Fractionation of Crude Soybean Lecithin with Aqueous Ethanol

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ABSTRACT: Aqueous ethanol was used to fractionate soybean PC and PI, which have dissimilar solubilities in this solvent. The effects of oil and moisture contents of the crude lecithin, ethanol-to-lecithin ratio, and dispersion temperature on the efficiency of phospholipid (PL) fractionation were investigated. Yield, purity, recovery, and PL class composition were examined. Yield was defined as the amount of fractionated material, divided by the acetone-insoluble (AI) matter in the starting material; purity was the percentage of PL (PC + PE + PI) as quantified by HPLC in the fraction; and recovery was the amount of PL quantified relative to the quantity of AI matter. Higher oil contents significantly increased the yield of the PC fraction, but they significantly decreased yield, purity, and recovery of the PI fraction. They also significantly affected the PL composition of the PC fraction. Higher moisture contents significantly decreased the yield but slightly increased the purity of PC fractions. Higher temperatures significantly increased the yield and recovery of the PC fraction. They also affected the relative proportion of PL classes in the PC and PI fractions. The ethanol-to-lecithin ratio significantly affected yield, purity, and recovery as well as the relative proportions of PL in both PC and PI fractions. A combination of multiple fractionation and high–low temperature treatment was also examined. Fractionating twice with ethanol increased the purity of the PC fraction. High–low temperature fractionation increased the purity and PC percentage in the PC fraction.

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KEY WORDS: Ethanol fractionation, PC fraction, PI fraction, soybean lecithin.

Lecithin is a mixture of phospholipids (PL) containing PC, PE, and PI, as well as sphingolipids, TAG, FFA, and glyco-lipids (1). Soybeans are the predominant plant source of lecithin because of their abundant availability and because their lecithin has outstanding functionalities (2). Soybean lecithin is used as an emulsifier in the food, cosmetics, and pharmaceuticals industries. It typically contains about 18% PC, 14% PE, 9% PI, 5% phosphatidic acid (PA), 2% minor PL, 11% glycolipids, 5% complex sugars, and 37% neutral oil (3).

PC, PE, and PI have different head groups, and this gives them different polarities and different emulsification properties (4). Therefore, lecithin fractionation is desirable for certain applications. Ethanol can be used for fractionation of PC and PI because of their dissimilar solubilities in ethanol. PC is relatively more soluble in ethanol than is PI, so ethanol extraction yields a PC-enriched fraction (4). This PC-enriched fraction may be a better oil-in-water (o/w) emulsifier than the unfractionated lecithin, whereas the PI-enriched fraction can be used as a water-in-oil (w/o) emulsifier that is often used in the confectionery industry (5).

The effects of extraction time, solvent volume, ethanol concentration, and temperature on the fractionation of rapeseed lecithin were studied by Sosada (6). We examined whether and how other factors, such as oil and moisture contents of the original crude lecithin, in addition to temperature and ratio of ethanol to crude lecithin, affect the fractionation so as to determine the optimal fractionation conditions. Also, multiple ethanol fractionation and a high–low (H–L) temperature treatment combination were used to examine whether these treatments could improve fractionation.

EXPERIMENTAL PROCEDURES

Lecithin fractionation. Extruded-expelled oil from commercial soybeans was filtered to remove meal fines. Water, a level of 0.5% of the weight of oil, was metered into the oil stream at about 60°C. The mixture was passed through an in-line static mixer, and the hydrated lecithin was allowed to form and settle in a vessel. Two days later, the oil was pumped out and the crude lecithin collected. The crude lecithin was centrifuged at 950 × *g* for 15 min to remove some of the free oil, and the centrifuged material contained 1.36% moisture and 59% oil. This crude lecithin was then subjected to ethanol fractionation. The effects of oil and moisture contents of the starting material, temperature, and ratio of ethanol to lecithin (see descriptions in the following sections) on fractionation were investigated. When testing for one factor, the other factors were kept constant. After 1 h of lecithin–ethanol mixing, the mixture was centrifuged (950 × *g*) and separated into two phases: The upper phase, which was the PC-enriched fraction, was ethanol soluble, and the lower phase, which was PI-enriched, was ethanol insoluble. The two phases were then desolventized and dried, yielding the PC and PI fractions. The PI fraction was further deoiled with acetone, according to AOCS Official Method Ja 4-46, procedures 1–5 (7). Three parameters were compared for the fractionation: yield, PL purity, and recovery, as defined in Equations 1–3:

$$\text{yield} = 100 \times \frac{\text{the amount of fractionated material}}{\text{acetone-insoluble (AI) matter in the starting material}} \quad [1]$$

$$\text{PL purity} = 100 \times \frac{\text{total PL (PC + PE + PI) quantified by HPLC}}{\text{amount of fractionated material}} \quad [2]$$

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$$\text{recovery} = \frac{100 \times \text{total PL}}{\text{AI matter}} = \text{yield} \times \text{PL purity}/100 \quad [3]$$

Yields >100% may be obtained when the fractionated material contains significant amounts of oil. PL profiles of all the fractions were obtained by HPLC analysis as described by Wu and Wang (8).

Effect of oil content on lecithin fractionation. Crude lecithin was deoiled with acetone, yielding a powdered lecithin after vacuum drying. This material was regarded as 0% oil content-lecithin. Oil recovered from the acetone phase was added back to the deoiled lecithin to levels of 40 and 80% oil. The original lecithin, which contained 59% oil, and the oil-free lecithin were also used in this experiment. Ethanol of 95% was added to the material in a 5:1 ratio (95% ethanol/moisture-free lecithin) and the mixture was stirred periodically at 22°C for 60 min. Separation was performed as described above.

