Soybean Lecithin Fractionation and Functionality

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ABSTRACT: Soybean lecithin contains primarily PC, PE, and PI. Fractionation of these phospholipids (PL) is desirable for certain applications. Ethanol was used to fractionate PC and PI, which have different solubilities in this solvent. Various concentrations of ethanol (90, 95, and 100%) and ethanol/gum ratios (0.5, 1.0, 1.5, 2.0, and 2.5) were used. Ethanol concentration significantly influenced the yield of the PC-enriched fraction and the PC and PI fractionation: The highest ethanol concentration resulted in the highest yield of PC fraction, the most PC in the PC fraction, and the most PI in the PI fraction. The ethanol/gum ratio significantly affected the yield of PC-enriched fraction, but did not affect the relative PL composition of the PC-enriched fraction. Ethanol of 90% concentration with a solvent/gum ratio of 3 was used for further large-scale fractionation. Such fractionation resulted in a PC-enriched fraction containing 73% PC, 24% PE, and 3% PI based on the total PL content, whereas the PI fraction contained 26% PC, 35% PE, and 39% PI. Functional properties of these two purified fractions, i.e., surface tension reduction, emulsion stability, and oxidative stability, were investigated. The PI-enriched fraction had a much lower critical micelle concentration than the PC-enriched fraction, which suggests the PI-enriched fraction has a higher surface tension reduction capability. For the emulsion stability test, the PI-enriched fraction performed better than the PC fraction in both water-in-oil and oil-in-water emulsions. An oxidative stability test showed that these PL were very stable to lipid oxidation.

Paper no. J10406 in *JAOCS 80,* 319–326 (April 2003).

KEY WORDS: Critical micelle concentration, ethanol fractionation, emulsion stability, oxidative stability, soybean lecithin functionality.

Lecithin is widely used in the food, pharmaceutical, and cosmetic industries. The percent distributions of lecithin products among the various sectors are: margarine, 25–30%; baking chocolate and ice cream, 25–30%; technical products, 10–20%; cosmetics, 3–5%; and pharmaceuticals, 3% (1). Soybean is the predominant oilseed source of lecithin owing to its ready availability as well as the outstanding functionalities of its lecithin. Crude soybean lecithin is obtained as a by-product of soybean oil processing. It typically contains 18% PC, 14% PE, 9% PI, 5% PA, 2% minor phospholipids (PL), 11% glycolipids, 5% complex sugars, and 37% neutral oil (2). Deoiling of crude lecithin is considered necessary in making high-purity lecithin products. Acetone is currently used in industry for the separation of neutral oil and PL, based on the fact that neutral oil is soluble in acetone but PL are not. Deoiled soybean lecithin contains 23% PC, 20% PE, 14% PI, 8% PA, 8% minor PL, 15% glycolipids, 8% complex sugars, and 3% neutral lipids (3). PC, PE, and PI proportions are each increased about 5% compared with the crude lecithin. Owing to the different functional properties of the various PL classes (4), it is desirable to fractionate the deoiled lecithin further with ethanol or ethanol/water systems. Since PC is relatively more soluble in ethanol than PI, an ethanol extraction will yield a PC-enriched fraction (4). This PC-enriched fraction is believed to be a better oil-in-water (o/w) emulsifier, whereas a PI-enriched fraction can be used as a water-in-oil emulsifier, for example, in the confectionery industry (5).

Lecithin recovered from soybean oil that has been extracted by an extruding-expelling (E-E) process is not solvent-treated. There is a general interest in obtaining purified or fractionated lecithin without solvents or chemicals. If ethanol fractionation of lecithin could be conducted without the initial acetone deoiling of the crude gum, the lecithin products obtained might have increased value. Our research hypothesis is that the oil contained in crude gum may act as a co-solvent in ethanol fractionation, and the separation of PC from PI may be improved compared with the fractionation of deoiled gum.

The PL molecule contains both a lipophilic fatty acyl group and a hydrophilic head group, and this amphiphilic structure makes it a good surface-tension-reducing agent and thus a good emulsifier (6). The critical micelle concentration (CMC) is usually used as an indication of the effectiveness of surface-active agents (surfactant or emulsifier) (7). Emulsion stability refers to the ability of an emulsion to resist changes in its physical properties over time: The more stable the emulsion, the more slowly the phases separate (8). PC- and PI-enriched fractions were used as emulsifiers for creating both o/w and w/o emulsions for the emulsion stability test. There is also a concern about the oxidative stability of PL, which are believed to be more unsaturated than the soybean oil from which they are recovered (9). The fractionated products will have different FA compositions; the PC-enriched fraction will be more unsaturated and the PI-enriched fraction more saturated. It is important to quantify the oxidative stability of these products.

In this study, crude lecithin was fractionated, and the PC- and PI-enriched fractions were tested for functionality, e.g., surface tension reduction, emulsion stability, and oxidative stability.

