Extraction of Egg-Yolk Lecithin

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ABSTRACT: In this research, the extraction of egg-yolk lecithin with ethanol was studied. Extraction was performed with deoiled and undeoiled yolks and with heated and unheated yolks. The yield of the extracted fraction relative to the initial material, phospholipid (PL) purity, and cholesterol content of both the PC-enriched fractions and the remaining PL fractions were determined. The yield and PL purity of the PC-enriched fractions obtained from the undeoiled yolks were 23.9 and 35.7%, and those obtained from deoiled yolks were 13.5 and 53.3%. The recovery of total PL in the two fractions was higher from the undeoiled (70%) than from the deoiled yolks (60%). However, heating had a negligible effect on PL extraction. Better enrichment of PC was observed by extraction from the undeoiled than from the deoiled yolks. The cholesterol content of the PC-enriched fraction obtained from the undeoiled yolks was much higher than that from the deoiled yolks.

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Egg yolks typically contain 43.8% solids, of which 34.3% is protein, 55.8% is total lipids, 3.4% is ash, and 3.6% is carbohydrates (1). Phospholipids (PL) represent approximately 10% of the wet weight of egg yolks (2). The main components of egg-yolk lecithin are PC (80.5%) and PE (11.7%). The extraction of total lipids or PL from yolks is desirable because of the unique properties and valuable applications of these products (3). Egg-yolk PC is reported to significantly lower cholesterol absorption in rats compared with soybean PC (4). Egg-yolk lecithin contains relatively more saturated FA than does soybean lecithin, so it may have better oxidative stability. The antioxidant activity of egg-yolk PL was also reported in a recent study (5).

Information on PL extraction from egg yolks is limited (3,6–12). The most common scheme uses dried yolks and an initial deoiling step, and the PL are then extracted from the deoiled material with ethanol. Alternatively, the yolks are first extracted with ethanol and then acetone is used to remove the oil from the ethanol extract. No quantitative comparison of these two different schemes, i.e., extraction with and without the presence of oil, has been reported. From previous findings in our laboratory (13), when oil was contained in the raw material, this tended to result in higher PL recovery. Dried yolks are typically used as the starting material because of the belief that lipids and protein in fresh egg yolks are intimately associated, so the lipids are not easily extractable with a nonpolar solvent (14). Yolk heating and drying is energy intensive, and the heat-denatured proteins are usually less functional when used in food systems. It is not known quantitatively how heating egg yolks will affect PL extraction. Extraction of the lipids from raw and cooked eggs has been investigated in different studies, but a paired study is lacking. Therefore, the objectives of this experiment were to examine how heating egg yolks would affect PL extraction and whether it is feasible and beneficial to extract PL directly from the whole yolks without first deoiling them.

MATERIALS AND METHODS

Egg yolk preparation. Grade A Monty eggs (Monty Produce, Monticello, IA) were purchased from a local grocery store. Ten eggs were heated in boiling water for 15 min, and the egg yolks were separated from the whites. The complete semiliquid-to-solid transformation was taken as an indictor that the yolk protein was heat denatured. Another 10 raw eggs were carefully broken to separate the yolks from the whites, and the combined yolks were kept in a cold room (5°C) before use. The moisture content of the heated and unheated egg yolks was determined by using a conventional oven-drying method at 100°C for 4 h.

Lecithin extraction from undeoiled egg yolks. A flow chart of the lecithin extraction procedure is outlined in Figure 1. Ethanol (100%) was added to approximately 5 g (accurately weighed) of heated or unheated egg yolks to a final 5:1 ratio of solvent to egg yolks (wet weight). The mixture was stirred until the egg yolks were completely dispersed. The final concentration of ethanol was 91% after it was diluted with the water contained in the fresh yolks. The sample was then centrifuged at $400 \times g$ for 5 min. The PC-enriched fraction (supernatant) was transferred to a previously weighed round-bottomed flask and the ethanol was removed by rotary evaporation. The dried PC-enriched fraction was weighed and transferred to a 15-mL vial with 10 mL of chloroform/methanol (2:1, vol/vol) for HPLC analysis. The residual egg yolk was dried at ambient temperature for 2 d and then deoiled with acetone using AOCS Official Method Ja 4-46 (15). The acetone extract was kept for further quantification of the neutral oil. After acetone deoiling, 40 mL of chloroform/methanol (2:1, vol/vol) was used to extract residual lipids from the yolks. Water-saturated butanol also was used to extract any remaining polar lipids from the yolk residual. The combined lipids were washed using the method of Folch *et al*. (16). This fraction was referred to as the remaining PL fraction.