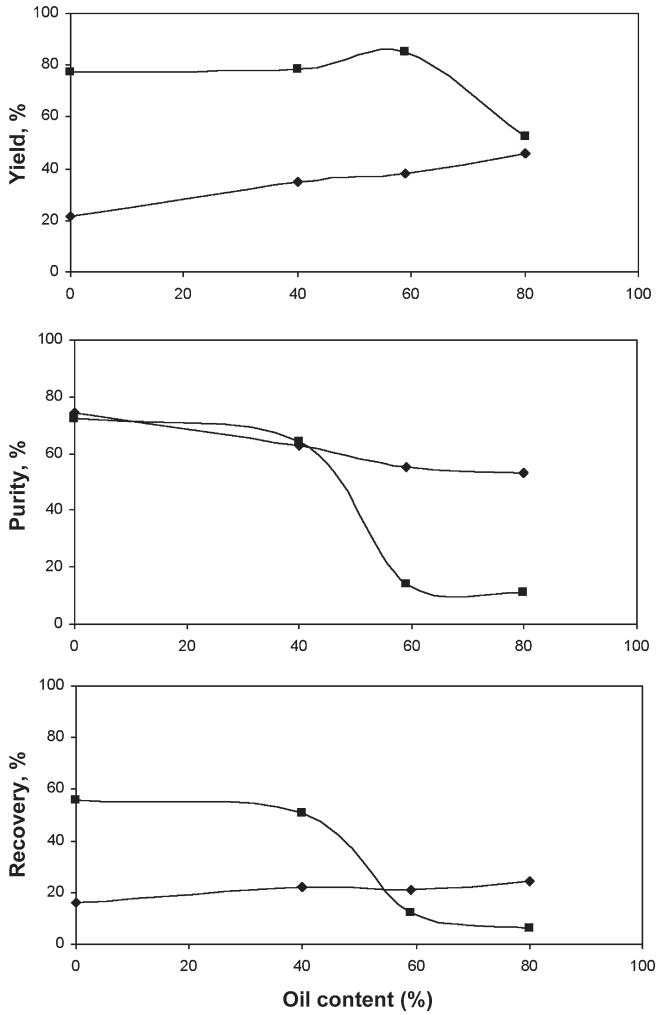

FIG. 1. Yield, purity, and recovery of lecithin fractions (◆, PC fraction; ■, PI fraction) at various oil contents. Yield = 100 × amount of fractionated material/acetone-insoluble (AI) matter in the starting material; purity = 100 × PL (PC + PE + PI) as quantified by HPLC/amount of fractionated material; recovery = 100 × amount of PL/AI matter. PL, phospholipids.

Effect of moisture content on lecithin fractionation. The 59% oil content-lecithin was fractionated at 22°C with ethanol of 84, 89, 95, and 99% concentrations at a 5:1 ratio (95% ethanol/moisture-free lecithin) to make moisture contents of lecithin equivalent to 49, 38, 24, and 5%.

Effect of temperature on lecithin fractionation. The 59% oil content-lecithin was fractionated with 95% ethanol at a 5:1 ratio (95% ethanol/moisture-free lecithin) at 5, 22, 40, and 65°C. Samples were centrifuged promptly so that the temperature was closely maintained.

Effect of ethanol/lecithin ratio on lecithin fractionation. The 59% oil content-lecithin was fractionated with 95% ethanol at 22°C. Seven ratios of ethanol to gum were used: 1, 3, 6, 9, 12, 15, and 18. Centrifuge tubes of 50-mL capacity were used for ratios of 1, 3, and 6. Centrifuge bottles of 250-mL capacity were used for the other ratios.

Effect of multiple fractionations on lecithin fractionation. PC and PI fractions recovered from crude lecithin still contained oil. The PI fraction may be deoiled with acetone, but for the PC fraction, acetone deoiling may be undesirable or unnecessary. Therefore, multiple ethanol fractionations of the PC fraction were used to increase its purity. Crude lecithin with 59% oil and 1.36% moisture was fractionated at 40°C by using a 5:1 ratio (95% ethanol/lecithin). After the first fractionation, both PC and PI fractions were dispersed in ethanol again and fractionated as described above. Then the ethanol-soluble and -insoluble fractions from the second fractionation

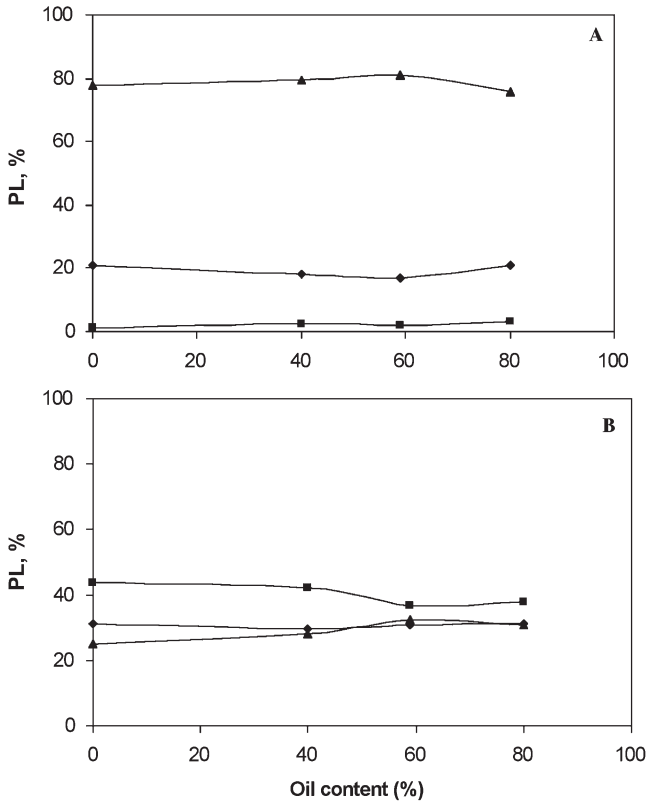

FIG. 2. PL proportions of the PC and PI fractions after fractionation at various oil contents. (A) PC fraction; (B) PI fraction. ◆ PE; ■ PI; ▲ PC. For abbreviation see Figure 1.