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EXPERIMENTAL PROCEDURES

Fractionation of crude gum with ethanol of various concentrations and at various ratios of ethanol to gum. E-E oil from a commercial soybean was filtered to remove meal fines. Water at 0.3% of oil level was metered in the oil stream at about 60°C. The mixture was passed through an in-line static mixer, and the gum was allowed to settle in a vessel. Two days later, the oil was pumped out and the crude gum was collected. The crude gum was centrifuged at 950 × *g* for 15 min, and about 40% of the weight was removed as oil. The residual gum after centrifugation contained 4% moisture. A factorial experimental design was used for this experiment, with ethanol concentration (three levels) and solvent/gum ratio (five levels) as two factors. About 3 g of gum was weighed into each centrifuge tube, then ethanol (three concentrations of ethanol: 90, 95, and 100%, at five ratios of ethanol to gum: 0.5, 1.0, 1.5, 2.0, 2.5) was added. The tube was heated in a water bath at 60–70°C for 1 h and was stirred every 10 min during heating for maximal dissolution of PC in ethanol, then centrifuged at 950 × *g* for 5 min. The upper clear phase (ethanol phase) was poured off into another tube. The residual lower phase was deoiled with acetone according to AOCS Official Method Ja 4-46, procedures 1–5 (10). Both the upper (PC-enriched fraction) and lower (PI-enriched fraction) phases were dried before PL were quantified by HPLC. Duplicate treatments were performed.

Fractionation of crude and deoiled gum. Two types of initial material were used to examine the influence of oil on the effectiveness of fractionation: crude gum and deoiled gum. Crude gum was obtained from the same source and procedures as described above. Deoiling was performed according to AOCS Official Method Ja 4-46, procedures 1–5 (10). Aqueous ethanol (90%) with an ethanol/gum ratio of 3:1 was used to fractionate PC and PI. The fractionation was conducted using a procedure similar to that discussed above. Duplicate treatments were performed. The fractionation of deoiled gum was designated as sequence 1 (seq 1), and the direct fractionation of crude gum was designated as sequence 2 (seq 2).

HPLC quantification of total PL and of PL class. A Beckman-Coulter (Fullerton, CA) HPLC system with auto sampler 508, solvent delivery module 126, silica column (250 × 2.1 mm i.d.; Alltech, Deerfield, IL), and ELSD (ELSD 2000; Alltech) was used for the PL class composition analysis. Two mobile phases with a gradient program were used: A is chloroform/methanol/ammonium hydroxide (80:19.5:0.5, by vol); B is chloroform/methanol/water/ammonium hydroxide (60:34:5.5:0.5, by vol) (11). Flow rate was 0.3 mL/min. Nitrogen gas with a flow rate of 1.6 L/min was used to evaporate the solvent in the heated chamber of the ELSD at 50°C.

Surface tension reduction test of fractionated PL products. PC- and PI-enriched fractions from ethanol fractionation were deoiled again with acetone to obtain relatively oil-free PL samples for functional property testing. The concentration of PL aqueous dispersion was calculated based on the total PL in the purified sample. The PL were individually dispersed in water at high concentrations and then diluted with water. Surface tensions at each concentration were determined with a FACE Automatic Surface Tensiometer (model CBVP-Z; Tantec Inc., Schaumburg, IL). Surface tensions were plotted against concentrations. The initial reduction portion and the stabilized or horizontal portion of the curve were analyzed separately to obtain the linear trend of each portion, and the intercept where the surface tension started to become constant with increase in concentration was considered as the CMC. Data were statistically analyzed, with the General Linear Model of the SAS program, to examine the effects of sample type on CMC (12). Least significant differences (LSD) at $P = 0.05$ were calculated to compare treatment differences. A commercial lecithin (Fisher Scientific, Pittsburgh, PA) with an acetone-insoluble value of 97% was also employed in the surface tension reduction test for comparison.

Emulsion stability. Both PC- and PI-enriched fractions as well as the commercial lecithin were used as emulsifiers for making w/o and o/w emulsions. For both types of emulsions, two percentage levels of emulsifier relative to the discontinuous phase were used: 5 and 10%. For o/w emulsion, the proportion of oil to water was 1:9; for w/o emulsion, the proportion of water to oil was 2:8. Emulsions were created by dispersing the emulsifier thoroughly in oil, then adding water and blending with a mixing vessel having a 50-mL capacity at high speed for 3 min. Emulsions were then transferred to 10-mL pipettes with sealed bottoms. The pipettes were placed vertically, and the volume of separated discontinuous phase was recorded periodically. The Michaelis–Menton equation was used to model the data and parameterize the stability profile (13). The reciprocal of percentage of separated oil to total oil was plotted against the reciprocal of time. The maximum percentage of separated oil to total oil (referred to as P_{max}) and the time used for reaching half of P_{max} (referred to as $T_{1/2}$) were calculated with the equation. A factorial experimental design with the SAS program was used to examine the effects of emulsifier type and percentage of lecithin on the stability of emulsion (12).

Oxidative stability evaluation. PL samples were dissolved in mineral oil so that the lecithin was completely dispersed, making oxidation more uniform than if lecithin were in a solid state. Mineral oil, also known as petrolatum liquid, is an oxidatively stable mixture of hydrocarbons derived from petroleum. The mixture was placed in an oven at 90°C for several days with periodic sampling. A Stamm test (14) was used for quantifying peroxides for both the PC- and PI-enriched fractions. Compared with the iodometric method, the Stamm test uses much less of the lipid sample. With the Stamm method, peroxides from lipid oxidation react with diphenylcarbohydrazide and produce diphenylcarbazone. The absorbance of diphenylcarbazone can be quantified by a spectrophotometer at 565 nm. Commercial lecithin also was used for the oxidation test, with both the AOCS official iodometric method Cd 8-53 (10) and the Stamm test so that the reliability of the Stamm test could be validated.

