and reported that the most hydratable phospholipids, PC and PE, were the primary constituents which precipitated during the storage period. During commercial degumming PC and PE may entrain other, less hydratable phospholipids; therefore, self degumming may reduce the efficiency of the commercial degumming process. The objective of this study was to examine the self degumming properties of phospholipids in soybean oil and the effect of self degumming on water degumming.

MATERIALS AND METHODS

Sample Preparation

Approximately 12 l of freshly extracted crude soybean oil was obtained from a commercial soybean oil refinery in Lincoln, Nebraska and immediately divided into ca. 350 g aliquots and stored at 24 C and 5 C for 0, 1, 3, 12, 24 and 48 hr and 1 and 2 weeks in capped 400 ml French square jars. Fractions of ca. 3 g crude oil or ca. 5 g degummed oil were dissolved to 10 ml in hexane:2-propanol (1:1) and filtered through 0.45 μm Nylon 66 disposable filter units (Rainin Instrument Company Inc., Woburn, Massachusetts) for HPLC analysis.

Degumming

Duplicate aliquots of the original 350 g sample were degummed according to the method of Al-Kahtani et al. (14) as modified by Handel and Winters (15). Crude oil (147.25 g) and 2.75 g deionized distilled water were stirred in a 250 ml round-bottomed flask at 400 rpm and 60 C for 15 min using a Fisher Stedi-Speed Stirrer (Fisher Scientific, Pittsburgh, Pennsylvania). The oil was then centrifuged for 10 min at 4,000 rpm (2,706 × g) in a Sorvall SS-3 Superspeed centrifuge (Sorvall Inc., Norwalk, Connecticut). The upper degummed oil layer was used for further analysis.

Phosphorus Determination

Total phosphorus was determined by spotting (in duplicate) 30 μl from crude fractions and 100 μl from degummed fractions onto 10 × 10 cm, LHP-K HPTLC plates (Whatman Inc., Clifton, New Jersey). The preadsorbent layer was not used due to the presence of high levels of phosphorus. The plates were then sprayed with 0.6% potassium dichromate in 55% (by weight) sulfuric acid and charred for 10 min at 140 C. The spots were scraped into 30 ml micro-Kjeldahl flasks (Kontes Scientific Glassware/Instruments, Vineland, New Jersey), and phosphorus content was determined by the method of Rouser et al. (16) using half amounts of reagents.

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High Performance Liquid Chromatography

An Optilab 5931 liquid chromatograph with 5902 interference refractometer detector (Tecator Inc., Herndon, Virginia) was used for the separation and identification of phospholipids. Conditions which provide optimum separation were: (i) mobile phase—hexane:2-propanol:deionized distilled water (72:55:5, v/v/v); (ii) stationary phase—Zorbax Sil, 5 μm particle diameter, 250 mm x 4.6 mm (DuPont Company, Wilmington, Delaware); (iii) flow rate—1.64 ml/min, and (iv) temperature—32 C. Hexane (Burdick and Jackson Laboratories Inc., Muskegon, Michigan) and 2-propanol (Mallinckrodt Inc., Paris, Kentucky) were HPLC grade. Individual phospholipids (PC, PE, PI, PA) were identified by comparison with the retention times of known phospholipid standards (P-L Biochemicals Inc., Milwaukee, Wisconsin). Identification was confirmed by the use of Rf values from HPTLC as described by Racicot and Handel (9). The phospholipids were separated via HPLC by injecting (in duplicate) 100 μl aliquots from the crude and degummed fractions. The eluting phospholipids were collected individually in 30 ml micro-Kjeldahl flasks. These fractions were dried under a stream of filtered air, and the phospholipids were quantitated by phosphorus analysis using the method of Rouser et al. (16).

Moisture Determination

Moisture content was determined by placing 50 g samples of crude oil in a vacuum oven for 1 hr at 100 C and 50 mm Hg (6.7 KPa).

RESULTS AND DISCUSSION

The reduction in total phosphorus content of crude oil stored for two weeks at 24 C and 5 C is illustrated in Figure 1. Over the two-week period 41% of the phosphorus precipitated from the oil stored at 24 C compared to a 34% reduction in total phosphorus in oil stored at 5 C. The phospholipids apparently are more stable and resistant to self degumming in crude oil stored at lower temperatures. Arutyunyan (10) reported that phospholipids in crude oil aggregated as a result of internal moisture, and additional precipitation over time occurred as a result of adsorption of moisture from the atmosphere. The moisture content of the crude oil in this study was 0.17%.