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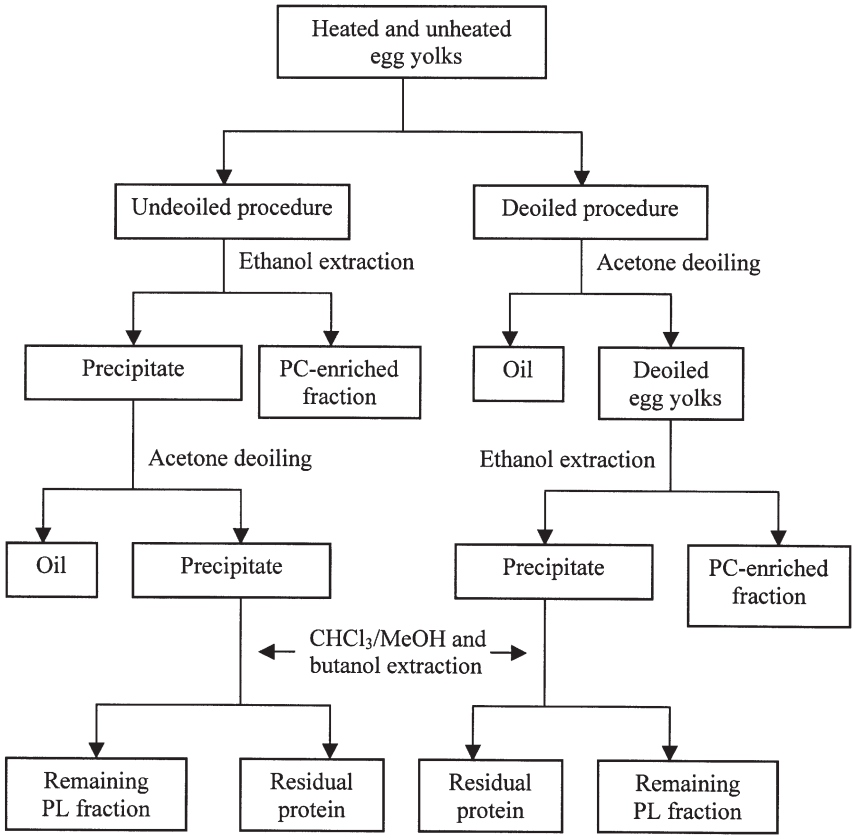

FIG. 1. Extraction of phospholipids from heated and unheated egg yolks by undeoiled and deoiled procedures.

Lecithin extraction from deoiled egg yolks. Acetone was used to deoil 5 g of egg yolks (heated or unheated) following the AOCS Official Method described above. The supernatant obtained after centrifugation was kept for quantification of the neutral oil. The precipitate was dried at ambient temperature for 2 d, then aqueous ethanol of a 91% concentration was used at a 5:1 ratio of solvent to egg yolk to extract PL as described above. Extraction of the remaining PL fraction was also done with chloroform/methanol (2:1, vol/vol) and water-saturated butanol as outlined above.

The parameters measured and used for evaluating the effectiveness of extraction were the yield of the fraction, the purity and recovery of the PL, and the cholesterol content of both the PC-enriched fraction and the remaining PL fractions. The yield was defined as the percentage of fraction obtained relative to the total dry weight of the starting material. Purity was defined as the percentage of total PL in a fraction as quantified by HPLC. Recovery was the amount of total PL in a fraction divided by the total PL in the starting material. The cholesterol content was the percentage of cholesterol in the fraction, and it was measured by saponification and then GC quantification as described in the following section.

Quantification of PL by HPLC. A Shimadzu HPLC system with an LC-600 liquid delivery module, silica column (250 mm length, 2.1 mm i.d.; Alltech, Deerfield, IL), Varex IIA ELSD, and CR501 Chromatopac integrator was used to quantify the PL. An isocratic elution with chloroform/methanol/water/acetic acid (63.5:32:4:0.5, by vol) at a flow rate of 0.5 mL/min was used. Nitrogen, at a flow rate of 2.3 L/min, was applied to evaporate the solvent in the 115°C drift tube of the ELSD. An injection loop with a 20-μL volume was used. External standard curves were established to quantify the PL.

Cholesterol quantification by GC. About 100 mg of the lipid sample was accurately weighed and transferred into a glass vial. Two milliliters of 1 N potassium hydroxide (in 95% ethanol) was added to the sample, which was then placed in a boiling water bath for 2 h. 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 extract the unsaponifiable materials. The ether extract was then washed with distilled water and evaporated under nitrogen. The crude unsaponifiable matter was dissolved in 1 mL of hexane containing cholestane as an internal standard, and duplicate samples were injected into a Hewlett-Packard 5890 Series II GC system equipped with a fused-silica capillary column (SAC-5, 30 m × 0.25 mm × 0.25 μm film thickness; Supelco, Bellefonte, PA). The GC oven temperature was 285°C, and the injector and detector temperatures were 300°C.

