Egg-Yolk Lipid Fractionation and Lecithin Characterization

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ABSTRACT: Egg-yolk lecithin has phospholipid (PL) classes and a FA composition that differ from soybean lecithin and may have unique functional properties. The purposes of this research were to develop an effective method for extracting a sufficient amount of lecithin from fresh egg yolks and to evaluate its functional properties. Ethanol was used to dehydrate and partially extract the PL, after which hexane was used to extract the total lipids. A phase separation of the combined extracts resulted in neutral and polar lipid fractions. An acetone precipitation of PL from the final polar lipid fraction was necessary to remove the residual neutral lipids, especially cholesterol. The purity of PL in the lecithin product was 95%. Surface tension reduction, emulsion stability, and oxidative stability studies were conducted to characterize the functional properties of egg-yolk lecithin. Egg-yolk lecithin and soy lecithin had similar surface activities, as evaluated by the surface tension reduction in an aqueous system and the critical micelle concentration. Soybean lecithin created a more stable emulsion than egg-yolk lecithin. However, egg-yolk lecithin was more oxidatively stable than soybean lecithin.

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KEY WORDS: Egg-yolk lecithin, emulsion stability, functionality, phospholipids, oxidative stability, surface tension.

Egg yolks are a good source of phospholipids (PL). PL represent approximately 10% of the wet weight of the egg yolk (1), equivalent to about 22% of the total egg yolk solids. The main components of egg-yolk lecithin are PC (80.5%) and PE (11.7%). Egg-yolk lecithin also contains lysophosphatidylcholine, sphingomyelin, and neutral lipids in minor quantities. The extraction of the total lipids or the PL from yolks is desirable because of the unique properties and valuable applications of these products (2). Yolk lecithin is used in the pharmaceutical and cosmetic industries as an emulsifier, and it is not as commonly used in foods as soy lecithin, because of its commercial availability and unfamiliar functional properties. It was recently reported that dietary egg-yolk PC can significantly lower cholesterol absorption in rats compared with soybean PC (3). In addition, egg-yolk lecithin contains relatively more saturated FA than does soybean lecithin, and it may have better oxidative stability than soybean lecithin. Therefore, yolk lecithin may have certain unique applications in foods.

According to the limited information available in the literature, egg-yolk lecithin is primarily extracted with solvents such as diethyl ether, hexane, chloroform, and ethanol. However, some of these solvents are considered undesirable because of environmental and health concerns (2,4). Earlier research focused mainly on total lipid extraction and cholesterol removal. Sequential extractions with various solvents or a multisolvent system were investigated (5,6). Other PL fractionation techniques, such as ethanol extraction and then low-temperature crystallization to remove the solidified neutral oil (7) and ultrafiltration to isolate egg PL (8), have been investigated. Hruschka *et al*. (9) described a procedure using low and high concentrations of aqueous ethanol in several steps to extract yolk PL, and they achieved 47% PL purity.

As discussed in a companion paper, the most common scheme for PL fractionation uses dried yolks and employs an initial deoiling step. The PL are then extracted from the deoiled material with ethanol. In our previous research (reported in the companion paper in this issue), we investigated PL extraction from heated and unheated and from deoiled and undeoiled egg yolks. The ethanol-extracted PL fraction had a low PL purity, and the recovery of total PL in this extract was also relatively low.

A simple and economical method to produce yolk oil and lecithin directly from fresh yolks and without using a hazardous solvent is still needed. The objectives of this research were to develop a method for the fractionation of egg-yolk lipids and to study the functional properties of egg-yolk lecithin.

MATERIALS AND METHODS

Egg yolk preparation and chemicals. Fresh farm eggs distributed by Boomsma's Inc. (Alden, IA) were purchased from a local grocery store. Eggshells were carefully broken, the yolks were separated from the egg whites, and the egg yolks were stored in a refrigerator (5°C) until use. The moisture content of the combined egg yolks was determined in an oven at 100°C for 4 h. The solvents and chemicals used in the PL extraction, PL quantification, and cholesterol determination were aqueous ethanol (87, 90, 95, and 100%), hexane, acetone, chloroform, methanol, diethyl ether, ammonium hydroxide, and 1 N potassium hydroxide (in 95% aqueous ethanol).

