EGG-YOLK LECITHIN FRACTIONATION AND CHARACTERIZATION

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Abstract

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

Key words: Egg-yolk lecithin, emulsion stability, functionality, phospholipids, oxidative stability, surface tension.
Egg yolk is a good source of phospholipids (PLs) representing approximately 10% of the wet weight of egg yolk (1), which is equivalent to about 22% of the total egg yolk solid. The main components of egg-yolk lecithin are phosphatidylcholine (PC, 80.5%) and phosphatidylethanolamine (PE, 11.7%). Egg-yolk lecithin also contains lysophosphatidylcholine (LPC), sphingomyelin (SM), and neutral lipids in minor quantities. The extraction of the total lipids or the PLs from yolk is desirable because of the unique properties and valuable applications of these products (2). The oil is used in nutritional supplements, baby foods, and parenteral feeding because of its relatively high ω-3 fatty acid content. The PLs are used in pharmaceutical and cosmetic industries as an emulsifier. Yolk lecithin is not as commonly used in food as soy lecithin, because of the 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). Egg-yolk lecithin contains relatively more saturated fatty acids than soybean lecithin does and it may have better oxidative stability than soybean lecithin. Therefore, yolk lecithin may have certain unique applications in foods.

According to limited information 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 mainly focused on total lipid extraction and cholesterol removal. Sequential extractions with various solvents or multi-solvent 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 PLs (8) have been investigated. Hruschka et al. (9) described a procedure of using low and high concentrations of aqueous ethanol in several steps to extract yolk PLs and they achieved 47% PL purity.

The most common scheme of PL fractionation uses dried yolk and employs an initial deoiling step. The PLs are then extracted from the deoiled material with ethanol. Dried yolk was used as starting material because of a general belief that lipid and protein in fresh yolk are intimately associated and the lipids are not easily extractable with non-polar solvent (10). Yolk drying is energy intensive and if the proteins are heat-denatured, they are usually less functional when used in food systems.
A rapid and economical method to produce yolk oil and lecithin directly from fresh yolk and without using hazardous solvent is still needed. The objectives of this research were to develop a method for the fractionation of egg-yolk lecithin and to compare its functional properties with those of soy lecithin.

Material 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 and yolk was separated from the egg white, and the egg yolk was stored in a refrigerator (5°C) until use. Moisture content of the combined egg yolk was determined in an oven at 100°C for 4 hours. 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 lecithin extraction and fractionation by solvent partition.** The multiple-step procedure of lecithin extraction from fresh egg yolk is outlined in Figure 1. For the initial extraction, 100 mL ethanol (95%) was added to 30 g of fresh egg yolk in a 200-mL centrifuge bottle and stirred until the egg yolk was completely dispersed. Then, the mixture was centrifuged at 1900 x g for 5 minutes, and the supernatant containing water, some polar and neutral lipids was transferred to a separatory funnel. Neutral 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 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 one hour for phase separation. The ethanol phase was removed and the hexane phase was mixed with additional 50 mL ethanol (90%) and left for phase separation. Hexane was removed by rotary evaporation, leaving behind the neutral lipids (NL-1) which were determined gravimetrically. The ethanol phase was combined with the previous ethanol phase, and the solvent was evaporated. The remaining polar 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 PLs. Then, the centrifuge bottle was placed in an ice-water
bath for 15 minutes and centrifuged at 1500 x g. The supernatant was removed and solvent was 
evaporated by rotary evaporation. This fraction should contain any neutral oil and cholesterol 
that was readily extracted into ethanol and it was named NL-2. The precipitate was the purified 
PLs.

**Quantification of PLs by HPLC.** Quantification of PLs in samples was done using a 
Beckman-Coulter (Fullerton, CA) high performance liquid chromatography (HPLC) system 
equipped with auto sampler 508, solvent delivery module 126, diol normal-phase silica column 
(250 mm x 4.6 mm i.d., with integral guard column, Advanced Separations Technologies, 
Whippany, NJ), and an evaporative light scattering detector (ELSD 2000; Alltech, Deerfield, IL). 
A gradient program with two mobile phases at a flow rate of 1 mL/min was used: A was 
chloroform/methanol/ammonium hydroxide (80:19:1, v/v), and B was 
chloroform/methanol/water/ammonium hydroxide (50:48:1:1, v/v). The gradient program used is 
shown in Figure 2. Nitrogen with a flow rate of 1.7 L/min was used to evaporate solvent in draft 
tube at 60°C. Standard curves for the two major PL classes in egg-yolk lecithin, i.e., PE and PC, 
were established.