TABLE 1
***P*- and $LSD_{0.05}$ Values for Yield, Purity, and Recovery of PC and PI Fractions as Affected by Oil and Moisture Contents, Temperature, and Ethanol-to-Lecithin Ratio**

Oil content							
PC-Frac.	Yield	Purity	Recovery	PI-Frac.	Yield	Purity	Recovery
P-value	0.008	0.062	0.097	*P*-value	0.002	0.004	0.004
$LSD_{0.05}$	6.002	15.843	6.802	$LSD_{0.05}$	9.109	24.347	19.913
Moisture content							
PC-Frac.	Yield	Purity	Recovery	PI-Frac.	Yield	Purity	Recovery
P-value	0.000	0.006	0.401	*P*-value	0.012	0.829	0.301
$LSD_{0.05}$	3.157	10.496	8.687	$LSD_{0.05}$	4.541	9.642	7.206
Temperature							
PC-Frac.	Yield	Purity	Recovery	PI-Frac.	Yield	Purity	Recovery
P-value	0.000	0.111	0.002	*P*-value	0.000	0.752	0.047
$LSD_{0.05}$	4.628	22.21	10.017	$LSD_{0.05}$	2.518	25.932	17.941
Ratio							
PC-Frac.	Yield	Purity	Recovery	PI-Frac.	Yield	Purity	Recovery
P-value	0.000	<0.000	0.002	*P*-value	0.001	0.000	0.014
$LSD_{0.05}$	33.784	2.764	23.949	$LSD_{0.05}$	33.515	19.781	35.154

TABLE 2
***P*- and $LSD_{0.05}$ Values for Relative Proportions of PE, PI, and PC in the PC and PI Fractions as Affected by Oil and Moisture Contents, Temperature, and Ethanol-to-Lecithin Ratio**

Oil content							
PC-Frac.	PE	PI	PC	PI-Frac.	PE	PI	PC
P-value	0.000	0.000	0.000	*P*-value	0.193	0.195	0.236
$LSD_{0.05}$	2.430	0.329	2.682	$LSD_{0.05}$	1.774	8.408	8.730
Moisture content							
PC-Frac.	PE	PI	PC	PI-Frac.	PE	PI	PC
P-value	0.915	0.581	0.504	*P*-value	0.170	0.002	0.001
$LSD_{0.05}$	11.505	12.380	15.819	$LSD_{0.05}$	4.815	3.384	3.954
Temperature							
PC-Frac.	PE	PI	PC	PI-Frac.	PE	PI	PC
P-value	0.024	0.405	0.036	*P*-value	0.330	0.005	0.003
$LSD_{0.05}$	7.580	3.418	8.564	$LSD_{0.05}$	7.117	11.435	8.945
Ratio							
PC-Frac.	PE	PI	PC	PI-Frac.	PE	PI	PC
P-value	0.000	0.000	0.000	*P*-value	0.000	0.004	0.000
$LSD_{0.05}$	2.430	0.329	2.682	$LSD_{0.05}$	1.629	3.438	2.904

were combined accordingly. The third fractionation was done the same way as the second.

Effect of H–L temperature treatment on lecithin fractionation. The crude lecithin of 59% oil content and 1.36% moisture content was fractionated with 95% ethanol at a 5:1 ratio (ethanol/lecithin). Fractionation was performed at 40°C in a water bath for 1 h, and the mixture was then put into an ice-bath for 15 min. This H–L temperature treatment was carried out because oil solubility in ethanol may change more with temperature than the solubility of PL; thus, this low temperature treatment may increase the purity of the PC fraction.

Large-scale fractionation. Crude soybean lecithin (1182 g; 1.36% moisture, 59% oil) was fractionated with 95% ethanol at a ratio of ethanol/lecithin of 5:1 at room temperature. After the first fractionation, half of the PC fraction was fractionated again using the same conditions, and the other fraction was