Analytical-scale lipid extraction and fractionation by solvent partition. The multiple-step procedure for extracting lipids from fresh egg yolks is outlined in Figure 1. For the initial extraction, 100 mL of ethanol (95%) was added to 30 g of fresh

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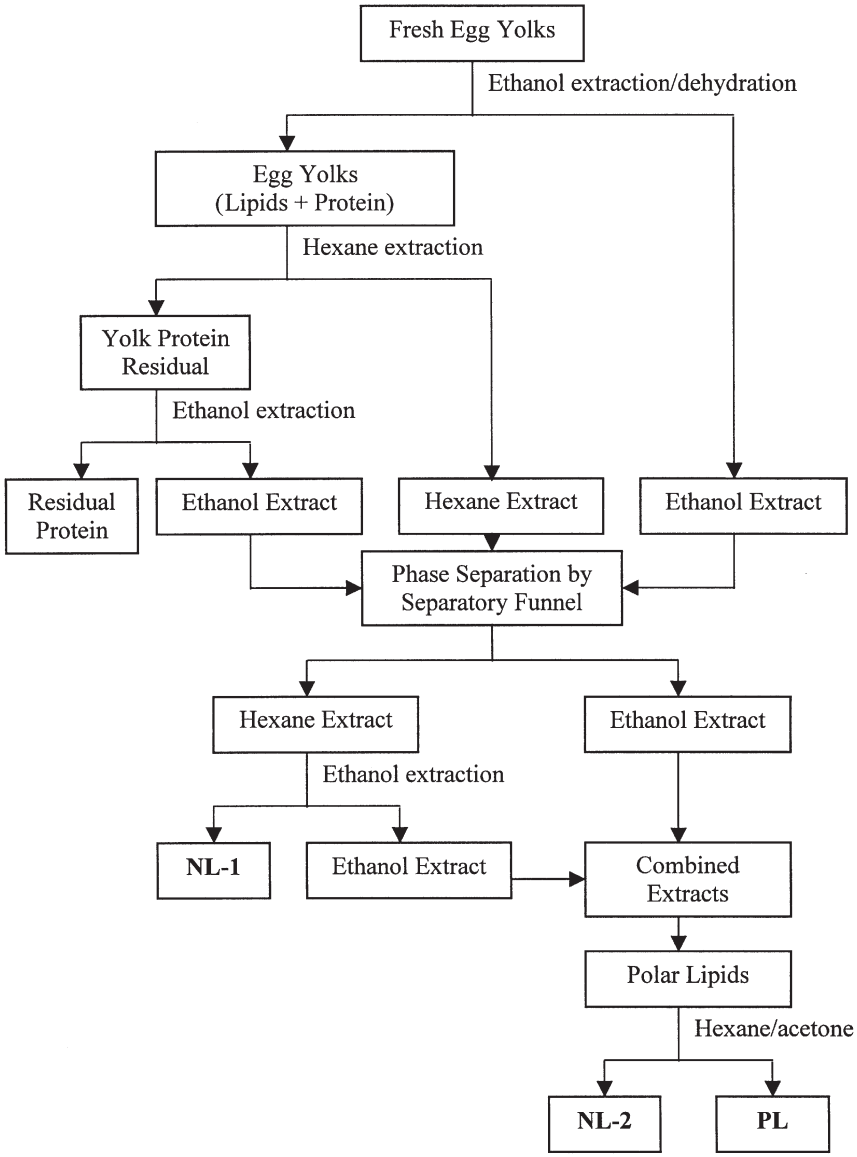

FIG. 1. Flow chart of lipid extraction from fresh egg yolks. NL-1, main neutral lipid fraction; NL-2, high-cholesterol neutral lipid fraction; PL, phospholipids.

egg yolks in a 200-mL centrifuge bottle and stirred until the egg yolks were completely dispersed. The mixture was then centrifuged at 1900 × *g* for 5 min, and the supernatant containing water, some polar lipids, and some neutral lipids was transferred to a separatory funnel. Lipids from the precipitate were extracted twice with 50 mL of hexane. The hexane extracts were transferred to the same separatory funnel. The protein precipitate was extracted two times with 50 mL of ethanol (95%) to remove any residual polar lipids. These ethanol extracts were combined with the previous ethanol and hexane extracts in the separatory funnel. The separatory funnel was then thoroughly but gently mixed and left to equilibrate for 1 h for phase separation. The ethanol phase was removed, and the hexane phase was mixed with an additional 50 mL of ethanol (90%) and left for phase separation. The hexane was removed from the hexane extract by rotary evaporation, leaving behind the neutral lipids (NL-1), which were determined gravimetrically. The ethanol phase was combined with the previous ethanol extract, and the solvent was evaporated. The remaining lipid material was dissolved in 35 mL of hexane and transferred to a 200-mL centrifuge bottle where 150 mL of chilled acetone (4°C) was added and carefully stirred to precipitate the PL. The centrifuge bottle was then placed in an ice-water bath for 15 min and centrifuged at 1500 × *g*. The supernatant was removed and the solvent was evaporated by rotary evaporation. This fraction should contain any neutral oil and cholesterol that was readily extracted into the ethanol, and was named NL-2. The precipitate was the purified PL.