**Characterization of PLs by TLC and GC.** The egg-yolk PL sample was dissolved in 
chloroform:methanol (2:1, v:v), and an aliquot of the solution was streaked on a 20 x 20 cm, 
500-µm thick Adsorbsil-plus preparative silica plate (Alltech), and the plate was developed 
with chloroform/methanol/acetic acid/water (100:45:5:2, v:v:v:v). Bands were visualized with of 
2’,7’-dichlorofluorescein (0.1% in methanol) spray and viewed under UV light. PC and PE bands 
were collected and transferred to glass vials in which the internal standard methyl heptadecanate 
(17:0) in hexane was previously transferred and the solvent evaporated under nitrogen. 
Approximately 1.5 mL of 0.5 M sodium methoxide was added, an amount sufficient to cover all 
the silica in the vials. The transesterification reaction was allowed for 40 minutes at 50°C. 
Distilled water was added to stop the reaction, and 1.0 mL hexane was used to extract the fatty 
acid methyl esters (FAME). About 1.0 µL of each extract was injected into a Hewlett-Packard 
(HP) 5890 Series II gas chromatography (GC) system with capillary column (SP-2330, 15 m x 
0.25 mm x 0.2-µm film thickness from Supelco, Bellefonte, PA) for fatty acid composition 
determination. GC oven temperature was 190°C, and injector and detector temperatures were 
230°C.
**Cholesterol quantification.** Cholesterol determination begun with the accurate weighing of about 100 mg of each lipid sample into a glass vial. Two mL of 1 N potassium hydroxide (in 95% ethanol) was added to each sample, which was then placed in a boiling water bath for two hours. After the saponification reaction, 5 mL of distilled water was added to the mixture, and three 5 mL portions of diethyl ether were used to the extract unsaponifiable materials. The combined ether extract was washed with distilled water, and the solvent was evaporated under nitrogen. The dried unsaponifiable matter was dissolved in 1 mL hexane containing a known amount of cholestane as internal standard. Duplicate samples were injected in a Hewlett Packard 5890 Series II GC system with fused silica capillary column (SAC-5, 30 m x 0.25 mm x 0.25-µm film thickness from Supelco, Bellefonte, PA). GC oven temperature was 285°C, and injector and detector temperatures were 300°C.

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

**Large-scale lecithin fractionation for functionality evaluation.** A large quantity of lecithin was needed for functionality studies. Changes in the analytical fractionation procedure were necessary to ensure feasibility of the scaled-up processing. There were three modifications. First, the ratio of egg yolk and total solvent was reduced from 1:16 (total solvent) in the analytical scale to 1:9.5 (proportionally reduced) in the large scale. Second, the ethanol concentration was changed. An early research of polar lipid isolation (11) demonstrated a nearly complete recovery of polar lipid 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 yolk would provide 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 needed to be reduced required the use of multiple times (three) of extraction in each step to ensure extraction efficiency. Three replicates were conducted in the production of lecithin. Parameters measured and calculated were the same as 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. Soy lecithin (Fisher Scientific, Pittsburg, PA, 99% purity) was used as a 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 critical micelle concentration (CMC) of the PLs.

**Emulsion stability.** Egg-yolk PL fractions and soybean lecithin were used as emulsifier to make oil in water (o/w) emulsions. The proportion of oil to water was 2:8 (v:v), whereas the emulsifier concentrations used were 5% and 10% of the oil weight. Emulsifier was dispersed in distilled water to facilitate its incorporation in 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 minutes. Then, emulsions were 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 soyb lecithin. To ensure an even and maximal exposure of the lecithin samples to oxygen, a modification of the process used by Wu and Wang (12) was made. Instead of using mineral oil as dispersing vehicle, glass beads (5 mm diameter) were used. A fixed number of glass beads 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 coating of the glass beads with 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 days with the removal of one vial every 48 hours. At the end of this term, peroxide quantification was done by a ferric thiocyanate method (13). Hydroperoxides in the samples were quantified by measuring the absorbance at 515 nm. Oxidized soybean oil was used to establish a standard curve as following: first, the actual peroxide value of the oxidized soybean oil was determined by the standard iodometric method, AOCS Cd 8-53 (14), then a stock solution of the oxidized soybean oil was prepared by dissolving known amount in ethanol-benzene (80:20). Different amounts of this solution were placed in a series of 10-mL volumetric flasks and color was
developed by adding the reagents as above. A linear standard curve was obtained by plotting the absorbance versus the micro-equivalent (µeq) of peroxide present.