RESULTS AND DISCUSSION

Effect of ethanol strength and solvent/gum ratio on PL fractionation. The yield of PC-enriched fraction, which is regarded as a high-value product in the pharmaceutical industry, was significantly affected by both ethanol concentration and solvent/gum ratio. There was no significant interaction between the two factors ($P > 0.05$). Table 1 shows the P and $LSD_{0.05}$ values from the SAS analysis of yield and PL content of the PC-enriched fraction and the PL proportions in PC- and PI-enriched fractions obtained from different ethanol concentrations and ratios. The effects of concentration and ratio on PL fractionation were different. The yield of PC-enriched fraction, which was the percentage of ethanol-extractable material in the original gum, ranged from 20 to 25% at the three ethanol concentrations (Fig. 1). But at different ethanol/gum ratios, they ranged from less than 10 to more than 30%. Table 1 also shows that the PL content in the PC-enriched fraction was not significantly affected by ethanol concentration or by solvent/gum ratio. The mean PL contents were 37.6 ± 2.1 and 37.6 ± 1.0% in PC fractions extracted with different ratios and concentrations, respectively. Similarly, the total PL content in the PI-enriched fraction was not significantly affected by ratio or concentration (data not shown). The mean PL contents were 61.3 ± 2.2% and 61.3 ± 1.2% in PI fractions extracted with different ratios and concentrations, respectively. For individual PL content, ethanol concentration significantly affected the proportion of all three PL in the PI-enriched fraction (acetone deoiled) as well as PE and PI in the PC-enriched fraction, with the highest ethanol concentration resulting in the most PC and the least PI in the PC fraction, and the most PI and the least PC in the PI fraction (Fig. 2). Although the solvent/gum ratio only affected PE content in the PC-enriched and PC and PI in the PI-enriched fractions, the higher the ratio of ethanol to gum, the more PI and the less PC were found in the PI-enriched fraction.

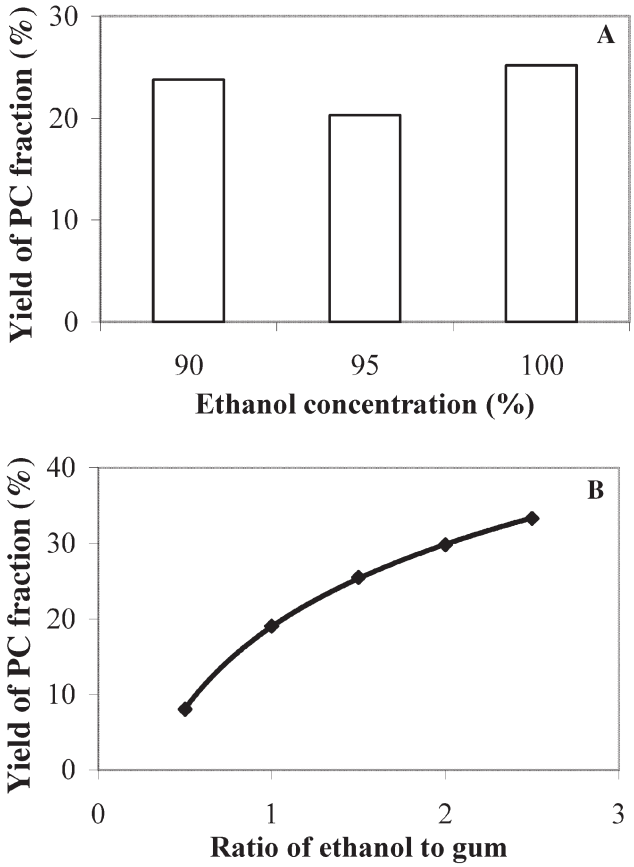


FIG. 1. Percentages of ethanol-extractable material in total gum (A) after fractionation with ethanol at various ethanol concentrations and (B) at five ratios of ethanol to gum.

TABLE 1
$LSD_{0.05}$ and *P* Values for the Effect of Ethanol Concentration and Solvent/Gum Ratio on Fractionation Yield and PL Class Proportion

	Yield of PC-enriched fraction		% PL in PC-enriched fraction	
	$LSD_{0.05}$[c]	*P* value	$LSD_{0.05}$	*P* value
Concentration[a]	3.56	0.0267	4.24	0.6315
Ratio[b]	4.59	<0.0001	5.47	0.2949

PC-enriched fraction

	% PC		% PI		% PE	
	$LSD_{0.05}$	*P* value	$LSD_{0.05}$	*P* value	$LSD_{0.05}$	*P* value
Concentration	4.41	0.2046	3.77	0.0218	1.33	0.0256
Ratio	5.7	0.7804	4.87	0.6307	1.72	0.0039

PI-enriched fraction

	% PC		% PI		% PE	
	$LSD_{0.05}$	*P* value	$LSD_{0.05}$	*P* value	$LSD_{0.05}$	*P* value
Concentration	0.98	<0.0001	2.3	0.0128	2.29	0.0237
Ratio	1.27	<0.0001	2.97	<0.0001	2.96	0.83

[a]Ethanol concentrations: 90, 95, and 100%.
[b]Ratio of ethanol to gum: 0.5,1,1.5, 2, and 2.5.
[c]$LSD_{0.05}$, least significant difference at $\alpha = 0.05$; PL, phospholipid.