Quantification of PL by HPLC. Quantification of PL in the samples was done using a Beckman-Coulter (Fullerton, CA)

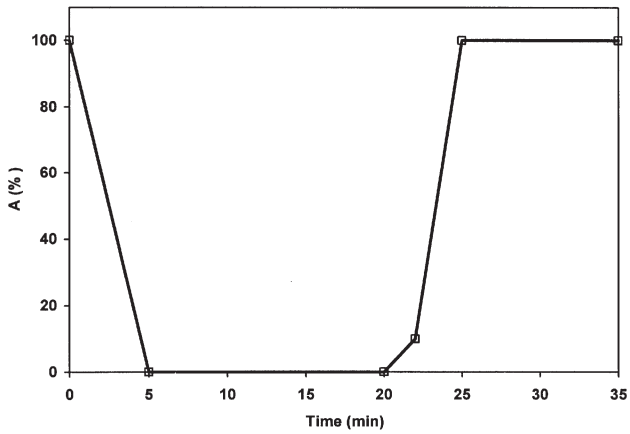

FIG. 2. Gradient program of the mobile phase for HPLC quantification of PL. Phase A: chloroform/methanol/ammonium hydroxide (80:19:1, by vol); phase B: chloroform/methanol/water/ammonium hydroxide (50:48:1:1, by vol). For abbreviation see Figure 1.

HPLC system equipped with a model 508 autosampler, model 126 solvent delivery module, a diol normal-phase silica column (250 mm × 4.6 mm i.d., with integral guard column; Advanced Separations Technologies, Whippany, NJ), and a model 2000 ELSD (Alltech, Deerfield, IL). A gradient program with two mobile phases at a flow rate of 1 mL/min was used: Phase A was chloroform/methanol/ammonium hydroxide (80:19:1, by vol), and phase B was chloroform/methanol/water/ammonium hydroxide (50:48:1:1, by vol). The gradient program used is shown in Figure 2. Nitrogen, at a flow rate of 1.7 L/min, was used to evaporate the solvent in the draft tube at 60°C. Standard curves were established for the two major PL classes in egg-yolk lecithin, i.e., PE and PC.

Characterization of PL by TLC and GC. The egg-yolk PL sample was dissolved in chloroform/methanol (2:1, vol/vol), and an aliquot of the solution was streaked on a 20 × 20 cm, 500-μm thick Adsorbsil-plus 1 preparative silica plate (Alltech); the plate was then developed with chloroform/methanol/acetic acid/water (100:45:5:2, by vol). Bands were visualized with 2′,7′-dichlorofluorescein spray (0.1% in methanol) and viewed under UV light. The PC and PE bands were collected and transferred to glass vials in which the internal standard methyl heptadecanate (17:0) in hexane had previously been transferred, and the solvent was evaporated under nitrogen. Approximately 1.5 mL of 0.5 M sodium methoxide was added in an amount sufficient to cover all the silica in the vials. The transesterification reaction was allowed to continue for 40 min at 50°C. Distilled water was added to stop the reaction, and 1.0 mL of hexane was used to extract the FAME. About 1.0 μL of each extract was injected into a Hewlett-Packard (HP) 5890 Series II GC system with an SP-2330 capillary column (15 m × 0.25 mm × 0.2-μm film thickness; Supelco, Bellefonte, PA) for determination of the FA composition. The GC oven temperature was 190°C, and the injector and detector temperatures were 230°C.

Cholesterol quantification. Cholesterol was determined as described in the companion paper in this issue.

The parameters used to evaluate the efficiency of the extraction were yield, purity of the PL fraction, cholesterol content, and PL distribution in the NL-1, NL-2, and PL fractions. The yield was the amount of fraction obtained divided by the total starting dry weight. The purity was the weight percentage of total PL in the fraction as quantified by HPLC. The cholesterol content was expressed as the weight percentage of cholesterol in the fraction. The PL distribution was the amount of PL in the fraction divided by the total PL in the starting material.

Large-scale lecithin fractionation for functionality evaluation. A large quantity of lecithin was needed for functionality studies; thus, changes in the analytical fractionation procedure were necessary to ensure feasibility of the scaled-up process. Three modifications were made: First, the ratio of egg yolk to total solvent was reduced from 1:16 (total solvent) in the analytical-scale process to 1:9.5 (proportionally reduced) in the large-scale process. Second, the ethanol concentration was changed. An early study of polar lipid isolation (10) demonstrated a nearly complete recovery of polar lipids when 87% aqueous ethanol was used in a petroleum ether–alcohol solvent partition of neutral and polar lipids. Therefore, we used 100 and 87% ethanol instead of 95 and 90% at the two steps of lipid extraction. The moisture contained in the egg yolks provided the correct amount of water to give the ethanol a final concentration of 87%. The third modification was the number of extractions in each stage. The fact that the amount of solvent was reduced required the use of multiple (three) extractions in each step to ensure extraction efficiency. Three replicates were conducted in the production of lecithin. The parameters measured and calculated were the same as those for the analytical procedure.