**Results and Discussion**

*Lecithin fractionation.* The moisture content of the fresh egg yolk was 49.4%. Yield percentages for each of the main fractions from the analytical and large scales are shown in Table 1. Reproducibility of the extraction is good, as indicated by the relatively small standard deviations. However, the yield differences between the two scales are statistically significant, although not considerable, possibly because of the smaller volume of solvent used in the large scale processing. The NL-1 yield of the large scale (21.7±0.1%) was significant higher than that from the analytical scale (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 from final acetone precipitation of PLs and removal of neutral lipid also indicated lower efficiency of the large scale compared with the analytical scale processing. However, significant amounts of solvents were saved by this lower solvent to egg yolk ratio.

On the dry weight basis, our yolk material contained 17.7% protein, 11.0% PLs (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 our total lipid content (66% of the dry weight) was higher than the reported 55.8%. We believe this difference is possibly because of variation in breed and feed of the hens.

*PL quantification by HPLC.* The use of a new HPLC column specific for PL characterization (vs. the silica column used by Wu and Wang (12)) reduced equilibration time between analyses, and sharper peaks were also 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 versus the amount of sample injected. The standard calibration equation for PE was Area = 125,160 x (Amount)\(^{1.61}\) (R\(^2\)=0.999) and that for PC was Area = 12,146 x (Amount)\(^{2.05}\) (R\(^2\)=0.995). Vegetable oil and cholesterol standards were also injected to identify each peak on the chromatogram (Figure 3). The first peak was identified as neutral oil, and the second peak was cholesterol. PL purity for the analytical-scale samples is slightly, but statistically significantly higher (95.9±0.3%) than the purity of the large-scale samples (94.9±0.1%), as seen
in Table 2. Overall, this new fractionation method for egg-yolk lecithin is much more efficient and effective than the methods of others. For example, the Hruschka’s patented procedure (9) in which low concentration aqueous ethanol was used to fractionate oil and polar lipids resulted in 47% purity of the PL fraction.

The PL content and distribution in each of the main fractions from the analytical and large-scale extractions are shown in Table 3. There was statistically less PLs lost in the NL-2 fraction during the large scale extraction than during analytical-scale extraction. It seemed that reduction of acetone used for precipitation reduced PL loss. PL distribution in the neutral and PL fractions of both scales was 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 cholesterol and neutral oil, and they were present at about 5%. However, our earlier HPLC analysis with silica column showed a much lower PL content because of a wide peak that gave inaccurate peak integration and poor reproducibility. Possible presence of other compounds in the PL fraction was tested by using TLC, and no other major unknown was identified. However, when the TLC plate was developed three times, the PE band split into two bands. We were able to identify one of the bands as PE plasmalogen by a simple acid hydrolysis test (15). Plasmalogen is a vinyl ether phospholipid (Figure 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 x 4 cm, 250-µm thickness, silica gel plate (Sigma-Aldrich, St. Louis MO). The plate was developed in hexane:diethyl ether (90:10, v:v) and then visualized with a potassium permanganate stain that is a mixture of potassium permanganate (1%) and potassium carbonate (6.7%) dispersed in 5% aqueous sodium hydroxide in water. Standard aldehydes (with C₈ and C₁₄ chain length) were used as references on the TLC plate. It was shown that two aldehydes having a chain length of approximately C₁₆ and C₁₈ were present in one of the two bands. This PE plasmalogen band (moved higher on TLC plate than the PE band) was also transesterified and the fatty acid methyl esters were quantified. The quantity of plasmalogen was then calculated assuming the carbon chain length linking by the ether bond was C₁₆. The plasmalogen content was estimated at about 3.6% of the total yolk PLs. Rhodes (16) reported that there was 0.9 % plasmalogen in yolk. More work is needed to fully
characterize and quantify this unique lipid in egg yolk. Recent studies showed that plasmalogen prevented cholesterol oxidation in membranes (17, 18).