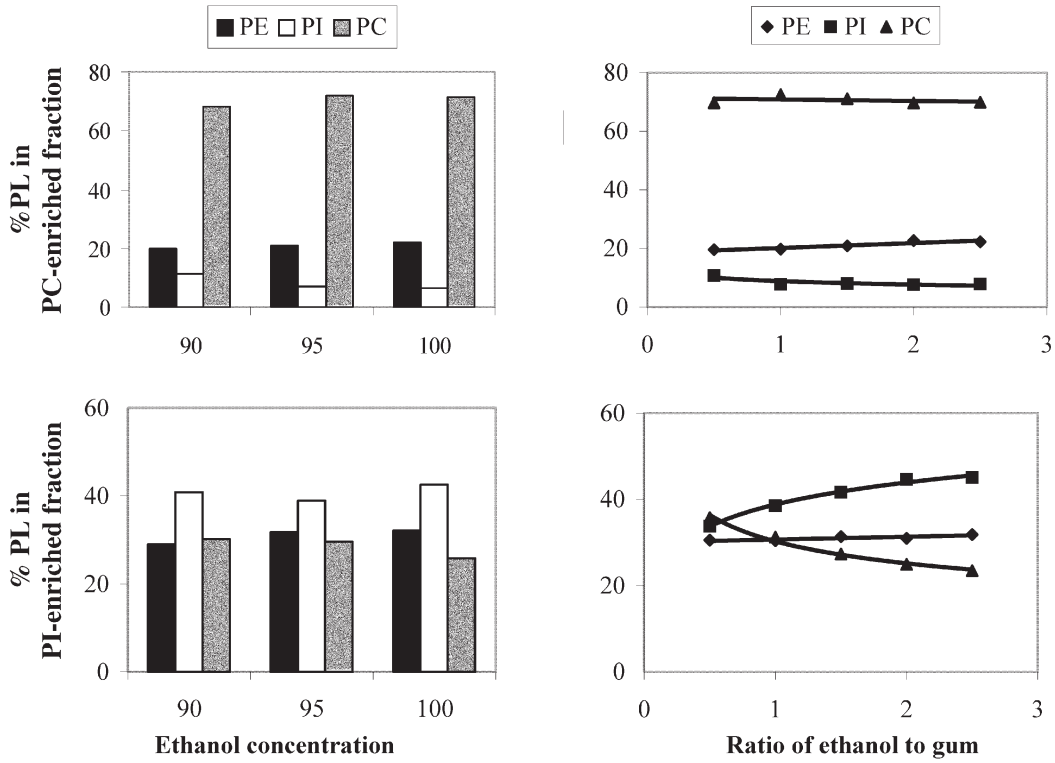
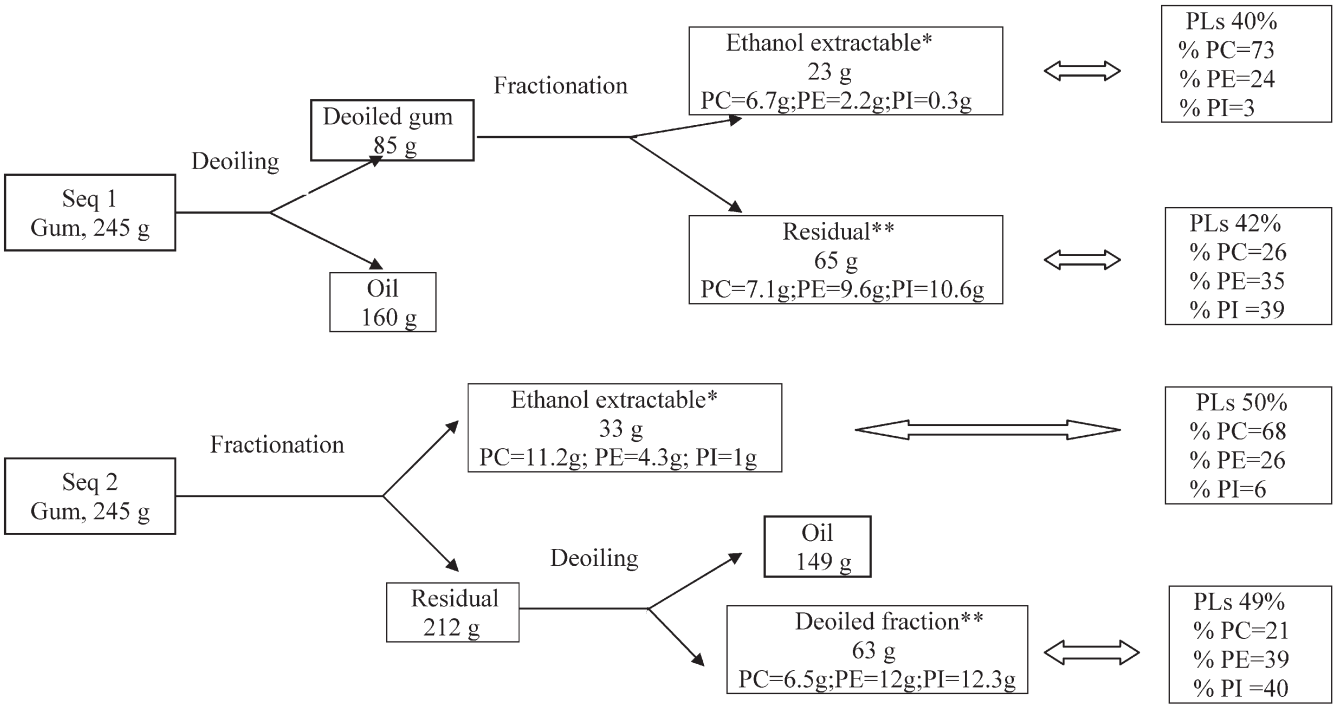