Surface tension reduction of egg-yolk lecithin. The PL fractions from the large-scale lecithin extraction were dispersed in distilled water at a high concentration and then diluted in series to obtain lower concentrations (25–0.1 mg/mL). Soy lecithin (Fisher Scientific, Pittsburg, PA; 99% purity) was used for comparison. Surface tension of the aqueous dispersion was determined at each concentration with a FACE Automatic Surface Tensiometer (Model CBVP-Z; Tantec Inc., Schaumburg, IL). The surface tension was plotted against the logarithm of the concentration to determine the critical micelle concentration (CMC) of the PL.

Emulsion stability. Egg-yolk PL fractions and soybean lecithin were used as emulsifiers to make oil-in-water (o/w) emulsions. The proportion of oil to water was 2:8 (vol/vol), whereas the emulsifier concentrations used were 5 and 10% of the oil weight. The emulsifier was dispersed in distilled water to facilitate its incorporation into the o/w emulsion. A stock solution (100 mg/mL) of each emulsifier was prepared. Water, emulsifier, and oil were blended with a Hamilton Beach® blender (Model 51101) for 3 min. The emulsions were then transferred to 50-mL graduated cylinders, and the volume of separated discontinuous phase (oil) was recorded periodically.

Oxidative stability of egg-yolk lecithin. A 20-mg/mL lecithin solution in hexane was prepared for each egg-yolk PL fraction as well as for the soy lecithin. To ensure an even and maximal exposure of the lecithin samples to oxygen, a modification was

made to the process used by Wu and Wang (11). Instead of using mineral oil as the dispersing vehicle, glass beads (5-mm diameter) were used. A fixed number of glass beads (30 pieces) were placed in 9 labeled 20-mL vials. Then 0.3 mL (6 mg) of the stock lecithin solution was dispensed into each vial. The vials were shaken to allow the glass beads to be coated with the lecithin solution. The hexane was then removed by blowing nitrogen into the vial. The vials were then placed in a conventional oven at 55°C for 16 d, with one vial removed every 48 h. At the end of this period, the peroxides were quantified by a ferric thiocyanate method (12). Hydroperoxides in the samples were quantified indirectly by measuring the absorbance at 515 nm. Oxidized soybean oil was used to establish a standard curve as follows: First, the actual PV of the oxidized soybean oil was determined by the standard iodometric method, AOCS Cd 8-53 (13), then a stock solution of the oxidized soybean oil was prepared by dissolving it in a known amount in ethanol/benzene (80:20). Different amounts of this solution were placed in a series of 10-mL volumetric flasks, and the color was developed by adding the ammonium thiocyanate (50 μL, 3.75 M) and ferrous chloride (100 μL, 0.014 M) reagents. A linear standard curve was obtained by plotting the absorbance vs. the microequivalent (μequiv) of peroxide present.

RESULTS AND DISCUSSION

Lipid fractionation. The moisture content of the fresh egg yolks was 49.4%. Yield percentages for each of the main fractions from the analytical- and large-scale extractions are shown in Table 1. Reproducibility of the extraction was generally good, as indicated by the relatively small SD. However, the yield differences between the two scales were statistically significant, although not considerable, possibly because of the smaller volume of solvent used in the large-scale process. The NL-1 yield of the large-scale extraction (21.7 ± 0.1%) was significantly higher than that of the analytical-scale process (19.9 ± 0.7%). This could be because of the insufficient separation of polar lipids from the neutral lipids. The yield of NL-2 fractions by the final acetone precipitation of PL and the removal of neutral lipids also indicated lower efficiency of the large-scale compared with the analytical-scale process. However, significant amounts of solvents were saved by this lower solvent-to-egg yolk ratio.

On a dry weight basis, our yolk material contained 17.7% protein, 11.0% PL (based on 95.4% purity), and 22.5% neutral oil. These values are similar to the values reported by the American Egg Board (www.aeb.org), except that our total lipid content (66% of the dry weight) was higher than the reported 55.8%. We believe this difference is possibly because of variations in the breeds and feed of the hens.

PL quantification by HPLC. The use of a new HPLC column specifically for PL characterization [compared with the silica column used by Wu and Wang (11)] reduced the equilibration time between analyses, and sharper peaks also were obtained. PE and PC standard curves were established by injecting different concentrations of PE and PC mixtures (15% PE and 85% PC) into the HPLC. An individual calibration equation was obtained for each standard by plotting the peak area vs. the amount of sample injected. The standard calibration equation for PE was area = 125,160 × $(\text{amount})^{1.61}$ (R^2 = 0.999), and that for PC was area = 12,146 × $(\text{amount})^{2.05}$ (R^2 = 0.995). Vegetable oil and cholesterol standards were also injected to identify each peak on the chromatogram (Fig. 3). The first two peaks were identified as neutral oil and cholesterol.