The fatty acid composition of PE and PC of egg-yolk lecithin was determined by GC, as shown in Table 4. There are more saturated fatty acids in egg-yolk lecithin than in soybean lecithin. The oxidative stability of a lipid may be predicted by calculating its oxidizability using the fatty acid composition (19). The oxidizability of the egg-yolk and soybean lecithin was calculated as 0.76 and 0.50, respectively.

**Cholesterol content in fractionated lecithin.** Cholesterol content in the PL fraction from the analytical scale (0.5%) was lower than those in the NL-1 and NL-2 fractions (Table 5), and particularly it was lower than the corresponding PL fraction from the large scale extraction (1.9%). From previous research, we found that cholesterol was very extractable with ethanol. It is evident that the last step in the procedure, i.e. acetone precipitation of the PLs, was necessary to remove the cholesterol from the PL fraction. It is obvious that the efficiency of cholesterol removal in the large scale process was not as high as in the analytical scale. 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 PLs maybe used to increase neutral lipid removal. In the analytical scale separation, 35.2% cholesterol was partitioned in the neutral oil fraction (NL-1), and 52.2% was removed during the final PL precipitation into NL-2. About 12.6% of the total cholesterol was retained in the lecithin product. In the large scale, processing, similar percentage of cholesterol (34.3%) was in the NL-1 product; however, the PL precipitation step removed much less cholesterol (28.3%) than that in the analytical scale, and much higher proportion of cholesterol (37.4%) went in the final lecithin product. The total cholesterol content in the initial material for the large scale processing was higher than that of the analytical scale, possibly because of different batches of egg used.

**Reduction of surface tension by egg-yolk lecithin in aqueous system.** Measuring surface tension reduction and critical micelle concentration (CMC) are common ways of characterizing emulsifiers or surfactants. Surface tension reduction of egg-yolk and soybean lecithin is shown in Figure 5. PL fractions and soy lecithin showed similar patterns. The surface tension was reduced as the concentration of the PLs increased, and was lowered to a minimal value and then became independent of concentrations. 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 when 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 soybean lecithin was 15.8 mg/mL. This CMC value for soybean lecithin was slightly different from the one reported by Wu and Wang (12) where the CMC for the same brand soybean lecithin was 13.6 mg/mL. 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 fluctuation in the graph could be because of the minor defects and the cleanliness of the platinum plate used with the surface tensiometer.

**Emulsion stability.** The emulsions created with egg-yolk PL fractions broke faster than the emulsion created with the soybean lecithin (Figure 6). The three o/w emulsions containing 5% egg-yolk lecithin samples showed a similar trend. They had about 13% oil separation at 240 minutes, while the soybean lecithin showed only 8% oil separation. A peculiar change was observed in the o/w emulsions containing 10% of the emulsifiers. It is typically believed that the more emulsifier we use, the more stable the emulsion will be. However, at 10% lecithin concentration, the percentage oil separated was higher (about 21% at 240 minutes) than at 5% concentration (13%). The emulsion containing 10% soybean lecithin had lower oil separation (6%) than at the 5% concentration (8%). We believe that this maybe because of the difference of these two lecithin products in their PL class and fatty acid compositions. Soybean lecithin contains phosphatidylinositol (PI, 21%), whereas egg-yolk lecithin does not. However, egg-yolk lecithin is rich in PC, and it should be a good o/w emulsifier. An optimal combination of various types of PLs may be needed for an effective emulsifier. More research should be conducted to compare these two lecithins when used at concentrations lower than 5%. It is clear that soybean lecithin performed much better than egg-yolk lecithin did 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 used at the last step of yolk lecithin extraction. Figure 7 shows lipid hydroperoxide generation and degradation with time. The starting peroxide values (PV) for all samples were relatively high (15-20 meq/kg), indicating that oxidation had occurred during the fractionation of egg lecithin or storage of soy lecithin.
Egg-yolk lecithins oxidized to a maximal PV value of 24 to 27 meq/kg at about 47 hours, whereas the highest PV for soybean lecithin was 46 meq/kg at about 96 hours. The PV declined because of peroxide decomposition and termination of the free radical chain reaction. A soybean oil was also oxidized and peroxides quantified under the identical conditions. It reached a maximal PV of about 400 meq/kg at 290 hours before declining. Therefore, hydroperoxides of PLs seem to decompose much more readily than those of the neutral oil. The lower degree of oxidation of egg-yolk lecithin than soybean lecithin may be explained by its high content of saturated fatty acids. The relatively high content of unsaturated fatty acids in soybean lecithin (Table 4) caused it to form more hydroperoxides and will probably cause a stronger rancid flavor when improperly used in foods.