FIG. 2. Relative percentages of phospholipid (PL) class in PC- and PI-enriched fractions after fractionation with ethanol at various ethanol concentrations and at five ratios of ethanol to gum.

Although variation of ethanol concentration gave a statistically significant difference in the total yields of the PC-enriched fraction, the difference was not great. Ethanol concentration significantly influenced PC and PI fractionation, with the highest concentration giving the best fractionation; in this case, that would mean the highest PC proportion in the PC fraction and the highest PI proportion in the PI fraction. Although the ethanol/gum ratio significantly affected the production of the PC-enriched fraction, it did not affect the relative contents of PC and PI in the PC-enriched fraction. In addition, the total PL content in the two fractions did not change significantly with ethanol/gum ratio. Therefore, the highest concentration of ethanol and highest ethanol/gum ratio could result in the best fractionation and the highest production of PC-enriched fraction.

Gum fractionation on a large scale and the effect of oil on fractionation. Fractionation of deoiled and crude gum is shown in Scheme 1. Seq 1 represents the process of first acetone-deoiling the crude gum (245 g), removing 160 g of oil, and then ethanol-fractionating the deoiled gum, resulting in 23 g of PC-enriched and 65 g of PI-enriched fractions. Note that the sum of the two fractions is 3 g more than the starting 85 g; the reason may be that one or both of the fractions were not dried properly. The total PL in the final products (PC- and PI-enriched fractions) were 36.5 g, accounting for 15% of the original gum amount. Seq 2 represents the process of direct ethanol fractionation of crude gum and then acetone-deoiling of the PI-enriched fraction, because that oil mainly remained in this fraction rather than in the PC-enriched fraction. With this process, 33 g of PC-enriched and 63 g of PI-enriched fractions were obtained, and 149 g of neutral oil was removed. Total PL in the final PC- and PI-enriched products were 47.3 g, accounting for 19.3% of the original gum amount. The yield of the PC-enriched fraction as well as the total PL remaining in the final products (i.e., purity) produced from the deoiled gum was less than that produced from crude gum. More PL were lost in seq 1 than in seq 2; the reason may be that, although PL are not soluble in acetone, some PL still can be lost or trapped in the oil when the oil is first removed by acetone. This was confirmed by the difference between the two methods in the amount of oil washed out by acetone. This difference equaled the amount of the PL lost in the final products of seq 1. Of the PL lost during acetone deoiling in seq 1, 3.9 g was PC, 4.5 g was PE, and 2.4 g was PI.

Based on the same amount of original gum, crude gum resulted in 10 g more PC-enriched fraction than did deoiled gum, with 10% more total PL in the former than in the latter. The extra PL in the PC-enriched fraction from crude gum were 4.5 g PC, 2.1 g PE, and 0.7 g PI. The two procedures resulted in a similar amount of the PI-enriched fraction, but the total PL contents were different. The PI fraction from the deoiled gum had 42% total PL, and the fraction from the crude gum had 49% total PL. The PI-enriched fraction from crude gum had 0.6 g less PC, 2.4 g more PE, and 1.7 g more PI than that from deoiled gum. Figure 3 shows the percentages of PL classes in the PC and PI fractions produced from seq 1 and seq