PL purity for the analytical-scale samples was slightly (but statistically significantly) higher (95.9 ± 0.3%) than the purity of the large-scale samples (94.9 ± 0.1%) (Table 2). Overall, this new fractionation method for egg-yolk lecithin was much more efficient and effective than the method used in our previous research (see the companion paper in this issue) and those used by others (2,4,7,9). For example, the procedure patented by Hruschka (9), in which low-concentration aqueous ethanol was used to fractionate the oil and polar lipids, resulted in 47% purity of the PL fraction.

The content and distribution of PL in each of the main fractions from the analytical- and large-scale extractions are shown in Table 3. Statistically less PL was lost from the NL-2 fraction during the large-scale extraction than during the analytical-scale extraction. It seemed that the reduction of acetone used for precipitation also reduced the loss of PL. The distributions of PL in the neutral and PL fractions on both scales were about 4 and 96%, respectively. Therefore, the total PL recovery achieved by this fractionation procedure was exceptional.

Identification of plasmalogen by TLC and GC. Our HPLC analysis with the new column showed that the non-PL materials were mainly cholesterol and neutral oil, and they were present at about 5%. However, our earlier HPLC analysis using a silica column showed a much lower PL content because of a wide peak that gave inaccurate peak integration and poor reproducibility.

TABLE 1
Yield of the Main Fractions from Analytical- and Large-Scale Extractions of Phospholipids (PL) from Fresh Egg Yolks[a]

Fractionation		NL-1	NL-2	PL	Protein
Analytical scale (n = 2, 30 g fresh, 15.3 g dry wt)	g, dry wt	6.0 ± 0.2	0.6 ± 0.6	3.6 ± 0.0	5.4 ± 0.1
	%	19.9 ± 0.7[b]	2.0 ± 0.1[a]	11.9 ± 0.0[a]	18.1 ± 0.3[a]
Large scale (n = 3, 180 g fresh, 91.8 g dry wt)	g, dry wt	39.0 ± 0.2	2.6 ± 0.1	20.0 ± 0.4	31.1 ± 0.3
	%	21.7 ± 0.1[a]	1.4 ± 0.1[b]	11.2 ± 0.2[b]	17.3 ± 0.1[b]
$LSD_{0.05}$		1.2	0.2	0.5	0.6

[a]Yield = 100 × quantity of PL fraction/quantity of initial yolk material. NL-1, main neutral lipid fraction; NL-2, high-cholesterol neutral lipid fraction. The same lowercase letter in the same column indicates there is no significant difference between the two scales of processing. $LSD_{0.05}$, least significant difference at P = 0.05. n, number of replicates in each scale.

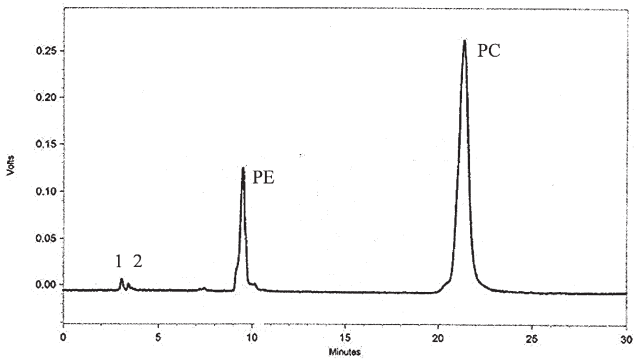

FIG. 3. HPLC chromatogram of an egg-yolk PL sample. Peaks 1 and 2 were identified as neutral oil and cholesterol, respectively. For abbreviation see Figure 1.

The possible presence of other compounds in the PL fraction was then tested by using TLC, and no other major unknown compounds were identified. However, when the TLC plate was developed three times, the PE band split into two. We were able to identify one of the bands as PE plasmalogen by a simple acid hydrolysis test (14). Plasmalogen is a vinyl ether PL (Fig. 4). The *sn*-1 position of glycerol is linked to the carbon chain by a vinyl ether bond instead of an ester bond. After acid hydrolysis, this vinyl ether bond is broken, forming an aldehyde. The acid hydrolysis products of the two separated PE bands were streaked on a 2 × 4 cm, 250-µm thickness silica gel plate (Sigma-Aldrich, St. Louis MO). The plate was developed in hexane/diethyl ether (90:10, vol/vol) and then visualized with a potassium permanganate stain, which is a mixture of potassium permanganate (1%) and potassium carbonate (6.7%) dispersed in 5% aqueous sodium hydroxide in water. Standard aldehydes (with C_8 and C_{14} chain lengths) were used as references on the TLC plate. These results showed that two aldehydes having chain lengths of approximately C_{16} and C_{18} were present in one of the two bands. This PE plasmalogen band (which moved higher on the TLC plate than the PE band) was also transesterified, and the FAME were quantified. The quantity of plasmalogen was then calculated, assuming the length of carbon chain linked by the ether bond was C_{16}. The plasmalogen content was estimated at about 3.6% of the total yolk PL. Rhodes and Lea (15) reported 0.9% plasmalogen in the yolk. More work is needed to fully characterize and quantify this unique lipid in the egg yolk. Recent studies have shown that plasmalogen prevents cholesterol oxidation in membranes (16,17).