To better understand the behavior of phospholipids in crude soybean oil, individual phospholipids from the crude oil fractions were separated by HPLC and quantitated by phosphorus analysis. Figure 2 shows the elution profile of the phospholipids in crude soybean oil. PE and PI are well resolved; however, PA occurs as a series of three small peaks from approximately 17 to 20 min, and PC elutes as two broad peaks at approximately 75 min. Yandrasitz et al. (17), using a similar solvent system and stationary phase, found PA to elute as several peaks in a broad region between PI and PC. These researchers attributed this phenomenon to the multiplicity of ionized forms exhibited by PA. The two broad peaks exhibited by PC may be due to the presence of multiple fatty acid species (17) and/or oxidized species of PC (18). Because of these peak shapes, quantitation by peak areas was difficult. Therefore, fractions were collected for quantitation by phosphorus analysis.

The concentrations of PE, PI, PA and PC in crude soybean oil stored for two weeks at 24 C are shown in Figure 3. The phospholipids behaved similarly at both storage temperatures (24 C and 5 C); PC and PE self-degummed most readily, and PI and PA precipitated at moderate rates. PC, in particular, precipitated to such an extent that it was present in the oil at lower levels than PE after two weeks of storage. It is interesting to note that all the phospholipids (at both temperatures) aggregate rapidly over the first three hr of storage and aggregate at a slower rate thereafter. This may be due to the availability of high concentrations of "free" phospholipids when the oil is initially stored; however, it also may be due to the type of aggregation. As previously discussed, Arutyunyan (10) concluded that phospholipids aggregated by interacting with available moisture in the oil. It is possible that the phospholipids rapidly tie up the available water in the oil over the first three hr. This would explain the rapid precipitation of the phospholipids during initial storage. After most of the water in the oil has been tied up, the major mode of precipitation would be by the formation of phospholipid-phospholipid complexes whereby the nonpolar "tails" would be directed toward the oil. Kanamoto et al. (19) studied the critical micelle concentration (CMC) and the P^{31} NMR spectra of PC, PE and PA in soybean oil to determine the relative degrees of hydratability of each respective phospholipid. PC was most hydratable, while PE and PA were less hydratable. However,

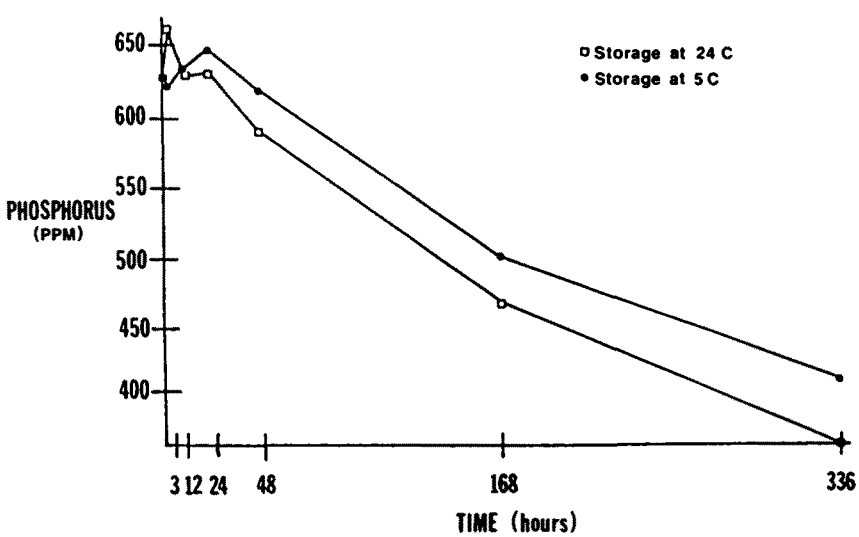

FIG. 1. Total phosphorus remaining in crude soybean oil during storage at 24 C and 5 C.

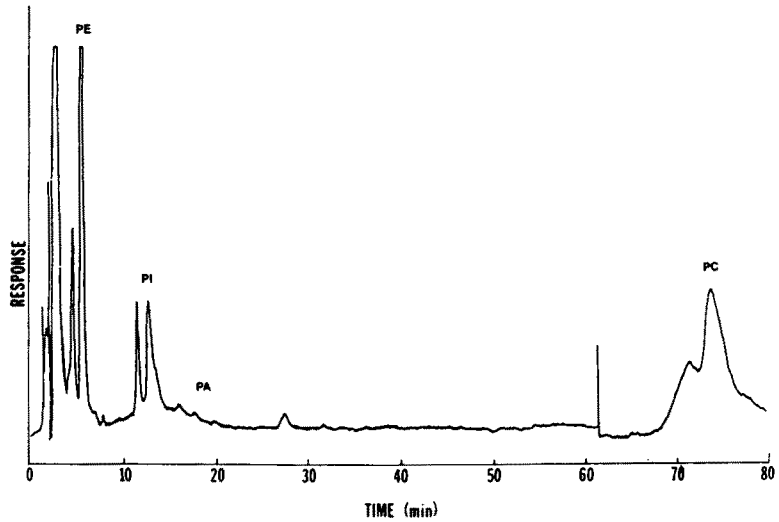

FIG. 2. HPLC separation of phospholipids in crude soybean oil (ca. 30 mg). Column, Dupont Zorbax Sil, 5 μm particle size, 250 mm × 4.6 mm; solvent system, hexane:2-propanol:deionized distilled water (73:55:5, v/v/v); flow rate, 1.64 ml/min; temperature, 32 C.