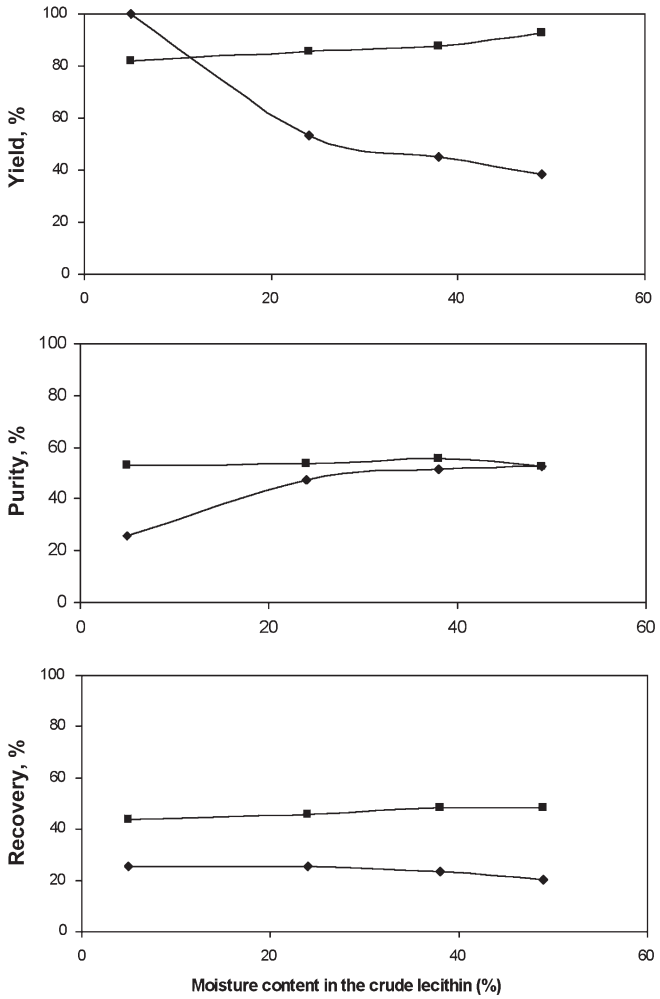

FIG. 3. Yield, purity, and recovery following lecithin fractionation at various moisture contents. ◆, PC fraction; ■, PI fraction.

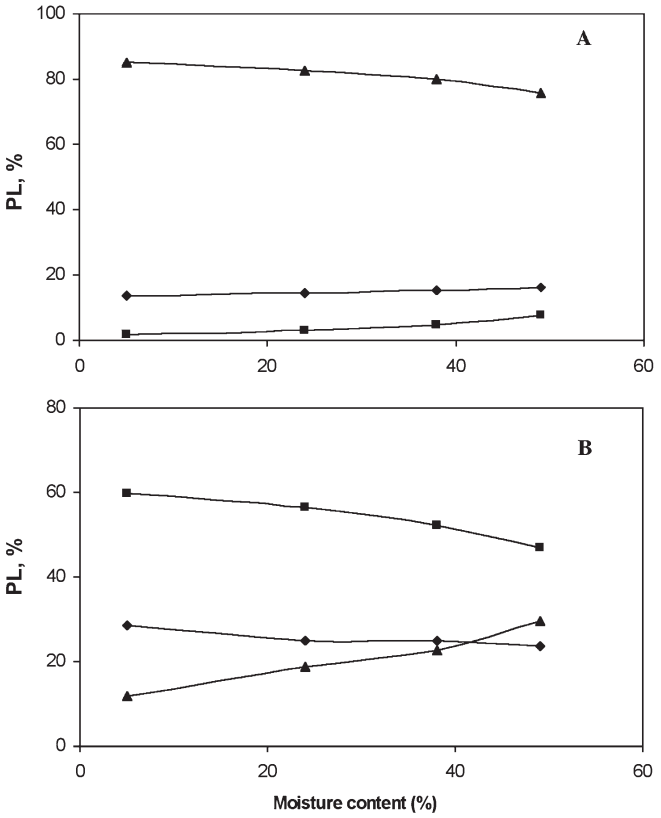

FIG. 4. PL proportions of the PC and PI fractions after fractionation at various moisture contents. (A) PC fraction; (B) PI fraction. ◆ PE; ■ PI; ▲ PC. For abbreviation see Figure 1.

fractionated with the high–low temperature treatment as described above. The PI fraction was also fractionated twice.

Statistical analysis. All experimental treatments were repeated two times. Statistical analysis was performed using the General Linear Model procedures of SAS 8.02 (9).

RESULTS AND DISCUSSION

Effect of oil content on lecithin fractionation. The yield of the PC fraction increased but its PL purity decreased with the increase in oil content, and this resulted in a slight increase in PL recovery with oil content (Fig. 1). The increase in PL yield between 0 and 40% oil content was significant, but the changes among 40, 59, and 80% oil content were not (Table 1). For the PI fraction, the yield at 0, 40, and 59% of oil contents did not show significant differences, but that at 80% decreased significantly (Fig. 1). The purity and PL recovery of the PI fraction with 0 and 40% oil contents were similar, and those with 59 and 80% initial oil contents were similar, but these higher oil content samples had much lower purity and recovery values than the lower oil content samples. It is possible that acetone washing resulted in significant loss in PL when the oil content was too high in the PI fraction (10). The recoveries of PL in the PC and PI fractions of 0 and 40% oil were all about 73% of the initial AI matter, but those from 59 and 80% oil content were only about 30%. PL class proportions in the PC fraction were significantly affected by oil content, whereas those in the PI fraction were not (Fig. 2, Table 2). There was no appreciable change in PL composition with oil content.

Therefore, fractionation with 40% oil in lecithin was considered optimal based on the preceding results. This level of oil content is also typical for commercial production. When a sufficient amount of water is added to crude oil for lecithin hydration, less oil will be entrapped than in the material we used, for which only a limited amount of water (0.5%) was used for degumming, which successfully removed almost all PL from this mechanically extracted oil.