SCHEME 1

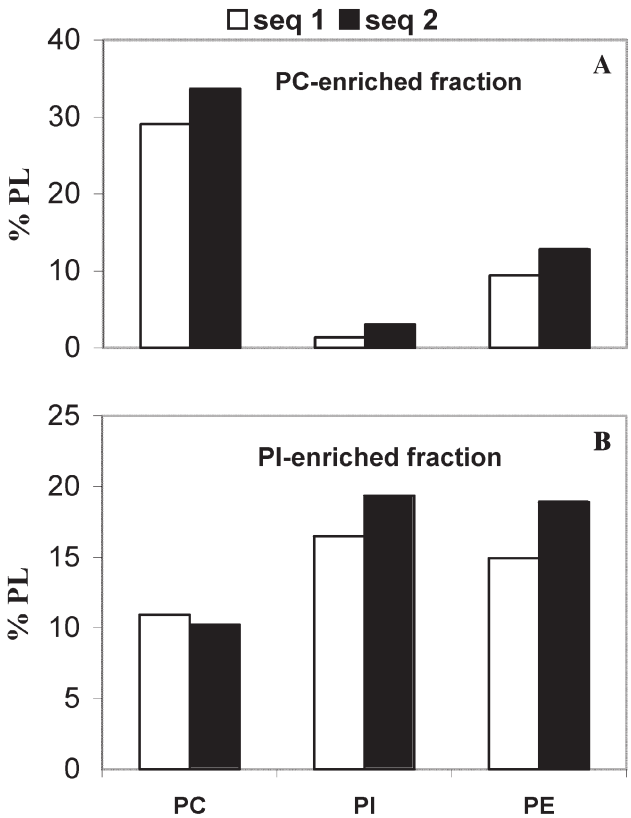


FIG. 3. PL class percentages in (A) PC-enriched and (B) PI-enriched fractions produced from seq 1 and seq 2. Seq 1 involved acetone deoiling before ethanol fractionation; seq 2 involved acetone deoiling of the PI fraction after ethanol fractionation. The *y*-axis designates the percentages of PL class in the total amount of either PC- or PI-enriched fraction. For abbreviation see Figure 2.

2. Seq 2 produced about 5% more PC in the PC-enriched fraction and about 3% more PI in the PI-enriched fraction.

We confirmed our hypothesis that seq 2 of fractionating crude gum is a more effective way of separating PC and PI, producing more PC in its PC-enriched fraction and more PI in its PI-enriched fraction. The reason may be that the oil contained in the crude gum acted as a co-solvent to trap the PI, improving the separation of PC and PI during ethanol fractionation. In addition, because the PC-enriched fraction obtained from crude gum was not subjected to undesirable organic solvent processing, it could maintain its "relatively natural" characteristics. Thus, small or large soybean processing plants that employ E-E technology or other physical processing methods not only could avoid the use of flammable acetone but also could improve both the quantity and quality of their ethanol-fractionated PC products.

Functionality study of fractionated lecithin products. Both the PC- and PI-enriched fractions contained only about 40 to 50% PL. To obtain more highly purified lecithin products for functionality tests, acetone washing was performed two more times for both the PC- and PI-enriched fractions. After this treatment, there was about 92% PL in the PC-enriched fraction, but still only about 65% PL in the PI-enriched fraction. With TLC analysis, we identified the impurities in these two fractions as lyso-PC, cerebrosides, MAG, DAG, and TAG. There were still compounds at the sample origin after TLC development that might have been glycolipids, but we did not have the standards to confirm this. Except for lyso-PC, all the other components are less polar than PL, allowing them to be more easily trapped in the PI-enriched fraction, which is less polar than the PC-enriched fraction. This result was in agree-

ment with our observation that the spot of lyso-PC of the PC-enriched fraction was relatively brighter than that of the PI-enriched fraction, whereas the other impurity spots of the PI-enriched fraction were relatively brighter than those of the PC-enriched fraction. This may result in a lower PL content in the PI-enriched fraction since PI had the majority of the impurities. But we cannot explain why these nonpolar impurities could not be removed by additional acetone washing. The purities of the lecithin products were considered when conducting functionality tests.

Surface tension reduction test of the PC and PI fractions. The surface tension reduction test showed significant differences between the two lecithin products. PC- and PI-enriched fractions reduced surface tension drastically at low concentrations, and then the surface tension stayed constant with increasing PL concentration (Fig. 4). Linear regression was applied separately for the initial reduction and the stabilized portions; the intercept of these two lines was regarded as the CMC. Mean CMC values for the duplicate surface tension tests for PC- and PI-enriched fractions were 2.67 and 0.72 mg/mL, respectively. Statistical analysis showed that there was a significant difference between the CMC of the two fractions (*P*-value was 0.0018) with the LSD value being 0.36 mg/mL. Above the CMC, the thermodynamic activity of an emulsifier does not increase with the addition of more emulsifier (7). So the smaller the CMC of the emulsifier, the better its emulsification ability. Commercial lecithin was employed in the surface tension reduction test for comparison with the PC- and PI-enriched fractions. Its CMC was 13.60 mg/mL, which was much higher than the two PL fractions. From our HPLC analysis, this commercial lecithin contained 52% PC, 27% PE, and 21% PI. Its PC percentage was between those of the PC- and PI-enriched fractions, and so was the PI percentage.

The results also showed that the surface tension of the system was reduced to a constant 38 mN/m when the CMC was reached by the PC-enriched fraction, whereas the PI-enriched fraction reduced it to 21 mN/m. This result showed that the PI-enriched lecithin had significantly better surface tension reduction capability than PC-enriched lecithin. The lower the surface tension could be reduced, the larger the amount of new interfacial area that could be created for a given amount of energy input (7), and the better emulsification capability of the emulsifier. In considering this degree of surface tension reduction and the CMC value, the PI-enriched fraction should be regarded as a better emulsifier than the PC-enriched fraction. Because both fractions are mixtures of PL as well as some other minor components, it is possible that the PL class proportion as well as the minor components of the PI-enriched fraction contributed to this functional property.