TABLE 2
PL Purity and Class Composition of Fractionated Egg-Yolk Lecithin[a]

Fractionation	Purity (%)	PE (%)	PC (%)
Analytical scale (n = 2)	95.9 ± 0.3[a]	19.1 ± 1.1[a]	80.9 ± 1.1[a]
Large scale (n = 3)	94.9 ± 0.1[b]	17.9 ± 1.1[a]	82.1 ± 1.1[a]
$LSD_{0.05}$	0.6	3.2	3.2

[a]Purity = 100 × total PL as quantified by HPLC/quantity of PL fraction. The same lowercase letter in the same column indicates there is no significant difference between the two scales of processing. *n*, number of replicates in each scale. For abbreviations see Table 1.

The FA composition of PE and PC of egg-yolk lecithin was determined by GC, as shown in Table 4. More saturated FA were found in egg-yolk lecithin than in soybean lecithin. The oxidative stability of a lipid can be predicted by calculating its oxidizability (18) using the FA composition (19). The oxidizability of egg-yolk and soybean lecithin was calculated as 0.76 and 0.50, respectively.

Cholesterol content in fractionated lipids. The cholesterol content in the PL fraction from the analytical-scale extraction (0.5%) was lower than those in the NL-1 and NL-2 fractions (Table 5), and in particular, it was lower than that in the corresponding PL fraction from the large-scale extraction (1.9%). In previous research (see the companion paper in this issue), we found that cholesterol was very extractable with ethanol. It was evident that the last step in the procedure, i.e., acetone precipitation of the PL, was absolutely necessary to remove the cholesterol from the PL fraction. It was obvious that the efficiency of cholesterol removal in the large-scale process was not as high as in the analytical-scale process. This might be because of the reduction in solvent use in the purification step, especially the last acetone precipitation step. Multiple acetone precipitations of the PL may be used in the future to increase neutral lipid removal. In the analytical-scale separation, 35.2% cholesterol was partitioned into the neutral oil fraction (NL-1), and 52.2% was removed during the final PL precipitation into

TABLE 3
PL Content and Distribution in the Main Fractions[a]

	Scale of fractionation	NL-1	NL-2	PL
PL content (%)	Analytical (n = 2)	1.8 ± 0.4[a]	7.9 ± 0.3[a]	95.9 ± 0.3[a]
	Large (n = 3)	1.7 ± 0.6[a]	4.3 ± 0.8[b]	94.9 ± 0.1[b]
$LSD_{0.05}$		1.5	1.9	0.5
PL distribution (%)	Analytical (n = 2)	2.9 ± 0.7[a]	1.3 ± 0.1[a]	95.9 ± 0.6[a]
	Large (n = 3)	3.1 ± 1.1[a]	0.5 ± 0.1[b]	96.4 ± 1.2[a]
$LSD_{0.05}$		2.9	0.2	3.0

[a]The same lowercase letter in the same column indicates there is no significant difference between the two scales of processing. *n*, number of replicates in each scale. For abbreviations see Table 1.

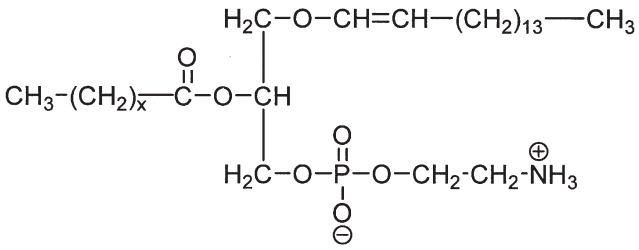

FIG. 4. PE plasmalogen identified in egg-yolk lecithin.

the NL-2 fraction. About 12.6% of the total cholesterol was retained in the lecithin product. In the large-scale process, a similar percentage of cholesterol (34.3%) was in the NL-1 product; however, the PL precipitation step removed much less cholesterol (28.3%) than in the analytical-scale process, and a much higher proportion of cholesterol (37.4%) went into the final lecithin product. The total cholesterol content in the initial material for the large-scale process was higher than that for the analytical-scale process, possibly because of the different batches of egg used.