Effect of moisture content on lecithin fractionation. The yield of PC fraction based on total initial AI matter (moisture-free, oil-free) decreased significantly with the increase in moisture content, but the purity significantly increased, thus resulting in a very slight decrease in PL recovery in the PC fraction (Table 1, Fig. 3). The yield of the PI fraction and the recovery of PL in this fraction both increased with the increase in moisture content, but the purities remained the same (Fig. 3). The total recoveries of PL in the two fractions added up to about 80% of the initial AI matter at all moisture con-

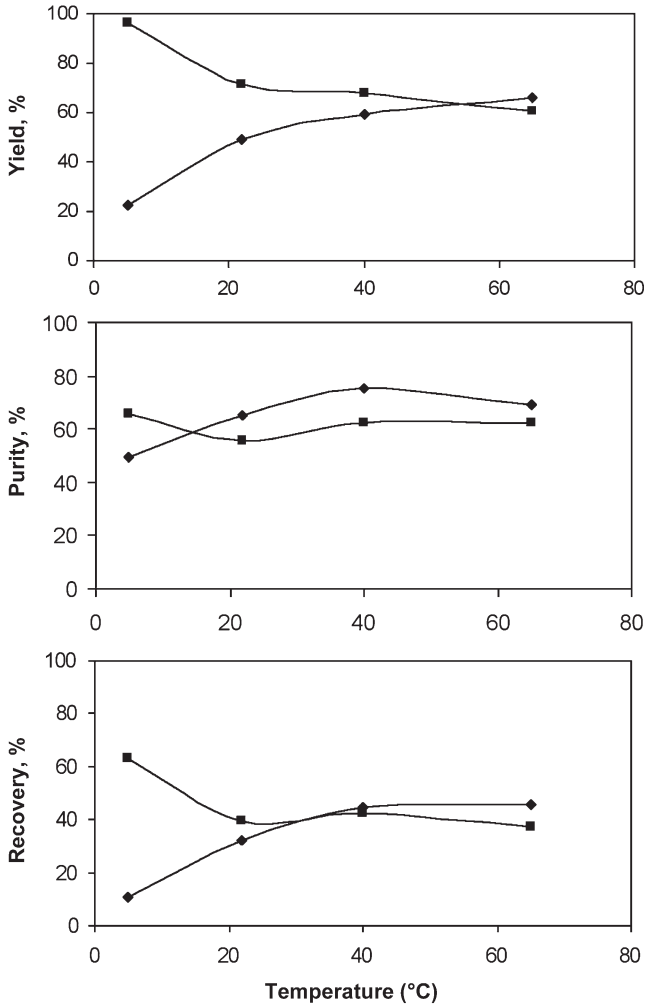

FIG. 5. Yield, purity, and recovery of lecithin fractions at various temperatures. ◆ PC; ■ PI.

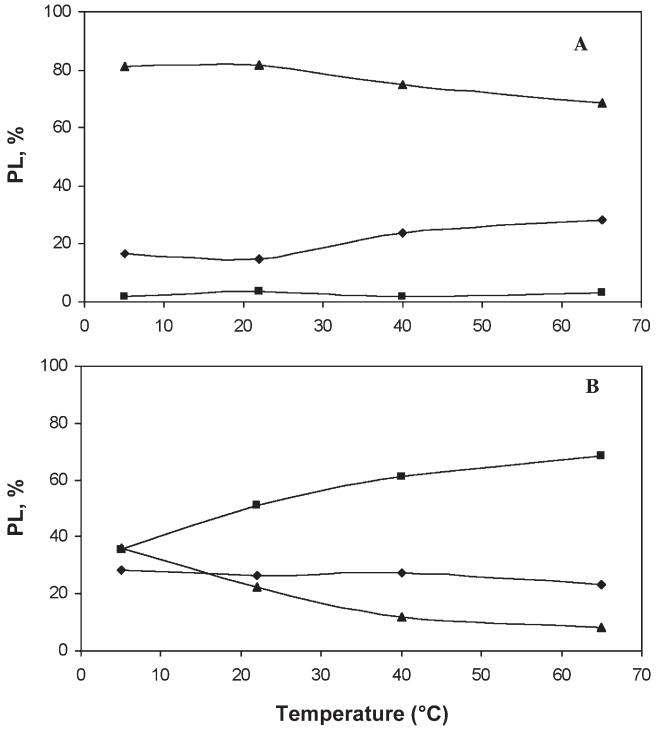

FIG. 6. PL proportions of the PC and PI fractions after fractionation at various temperatures. (A) PC fraction, (B) PI fraction. ◆ PE; ■ PI; ▲ PC. For abbreviation see Figure 1.

tent levels. The other 20% relative to AI matter may be PL other than PC, PE, and PI that were not detected or quantified with our HPLC program, and also some PL may be lost during the acetone deoiling of the PI fraction.

The relative proportions of PC in the PC fraction and PI in the PI fraction decreased with the increase in moisture content; with PC the change was significant, and with PI it was not (Fig 4., Table 2). Based on these results, a 24% moisture content was considered reasonable for fractionation. Such a moisture content can be obtained by mixing at a 1:5 lecithin-to-solvent ratio using typical soybean crude lecithin (1.36% of moisture content) and 95% ethanol. Ethanol at 95% reportedly resulted in the highest yield of the PC fraction as well as the highest PC percentage in the PC fraction for rapeseed lecithin fractionation (6).

Effect of temperature on lecithin fractionation. Yield, purity, and recovery of the PC fraction were all increased with the increase in temperature (Fig. 5). The increases in yield and recovery were significant, whereas that of purity was not (Table 1). Nevertheless, the purity tended to peak at 40°C, which was the highest purity (~75% PL) in this study. The yield and recovery of the PI fraction decreased significantly with the increase in temperature (Table 1, Fig 5). The purity of the PI fraction was not affected by temperature. The recovery of PL in the two fractions added up to about 80% of the initial AI matter. The relative proportion of PC in the PC fraction decreased slightly but statistically significantly with increase in temperature, but PI in the PI fraction significantly increased (Table 2, Fig. 6). Based on these observations, 40°C was judged to be the best fractionation temperature.