Fujita and Suzuki (15) reported that soy lyso-PL had different surface tension reduction capabilities when mixed with different FA. Saturated FA, such as palmitate and stearate, reduced surface tension the most. Our previous study illustrated that in soy lecithin PL, PI contained more saturated and less unsaturated FA than did PC and PE. Although the two systems were different—the former was PL mixed with FA, the latter was different FA esterified in PL—the contribution of the FA may be similar. So the increased palmitate and stearate content in the PI-enriched fraction may contribute to the PI-enriched fraction having better surface tension reduction capability.

Emulsion stability. Both PI- and PC-enriched fractions created o/w emulsions. Figure 5 shows how the percentage of

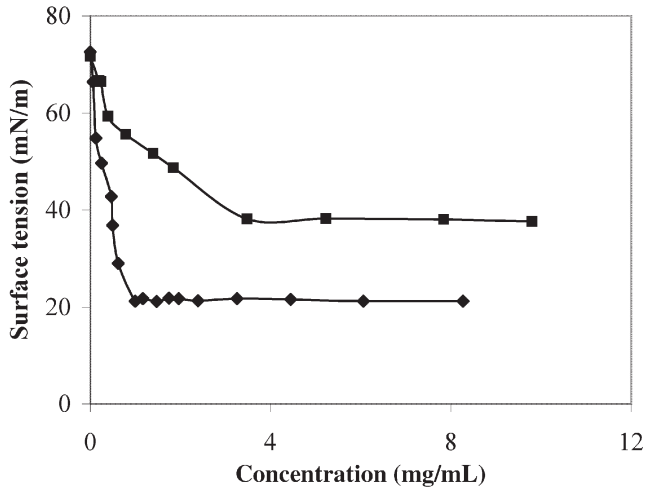


FIG. 4. Surface tension reduction of an aqueous system by PC- (■) and PI-enriched (◆) lecithin fractions.

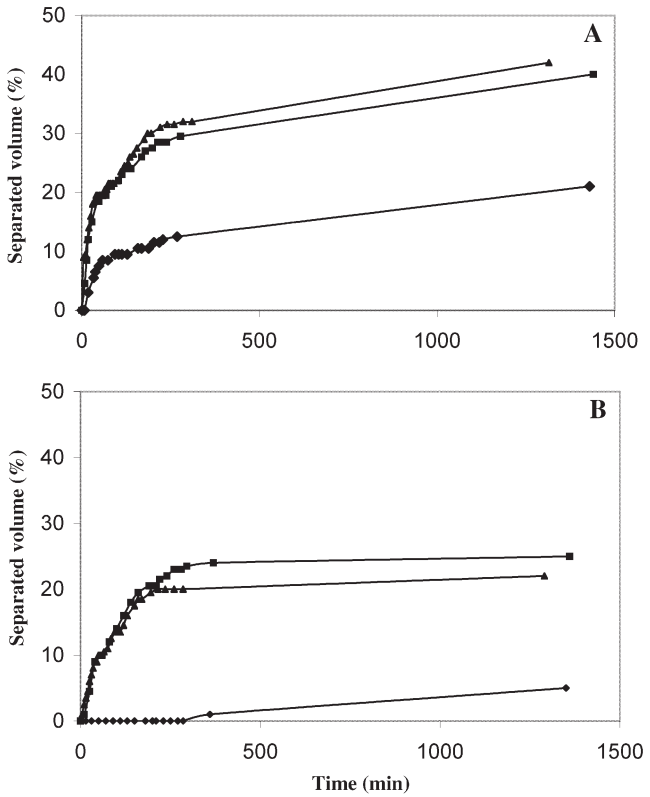


FIG. 5. Emulsion stability with 1:9 ratio of oil to water. (A) 5% lecithin in oil; (B) 10% lecithin in oil. The *y*-axis represents the separated oil volume as a percentage of the total volume. (■) PC-enriched fraction; (◆) PI-enriched fraction; (▲) commercial lecithin.

separated oil relative to total oil volume changed with time. The shape of the curves resembles an enzyme kinetics curve, which shows that the emulsion destabilized at a decreasing rate and eventually reached a relatively steady state. Therefore, the data were modeled with the Michaelis–Menton equation, and we obtained characteristic parameters, P_{max} and $T_{1/2}$, which can be used to compare treatment effects. P_{max} was the index of the maximum breaking of the emulsion, and $T_{1/2}$ was the index of the speed of emulsion breaking. The correlation coefficients of the experimental data and the data calculated with this model were 0.9730 and 0.9832 for 5 and 10% levels of PC-enriched fraction, 0.9619 for 5% level of PI-enriched fraction, and 0.8914 and 0.9877 for 5 and 10% levels of commercial lecithin. The 10% lecithin addition level seemed to create a better-fitting Michaelis–Menton curve than did the 5%. The Michaelis–Menton model was used merely for data fitting; this model was not intended to explain the mechanism of emulsion destabilization.