Reduction of surface tension by egg-yolk lecithin in an aqueous system. Measuring the surface tension reduction and CMC are common ways to characterize emulsifiers or surfactants. The surface tension reduction by egg-yolk and soybean lecithins is shown in Figure 5. The PL fractions and soybean lecithin showed similar patterns. The surface tension was reduced as the concentration of PL increased, and it was lowered to a minimal value and then became independent of the concentration. The curve from each sample was divided into two parts: One part was the near-linear reduction of surface tension with concentration, and the other part was the last few points at which the surface tension was relatively unchanged. A linear trend for each portion was determined, and the concentration at which these two lines intercepted was determined as the CMC. The mean of the CMC values for the egg-yolk PL fractions was 15.3 mg/mL, and that for the soybean lecithin was 15.8 mg/mL. This CMC value for soybean lecithin was slightly different from the one reported by Wu and Wang (11), where the CMC for the same brand of soybean lecithin was 13.6 mg/mL. The surface tension was reduced to a mean of 35.1 mN/m for the yolk PL fractions and 30.2 mN/m for the soybean lecithin. These results suggest that egg-yolk lecithin has a surface activity similar to soybean lecithin. Some of the data point fluctuations in the graph could be due to the minor defects in and the cleanliness of the platinum plate used with the surface tensiometer.

Emulsion stability. The emulsions created with the egg-yolk PL fractions broke faster than the emulsion created with the soybean lecithin (Fig. 6). The three o/w emulsions containing 5% egg-yolk lecithin samples showed a similar trend. They had about 13% oil separation at 240 min, whereas the soybean lecithin showed only 8% oil separation. A peculiarity was observed in the o/w emulsions containing 10% emulsifier. It is typically believed that the more emulsifier we use, the more stable the emulsion will be. However, at a 10% yolk lecithin concentration, the percentage of oil separated was higher (about 21% at 240 min) than at a 5% concentration (13%). The emulsion containing 10% soybean lecithin had lower oil separation (6%) than that at the 5% concentration (8%). We believe that this may be due to differences in the PL classes and FA compositions of these two lecithin products. Soybean lecithin

TABLE 4
FA Composition (%) of the Main PL Classes of Egg-Yolk and Soybean Lecithin

Source	PL	16:0	18:0	18:1	18:2	18:3	20:4
Soybean[a]	PE	16.0	8.3	6.8	57.3	11.7	—
	PI	22.2	19.3	6.1	43.4	9.3	—
	PC	11.2	11.9	8.6	58.6	9.9	—
Egg yolk	PE	25.0	26.8	20.7	16.3	—	11.2
	PC	35.0	13.4	30.4	18.0	—	3.2

[a]Soybean data from Hammond *et al.* (19). For abbreviation see Table 1.

TABLE 5
Cholesterol Distribution Among the Three Major Fractions[a]

Fractionation		NL-1	NL-2	PL
Analytical scale (n = 2)	Cholesterol quantity (mg from 15.3 g dry yolk)	52.1 ± 1.4	77.2 ± 27.8	18.6 ± 0.2
	Concentration in product (%)	0.9 ± 0.0[a]	13.0 ± 4.3 [a]	0.5 ± 0.0[a]
Large scale (n = 3)	Cholesterol quantity (mg from 91.8 g dry yolk)	347.8 ± 28.3	286.8 ± 27.8	379.9 ± 31.7
	Concentration in product (%)	0.9 ± 0.1[a]	10.8 ± 1.0[a]	1.9 ± 0.1[b]
$LSD_{0.05}$		0.2	13.4	0.3

[a]The same lowercase letter in the same column indicates there is no significant difference between the two scales of processing. For abbreviations see Table 1.

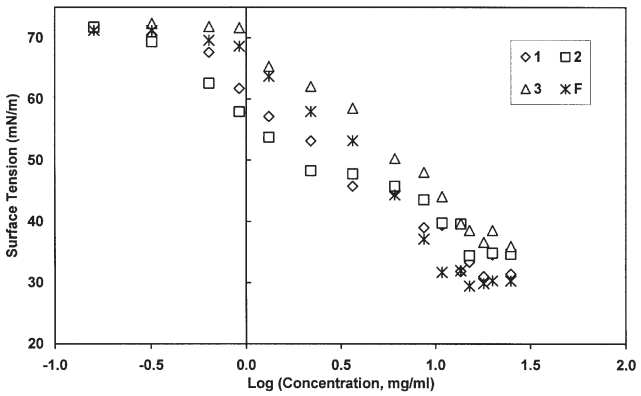

FIG. 5. Surface tension reduction of egg-yolk and soy lecithins. The curves labeled 1, 2, 3, and F are three egg-yolk lecithin products from three replicates of fractionation and a Fisher brand soybean lecithin.