In a similar study on rapeseed lecithin fractionation (6), the yield of the PC fraction was highest at 20°C, and the percentage of PC in the PC fraction was the lowest at this temperature, a result with which our data did not agree. In our study, the yield of the PC fraction increased with temperature, but the PC percentage in the PC fraction decreased slightly with the temperature increase.

Effect of ethanol-to-lecithin ratio on fractionation. The yield of the PC fraction decreased, whereas that of the PI fraction increased with the increase of ratio when the ratios were below 6 (Fig. 7). For higher ratios (>9), there was no significant change in the yield of the PC and PI fractions (Table 1, Fig. 7). The purity of fractions at various ratios did not differ appreciably when the ratio exceeded 6. PL recovery in both the PC and PI fractions increased as the ratio increased at low ratios. For higher ratios, there were no significant differences.

The sudden changes in yield and recovery values may be due to the differences in size of container and in the centrifugal used for fractionation (Fig. 7). When 250-mL containers were used for the higher-ratio treatment, the centrifuge speed was

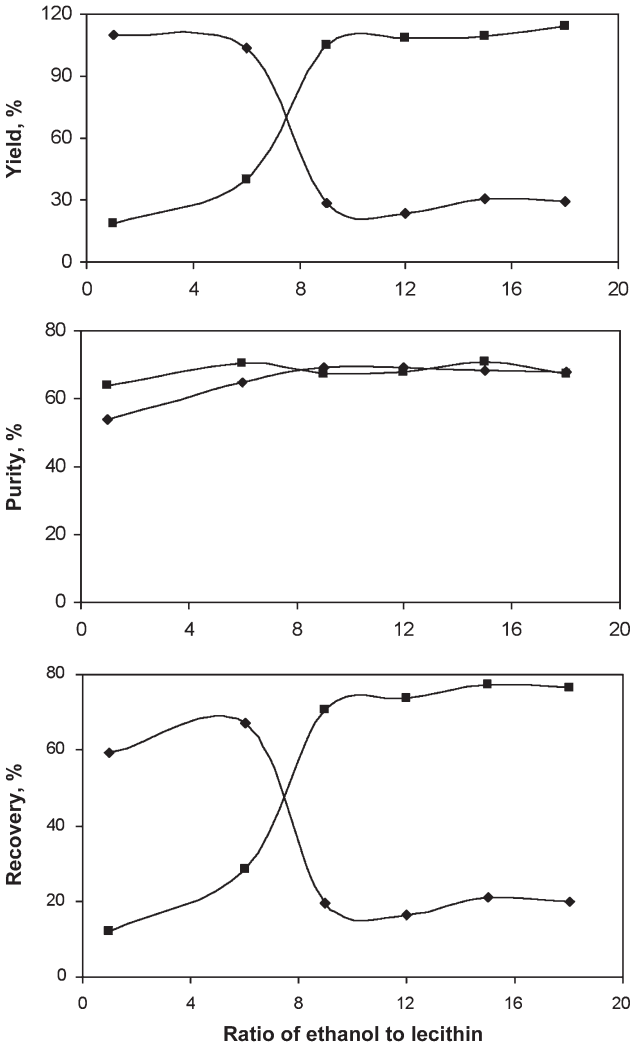

FIG. 7. Yield, purity, and recovery of lecithin fractions at various ethanol/lecithin ratios. ◆ PC; ■ PE.

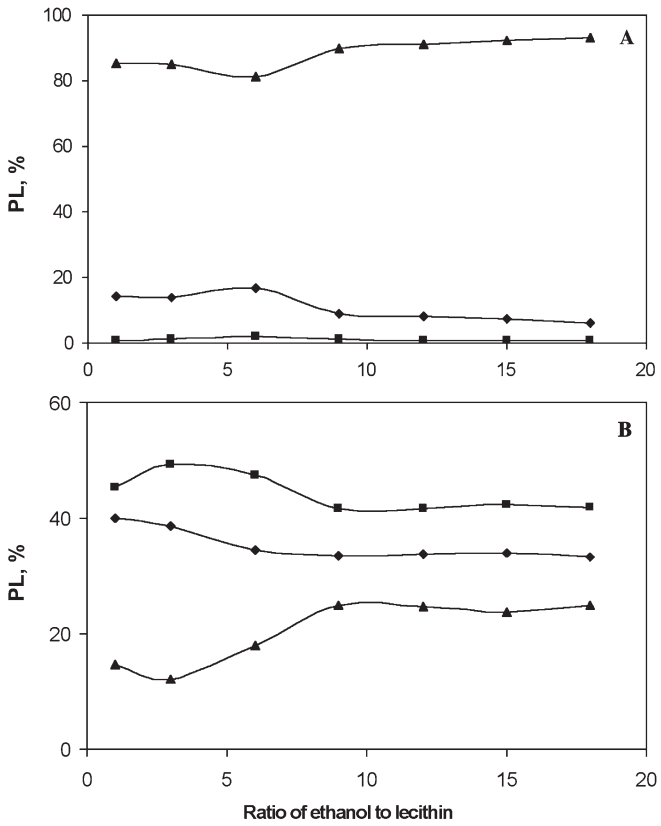

FIG. 8. PL proportions of the PC and PI fractions after fractionation at various ethanol/lecithin ratios. (A) PC fraction; (B) PI fraction. ◆ PE; ■ PI; ▲ PC. For abbreviation see Figure 1.

kept the same (3000 rpm) as with the 50-mL tubes. This resulted in a much higher *g* force ($8000 \times g$) than the intended $950 \times g$, as for the tubes. The higher centrifugal force may have caused the significant reduction in the yield of the PC fraction.