SAS analysis (12) showed that P_{max} and $T_{1/2}$ were significantly affected by the type and percentage of lecithin; there was a significant interaction between these two parameters. Values in Table 2, as well as Figure 6, show that the emulsion created by the PI-enriched fraction broke more slowly and to a lesser degree than that created by the PC-enriched fraction. These results suggest that the PI-enriched phase performed better than the PC-enriched fraction in an o/w emulsion and are in agreement with the results from the surface tension reduction measurement. But our observation is contrary to the results of others, which suggest that the PC-enriched fraction is an excellent o/w emulsifier (5). Commercial lecithin also was used for the emulsion stability test. Figure 5 shows that it performed similarly to the PC fraction at both the 5 and 10% levels.

For the w/o emulsion, the PI-enriched fraction created an emulsion at both the 5 and 10% levels of lecithin to water. These emulsions were very viscous: The texture and appearance were similar to yogurt. Owing to the small inside diameter of the pipettes and to the viscous nature of the emulsion, the separated water was difficult to accumulate into one continuous phase; thus, there was no obvious separated water phase to record in a similar time period as for o/w emulsions. Nevertheless, at 2.5, 20, and 44 h, an estimated 12, 60, and 68% of the emulsions had separated out as oil for the emulsion created with 10% lecithin to oil, whereas for 5% lecithin to oil, there was approximately 11, 60, and 71% separation, respectively. The two percentages of lecithin to oil did not result in a significant difference in the stability of the emulsion. The PC-enriched phase did not create an emulsion at either 5 or 10%. Instead, the lecithin hydrated and settled from the oil, a phenomenon very similar to the degumming of crude soybean oil. These results show that the PI-enriched fraction is better than the PC-enriched fraction when creating a w/o emulsion. This corresponds to the results of others. Theoretically, PI has a lower HLB (hydrophilic-lipophilic balance) number, which suggests a good w/o emulsifier (16). So the relatively higher PI content in the PI-enriched phase favors w/o emulsion formation. The commercial lecithin created viscous and yogurt-like emulsions at 5 and 10% levels, and the separation rates were similar to those of the PI-enriched fraction.

Oxidative stability test. The PV of the PC- and PI-enriched lecithin determined by the Stamm method fluctuated over the 170 h period at 90°C and did not show a definite trend. The PV (mean ± SD) of the PC- and PI-enriched fractions were 3.30 ± 2.19 and 2.82 ± 1.68 meq/kg, respectively, suggesting that the lecithin products were not oxidized under our test conditions.

PL are regarded both as primary antioxidants and as synergists (17), or only as antioxidant synergists (18). Because there was no tocopherol detected in our samples with TLC analysis, it is likely that the PL themselves behaved as antioxidants. The antioxidant characteristic of lecithin has been attributed to the metal-scavenging ability of the PL (8). The metal ions may be chelated by the head groups of PL, thus preventing their catalysis of FA chain oxidation. Our results suggest that the antioxidant properties of PC- and PI-enriched fractions are similar and that both are good antioxidants. This information is useful because the application of PC and PI fractions for other functionalities could lead to oxidative stabilization of both themselves and lipids in the system.

Commercial lecithin product was oxidized under the same conditions as the fractionated lecithin, and the PV was measured by both Stamm and standard iodometric methods. The two sets of PV were similar, thus validating the reliability of the Stamm test. The standard iodometric method needs about 5-g samples, whereas less than 100-mg samples are required by the Stamm test. Therefore, the Stamm method is a good choice for PV determination when only a small quantity of sample is available. This experiment showed that PV at 90°C decreased over time (in more than 100-h periods), from 3.21 to 0.58 with the Stamm method and from 2.28 to 0.87 with the iodometric method. The same oxidation conditions were used on soybean oil in an oxidative stability test with the iodometric method, which resulted in increased PV: 34.49 meq/kg at 48 h, 60.47 meq/kg at 96 h, and 93.45 meq/kg at 144 h. This confirmed that the mineral oil used in the system did not have any effect on the oxidative stability of soybean oil and lecithins.

TABLE 2
Mean Value and SD of P_{max} and $T_{1/2}$ for Emulsion Stability Test for PC- and PI-Enriched Fractions

	5%[a]	10%
P_{max}[b]		
PC-enriched fraction	0.3260 ± 0.0042	0.2655 ± 0.0233
PI-enriched fraction	0.1705 ± 0.0035	0.0360 ± 0.0014
Commercial lecithin	0.2674 ± 0.0079	0.2892 ± 0.0140
$T_{1/2}$[c]		
PC-enriched fraction	35.47 ± 0.93	53.59 ± 4.92
PI-enriched fraction	89.08 ± 15.04	551.25 ± 15.91
Commercial lecithin	12.73 ± 3.92	104.52 ± 2.74

[a]Ratio of weight of lecithin to weight of oil, expressed as a percentage.
[b]P_{max} is the maximum of separated oil volume to total oil volume, expressed as a percentage.
[c]$T_{1/2}$ is the time in minutes needed to reach ½ P_{max}.

ACKNOWLEDGMENTS

Journal Paper No. J-19902 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 0178, and supported by Hatch Act and State of Iowa funds and a grant provided by the Iowa Soybean Promotion Board.

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[Received July 29, 2002; accepted December 23, 2002]