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 (12), in which, lecithin was dispersed in an inert mineral oil. No increase of peroxide value was obtained in that study, possibly because of the limited exposure of PLs to oxygen.

The oxidative stability of lecithin has not been studied as much as for other neutral lipids. There are a few studies on analytical procedures for hydroperoxides PLs (21), however, the relatively stability of PLs from different sources and with different fatty acid composition has not been reported.

In summary, the extraction procedures established in this research were successful and effective in producing a high purity egg-yolk lecithin. The last purification step of the PL fraction with hexane and acetone not only removed neutral oil but also decreased the amount of cholesterol that was co-extracted with the polar lipids by ethanol. The quantitative method developed to study the oxidative stability of lecithins was successfully used in the study of PL oxidation. Egg-yolk lecithin proved to have a better oxidative stability than did soybean lecithin. The two types of lecithin had similar surface activity; however, soybean lecithin created a more stable emulsion than did egg-yolk lecithin.

Acknowledgement

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References


7. Sim, J.S., Extraction of fresh liquid egg yolk, Canadian patent 1,335,054 (1995).


Table 1. Yield of main fractions from analytical and large-scale extraction of PLs from fresh egg yolk

<table>
<thead>
<tr>
<th>Fractionation</th>
<th>NL-1 (g)</th>
<th>NL-2 (g)</th>
<th>PLs (g)</th>
<th>Protein (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analytical Scale (n=2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield from fresh egg yolk (15.3 g, dry weight), g</td>
<td>6.0±0.2</td>
<td>0.6±0.6</td>
<td>3.6±0.0</td>
<td>5.4±0.1</td>
</tr>
<tr>
<td>Yield from fresh egg yolk (30 g), %</td>
<td>19.9±0.7 b</td>
<td>2.0±0.1 a</td>
<td>11.9±0.0 a</td>
<td>18.1±0.3 a</td>
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<tr>
<td><strong>Large Scale (n=3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield from fresh egg yolk (91.8 g, dry weight), g</td>
<td>39.0±0.2</td>
<td>2.6±0.1</td>
<td>20.0±0.4</td>
<td>31.1±0.3</td>
</tr>
<tr>
<td>Yield from fresh egg yolk (180 g), %</td>
<td>21.7±0.1 a</td>
<td>1.4±0.1 b</td>
<td>11.2±0.2 b</td>
<td>17.3±0.1 b</td>
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<tr>
<td>LSD&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>1.2</td>
<td>0.2</td>
<td>0.5</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Yield = 100 x quantity of PL fraction / quantity of initial yolk material.

Abbreviations: NL-1, main neutral lipid fraction; NL-2, high cholesterol neutral lipid fraction; PLs, phospholipid fraction.

Same letter in the same column indicates there is no significant difference between the two scales of processing.

LSD<sub>0.05</sub>: Least Significant Difference at P=0.05.
n, number of replicates in each scale

Table 2. Phospholipid purity and class composition of fractionated egg-yolk lecithin

<table>
<thead>
<tr>
<th>Fractionation</th>
<th>Purity %</th>
<th>PE %</th>
<th>PC %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analytical Scale (n=2)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yield from fresh egg yolk (15.3 g, dry weight), g</td>
<td>95.9±0.3 a</td>
<td>19.1±1.1 a</td>
<td>80.9±1.1 a</td>
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<tr>
<td><strong>Large Scale (n=3)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yield from fresh egg yolk (91.8 g, dry weight), g</td>
<td>94.9±0.1 b</td>
<td>17.9±1.1 a</td>
<td>82.1±1.1 a</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>0.6</td>
<td>3.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Purity = 100 x total PLs as quantified by HPLC / quantity of PL fraction

Same letter in the same column indicates there is no significant difference between the two scales of processing.