The recovery of PL in the two fractions added up to about 90% of AI matter in the initial material. The ethanol-to-lecithin ratio significantly affected the individual PL proportions for both PC and PI fractions (Fig. 8, Table 2), whereas those from same container size did not.

In Sosada's research (6), the PC fraction yield increased with an increase in ratio, and the percentage of PC decreased. However, there was no statistical analysis of those data, so we do not know whether the difference was significant. Our results suggest that there is no significant benefit to increasing the ratio of solvent to lecithin above 6.

Multiple fractionations. The yield of the PC fraction decreased significantly as the number of fractionations increased (Fig. 9, Table 3). The purity of the PC fraction increased significantly when fractionated twice but did not increase further with another fractionation. PL recovery in PC increased slightly with a second fractionation but decreased significantly with a third. Multiple fractionations did not affect the yields of the PI fraction. The purity of the PI fraction decreased slightly as the number of fractionations increased. PL recovery in the PI fractions decreased, but insignificantly, with multiple fractionations. In the PC fraction, the percentage of PC increased and PI decreased with multiple fractionation. In the PI fraction, the PI proportion increased and PC decreased with multiple fractionations (Fig. 10). Therefore, multiple fractionation is advantageous in improving the PL composition. Two fractionations are considered most effective in increasing the purity and recovery of the PC fraction, and in increasing the PC percentage in the PC fraction.

Effect of H–L temperature treatment on fractionation. Compared with fractionation at 40°C (using data from the single fractionation as the control), the yield of the PC fraction from the H–L temperature treatment was significantly lower and that of the PI fraction was significantly higher (Fig. 9). The purity of the PC fraction increased by 10% after the H–L temperature treatment compared with 40°C fractionation, and the purity of the PI fraction decreased slightly. The PL recovery for the PC fraction was significantly lower and that for the PI fraction was higher than the 40°C fractionation control. However, in the PC fraction, the percentage of PC increased and that of PI decreased to barely detectable levels (92.8 and 0.8%, respectively), so a much purer PC fraction was ob-

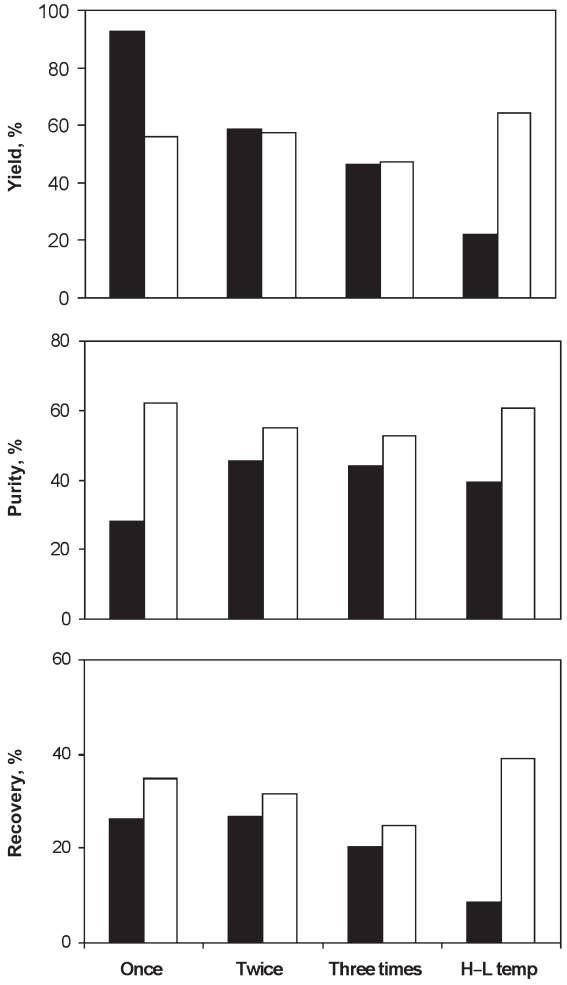

FIG. 9. Yield, purity, and recovery of lecithin fractions performed multiple times and with high–low (H–L) temperature treatment. ■ PC fraction; □ PI fraction.

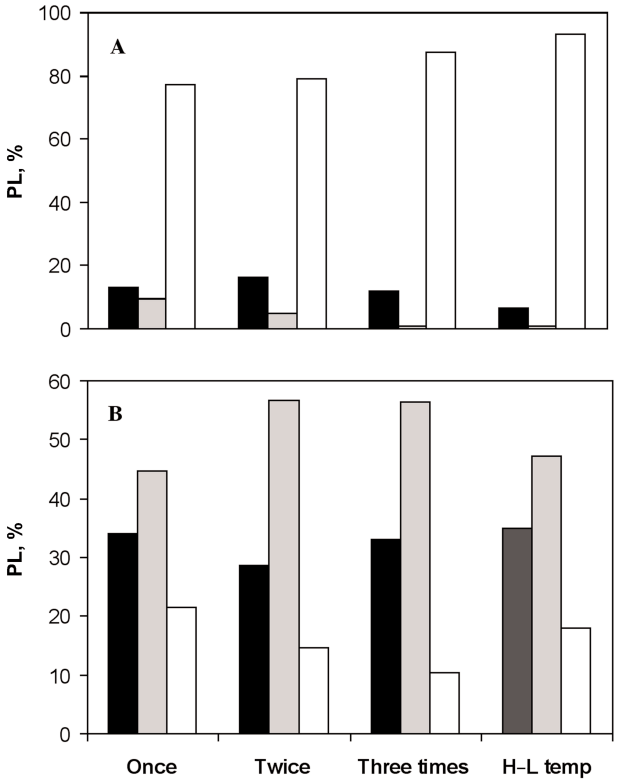

FIG. 10. PL proportions of the PC and PI fractions after fractionation multiple times and with H–L temperature treatment. (A) PC fraction; (B) PI fraction. PE, solid bars; PI, shaded bars; PC, open bars. For abbreviations see Figures 1 and 9.

tained (Fig. 10). The proportion of individual PL in the PI fraction changed little compared with fractionation at 40°C.