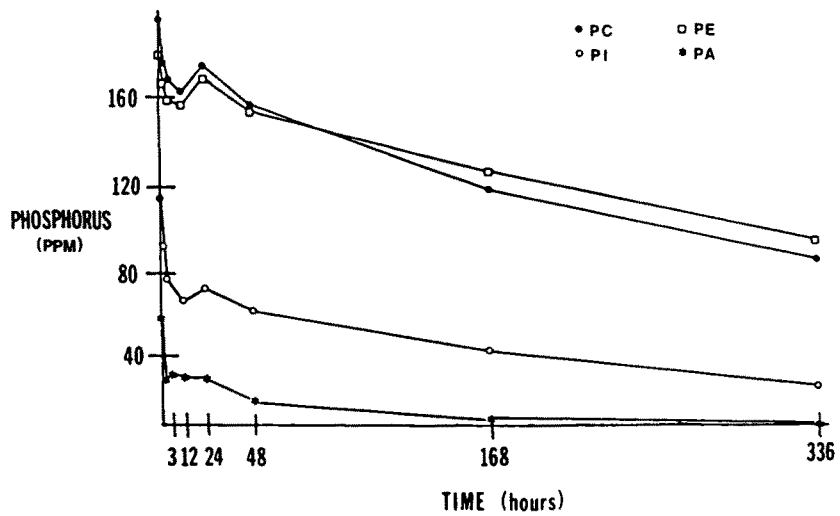

FIG. 3. Relative concentrations of PC, PE, PI and PA in crude soybean oil during storage at 24 C.

when PC was added with PE and PA, the hydratability of these two respective phospholipids increased. Kanamoto et al. (19) concluded that PC increased the hydratability of PE and PA by forming mixed micelles. This supports the conclusion that additional self-degumming over extended storage periods is a result of phospholipid-phospholipid interactions. In fact, the rapid rate at which PC self-degums over two weeks indicates that it may be interacting extensively with itself or with the other phospholipids in the system (more so than the other phospholipids are interacting with each other).

It is important next to consider how self-degumming affected degumming efficiency. The total phosphorus content of degummed oil made from crude oil stored for two weeks at 24 C and 5 C is shown in Figure 4. The amount of phosphorus remaining in degummed oil was lowest after 12 hr storage of crude oil at both 24 C and 5 C. As storage time of the crude oil increased, the amount of phosphorus remaining in the degummed oil also increased, gradually. Furthermore, the amount of phosphorus remaining in degummed oil made from crude oil stored at 24 C was greater than the amount remaining in degummed oil made from crude oil stored at 5 C. As mentioned previously, higher concentrations of phospholipids remained in the crude oil stored at 5 C than in the crude oil stored at 24 C; therefore, these results confirm Arutyunyan's (10) observations with sunflower oil that better degumming efficiency is achieved when phospholipids are present in greater amounts in the crude oil.

The concentrations of PE, PI, PA and PC in degummed oil made from crude oil stored for two weeks at 24 C are shown in Figure 5. Once again, the behavior of the individual phospholipids at both temperatures (24 C and 5 C) is very similar. PC was easily hydrated from the unstored sample (to ca. 1 μg/g oil) but remained in the degummed oil at consistently higher quantities as storage time increased.

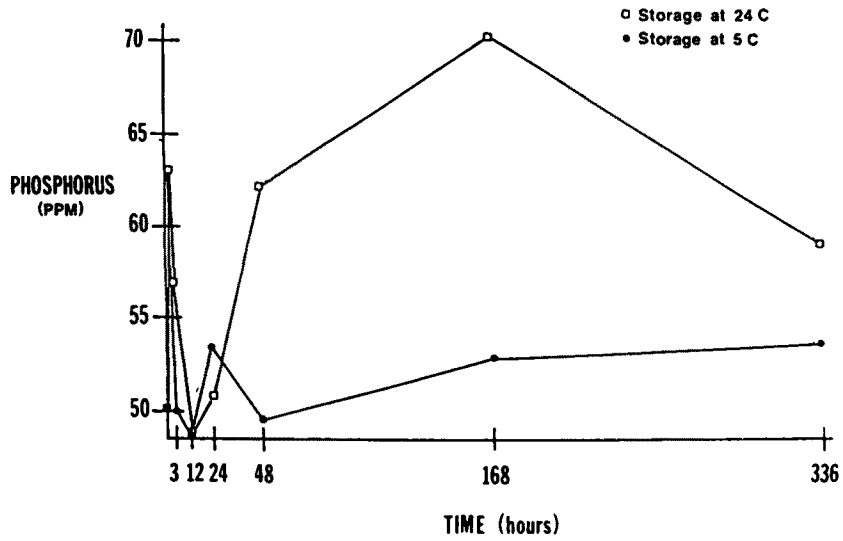

FIG. 4. Total phosphorus in degummed soybean oil made from crude oil stored at 24 C and 5 C.