See Table 1 footnotes.
Table 3. PL content and distribution in the main fractions

<table>
<thead>
<tr>
<th>Scale of fractionation</th>
<th>NL-1</th>
<th>NL-2</th>
<th>PLs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL content, %</td>
<td>Analytical (n=2)</td>
<td>1.8±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Large (n=3)</td>
<td>1.7±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3±0.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>1.5</td>
<td>1.9</td>
<td>0.5</td>
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</tbody>
</table>

| PL distribution, %     | Analytical (n=2) | 2.9±0.7<sup>a</sup> | 1.3±0.1<sup>a</sup> | 95.9±0.6<sup>a</sup> |
|                        | Large (n=3)  | 3.1±1.1<sup>a</sup> | 0.5±0.1<sup>b</sup> | 96.4±1.2<sup>a</sup> |
| LSD<sub>0.05</sub>     | 2.9        | 0.2        | 3.0        |

Same letter in the same column indicates there is no significant difference between the two scales of processing.
See Table 1 footnotes.

Table 4. Fatty acid composition (%) of the main PL classes of egg-yolk and soybean lecithin

<table>
<thead>
<tr>
<th>Source</th>
<th>PL</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>20:4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean&lt;sup&gt;1&lt;/sup&gt;</td>
<td>PE</td>
<td>16.0</td>
<td>8.3</td>
<td>6.8</td>
<td>57.3</td>
<td>11.7</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>22.2</td>
<td>19.3</td>
<td>6.1</td>
<td>43.4</td>
<td>9.3</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>11.2</td>
<td>11.9</td>
<td>8.6</td>
<td>58.6</td>
<td>9.9</td>
<td>--</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>PE</td>
<td>25.0</td>
<td>26.8</td>
<td>20.7</td>
<td>16.3</td>
<td>--</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>35.0</td>
<td>13.4</td>
<td>30.4</td>
<td>18.0</td>
<td>--</td>
<td>3.2</td>
</tr>
</tbody>
</table>

<sup>1</sup>Soybean data from Hammond et al. (20).
Table 5. Cholesterol distribution among the three major fractions

<table>
<thead>
<tr>
<th>Fractionation</th>
<th>NL-1</th>
<th>NL-2</th>
<th>PLs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analytical Scale</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol quantity,</td>
<td>52.1±1.4</td>
<td>77.2±27.8</td>
<td>18.6±0.2</td>
</tr>
<tr>
<td>mg from 15.3 g dry yolk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration in</td>
<td>0.9±0.0 a</td>
<td>13.0±4.3 a</td>
<td>0.5±0.0 a</td>
</tr>
<tr>
<td>Product, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Large Scale</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol quantity,</td>
<td>347.8±28.3</td>
<td>286.8±27.8</td>
<td>379.9±31.7</td>
</tr>
<tr>
<td>mg from 91.8 g dry yolk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration in</td>
<td>0.9±0.1 a</td>
<td>10.8±1.0 a</td>
<td>1.9±0.1 b</td>
</tr>
<tr>
<td>Product, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LSD</strong>&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>0.2</td>
<td>13.4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

4 Same letter in the same column indicates there is no significant difference between the two scales of processing.
6 See Table 1 footnotes.
Figure 1. Flow chart of lecithin extraction from fresh egg yolk
Figure 2. Gradient program of mobile phase for HPLC quantification of PLs.

Figure 3. HPLC chromatogram of an egg-yolk PL sample. Peak 1, and 2 were identified as neutral oil and cholesterol, respectively.
Figure 4. PE plasmalogen identified in egg-yolk lecithin

Figure 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.
Figure 6. Stability of emulsion (o/w) with 5% (A) and 10% lecithin (B) 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.
Figure 7. Peroxide values of lecithins determined by a ferric thiocyanate method. 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.