Therefore, H–L temperature fractionation greatly increased both the purity of the PC fraction and the percentage of PC in the PC fraction, although the yield and recovery of the PC fraction were low.

Large-scale fractionation. A flow chart of this fractionation is shown in Scheme 1. From 1182 g crude soybean lecithin, we obtained a PC fraction of 196 g after the first ethanol fraction had been combined with the ethanol extract from the PI fraction. The purity and relative proportions of PL of this fraction were similar to the result from the small-scale experiment. After a second ethanol fractionation, the purity of the PC fraction and the PC percentage in the PC fraction were increased compared with those from the first fractionation. The PL purity increased more (from 35.5 to 43.4%) than did the PC percentage (from 82.6 to 85.0%), a result very similar to that from the small-scale experiment. The purity of the PC fraction from the H–L temperature treatment was slightly higher than that from the first fractionation but lower than that from the second ethanol fractionation. This result is similar to

TABLE 3
***P*- and $LSD_{0.05}$ Values for Yield, Purity, Recovery, and PL Composition of PC and PI Fractions as Affected by Multiple Fraction and High–Low Temperature Treatment**

PL fractionation

PC-Frac.	Yield	Purity	Recovery	PI-Frac.	Yield	Purity	Recovery
P-value	0.000	0.005	0.000	*P*-value	0.134	0.041	0.139
$LSD_{0.05}$	10.284	7.488	3.181	$LSD_{0.05}$	13.377	6.540	11.546

PL composition (%)

PC-Frac.	Yield	Purity	Recovery	PI-Frac.	Yield	Purity	Recovery
P-value	0.017	0.000	0.004	*P*-value	0.142	0.008	0.002
$LSD_{0.05}$	4.691	2.073	6.381	$LSD_{0.05}$	6.500	6.007	2.912

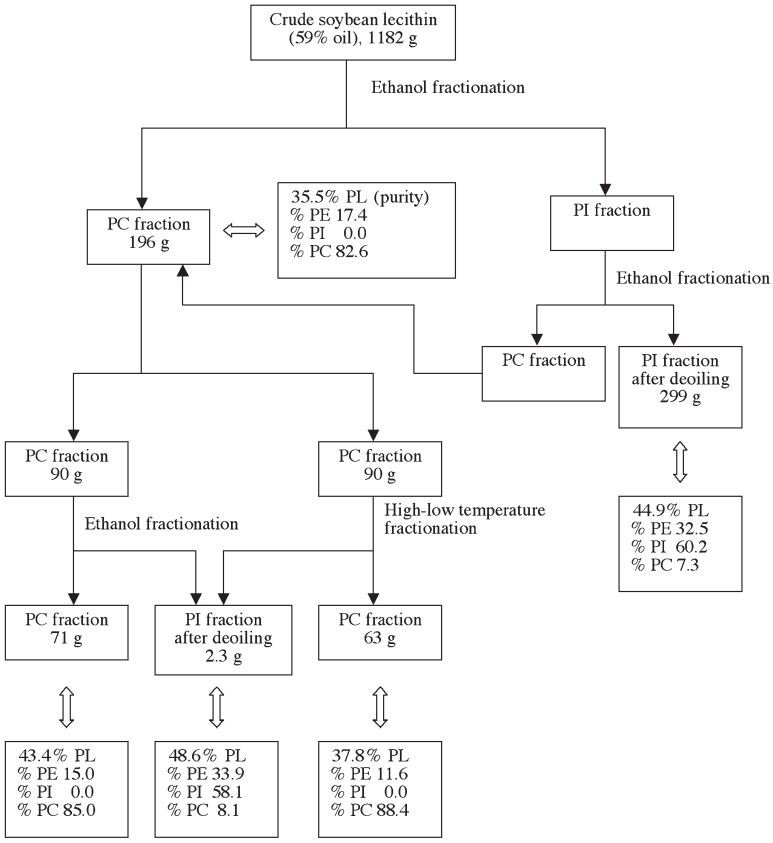

FIG. 11. Schematic of large-scale fractionation of crude lecithin with 95% ethanol at a ratio of ethanol to lecithin of 5:1 at room temperature. For abbreviation see Figure 1.

that of the analytical scale. The PC percentage in the PC fraction from the H–L temperature treatment was higher than that from the second fractionation, as expected. Both small- and large-scale fractionations have shown that the H–L temperature treatment can be used to increase the PC percentage in the PC fraction, but the recovery is low. On the other hand, multiple ethanol extractions of the PC fraction can result in a high yield and improved PC percentage.

A commercially prepared ethanol-soluble PC fraction from the deoiled lecithin contained 40–60% PC, and the ethanol-insoluble PI fraction contained 40–60% PI (11). In our fractionation of oil-containing lecithin, the ethanol-soluble PC fraction (twice extracted) contained 36.9% PC, 6.5% PE, and undetectable quantities of PI; the ethanol-insoluble PI fraction (after deoiling) contained 27.1% PI, 14.6% PE, and 3.3% PC.

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