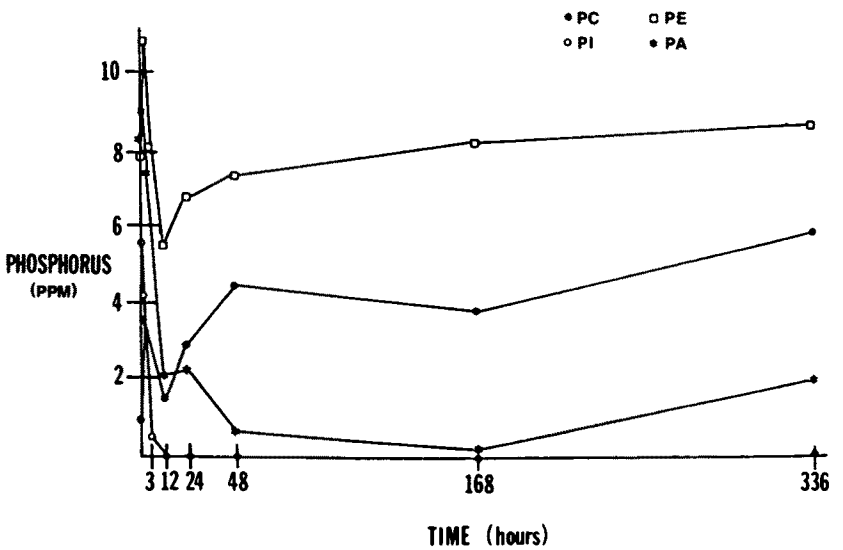

FIG. 5. Relative concentrations of PC, PE, PI and PA in degummed soybean oil made from crude oil stored at 24 C.

PA did not hydrate well from the unstored sample (ca. 8 μg/g oil) but fell off sharply over the first 12 hr of storage and remained in the degummed oil at low levels thereafter. PE behaved similarly to PA in that it decreased in concentration rapidly over the first 12 hr of storage; however, over the following two weeks the concentration of PE in degummed oil gradually increased. The PI concentration in degummed oil fell rapidly over the first 12 hr and remained in only trace amounts over the next two weeks. These results are consistent with those for total phosphorus in degummed oil in that the total phosphorus concentration was least at 12 hr storage. Once again, Kanamoto's (19) results can be used to explain the behavior of the phospholipids in the oil. At 0 hr PC has not had time to form mixed micelles with the other phospholipids; therefore, it degums well and the other phospholipids remain in the degummed oil made from unstored oil at high concentrations. As storage time increases to 12 hr, PC has time to form mixed micelles with the other phospholipids (simultaneous with precipitation of the phospholipids by moisture) and overall degumming efficiency increases. As storage time increased past 12 hr, total phosphorus increased in the degummed oil primarily as a result of an increase in the concentrations of PE and PC. Phospholipid-phospholipid interactions during extensive storage of the oil already have been discussed; however, as storage time increases the phospholipids also may have the opportunity to interact with other constituents of the oil which would interfere with degumming. One such possibility is phospholipid-protein interactions. Investigators have isolated protein from crude soybean oil (12) and amino acid residues from degummed soybean oil extracts (13). Because PE and PC are present in crude oil in the greatest quantities, it is reasonable to assume these phospholipids have the best opportunity to associate with

protein. Protein probably exists in soybean oil, not as highly ordered molecules with hydrophobic cores and hydrophilic shells, but as denatured hydrophobic peptides with infrequent hydrophilic regions. As storage time increases, phospholipids may interact with protein by electrostatic bonding between polar regions and hydrophobic interactions between nonpolar regions to form tightly interwoven, ordered systems. This type of interaction would make the phospholipids less susceptible to hydration and therefore decrease degumming efficiency.

Finally, it must be mentioned that all phosphorus-containing compounds have not been accounted for: primarily the lysophospholipids and the calcium and magnesium salts of PA. The calcium and magnesium salts of PA are stable and may be eluting with the neutral lipids during HPLC separation of the phospholipids. Therefore, it must be remembered that this discussion has been restricted to the hydratable or "free" forms of the phospholipids.

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