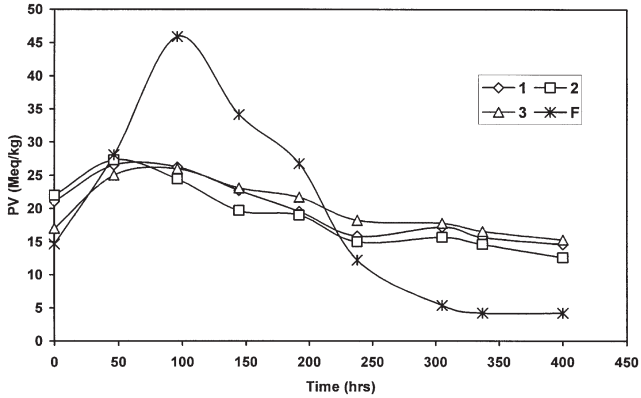

FIG. 7. PV of lecithins determined by the ferric thiocyanate method (12). The curves labeled 1, 2, 3, and F are three egg-yolk lecithin products from three replicates of fractionation and a Fisher brand soybean lecithin.

contains PI (21%), whereas egg-yolk lecithin does not. However, egg-yolk lecithin is rich in PC, and it is expected to be a good o/w emulsifier. An optimal combination of various types of PL may be needed for an effective emulsifier. More research should be conducted to compare these two lecithins when they are used at concentrations lower than 5%. Soybean lecithin clearly performed much better than egg-yolk lecithin in creating a stable o/w emulsion at the concentration studied.

Oxidative stability of egg-yolk lecithin. To remove any tocopherols that might be present, soy lecithin was precipitated with acetone using the same method as that used at the last step of egg-yolk lecithin extraction. Figure 7 shows lipid hydroperoxide generation and degradation with time. The starting PV for all samples were relatively high (15–20 mequiv/kg), indicating that oxidation had occurred during the fractionation of egg lecithin or the storage of soy lecithin. Egg-yolk lecithins oxidized to a maximal PV value of 24 to 27 mequiv/kg at about 47 h, whereas the highest PV for soybean lecithin was 46 mequiv/kg at about 96 h. The PV declined because of peroxide decomposition and termination of the free radical chain reaction. A soybean oil was also oxidized and the peroxides quantified under identical conditions. It reached a maximal PV of about 400 mequiv/kg at 290 h before declining. Therefore, hydroperoxides of the PL seem to decompose much more readily than those of the neutral oil. The lower degree of oxidation of egg-yolk lecithin compared with soybean lecithin may be explained by its high content of saturated FA. The relatively high content of unsaturated FA in soybean lecithin (Table 4) caused it to form more hydroperoxides and will probably cause a stronger rancid flavor when improperly used in foods.

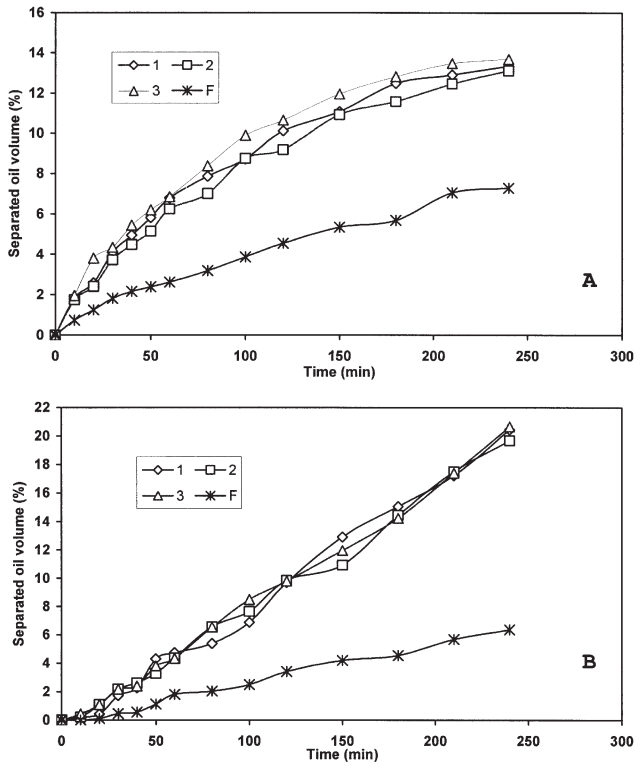

FIG. 6. Stability of emulsions (oil-in-water) with 5 (A) and 10% (B) lecithin relative to oil. The curves labeled 1, 2, 3, and F are three egg-yolk lecithin products from three replicates of fractionation and a Fisher brand soybean lecithin.

The method established in this research for studying the oxidative stability of lecithin is much more effective than the method used previously by Wu and Wang (11), in which lecithin was dispersed in an inert mineral oil. No increase in the PV was obtained in that study, possibly because of the limited exposure of PL to oxygen and because lecithin was less oxidizable using that method compared with soybean oil.

The oxidative stability of lecithin has not been studied as much as that of other neutral lipids. A few studies are available on analytical procedures for determining the hydroperoxides of PL (20); however, the relative stability of PL from different sources and with different FA compositions has not been reported.

The extraction procedures established in this research may be conveniently adopted by the industry to produce a high-purity egg-yolk lecithin. The last purification step of the PL fraction by

hexane and acetone is necessary to remove not only neutral oil but also cholesterol that is co-extracted with the ethanol. The quantitative method established to study the oxidative stability of lecithin is effective and easy to perform.

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