Initial composition of the egg yolks. To determine the composition profile of the initial yolk material, total lipids were

extracted with chloroform/methanol (2:1, vol/vol) and water-saturated butanol. A wash following the method of Folch *et al*. (16) was applied to remove the water-soluble contaminants. Neutral lipids were removed from the total lipids extracted by acetone, and the PL were quantified by HPLC. The total oil content was calculated as the oil obtained by acetone deoiling plus the non-PL portion of the deoiled PL fraction. The total PL content was the sum of the PL in the PL extract and in the oil fraction. The protein content was determined after drying and weighing the residual solids.

Statistical analysis. Data were analyzed with the General Linear Model (GLM) program of SAS (17). LSD values were used to examine whether there were significant treatment effects on PL extraction in this two-factor factorial treatment design, i.e., the presence of oil (undeoiled and deoiled yolks) and protein denaturation (heated and unheated yolks).

RESULTS AND DISCUSSION

The initial composition (%) of the egg yolks was as follows: moisture, 51.6; PL (PC + PE), 7.0; neutral lipids, 25.4 (TAG, 24.8; cholesterol, 0.6); residual protein, 16. On the basis of total lipids, 78.4% was neutral lipids (including 1.8% cholesterol) and 21.6% was PL. As reported in the literature, there is considerable variation in the composition of egg yolks, possibly because of differences in the breeds and feed of the hens. Tokarska and Clandinin (7) reported that the lipid profile of egg yolks was 65% neutral oil, 28.3% PL, and 5.2% cholesterol.

The results of lecithin extraction and quantification are presented in Tables 1 and 2, and the statistical analysis is shown in Table 3. The moisture content of the heated egg yolks was 49.9%, which was reduced slightly compared with that of the unheated yolks (51.6%).

Yield of the PL fractions. Heating had no effect on the yields of the PC-enriched fractions and the remaining PL fractions (Tables 1, 3). However, the yields of the PC-enriched fractions were significantly different between the undeoiled and the deoiled egg yolks, 23.9 and 13.5% (mean values), respectively. The mean yields of the remaining PL fractions were 4.2 and 5.9% from the undeoiled and deoiled materials, and this difference was also statistically significant. The higher yield from the undeoiled material was due to the extraction of a significant amount of oil into the ethanol-soluble fraction. Consequently, the purity of the PC-enriched fraction was much higher from the deoiled than from the undeoiled material. Conventionally, heating and protein denaturation of the oil-bearing material is believed to facilitate lipid extraction through the disruption of protein and oil interaction and the coalescence of oil droplets. However, the data shown here suggest that heating is unnecessary for PL extraction with ethanol, possibly because ethanol is a strong polar solvent that can disrupt protein–lipid interaction in the yolk lipoproteins.

Purity and recovery of the PL fractions. Heating did not affect the purity of the PC-enriched fraction, but it significantly affected the purity of the remaining PL fraction (Tables 1, 3). When protein was heated, the extraction of PL from the yolk into the remaining PL fraction was more complete than when the protein was not heated, regardless of whether the oil was removed before or after ethanol extraction. The presence of oil significantly decreased the purity of the PC-enriched fraction, with the purity being 35.7% from the undeoiled yolks and 53.3% from the deoiled yolks. When acetone deoiling was done after ethanol extraction, the remaining PL fraction was purer than when acetone deoiling was done before ethanol extraction (Table 1). The purity or PL content in these products was low because of the large amount of residual oil co-extracted with the PL. Other impurities present could be minor polar lipids that were not quantified in our HPLC analysis. Additional deoiling/purification with acetone may have to be used to further increase the purity. Alternatively, if acetone is undesirable in PL production because of environmental or safety concerns, multiple extractions with ethanol will improve the purity, as shown in our previous research (13).

PL recovery in the PC-enriched fraction was not affected by heating the yolks, but again, heating significantly increased PL recovery in the remaining PL fraction, with 9.1 and 8.1% recovery from the heated and unheated materials (mean values for deoiled and undeoiled yolks; see Tables 1, 3). Statistically, the presence of oil did not affect PL recovery in the PC-enriched fraction. However, there was a clear trend for the total

TABLE 1
Mean Value and SD of Yield, Purity, Recovery, and Cholesterol Content[a] of Fractionated Egg-Yolk Lecithin

		PC-enriched fraction		Remaining PL fraction	
	Yolk treatment	Undeoiled	Deoiled	Undeoiled	Deoiled
Yield (%)	Heated	24.4 ± 0.1	13.3 ± 0.4	4.0 ± 0.0	5.6 ± 0.4
	Unheated	23.4 ± 0.0	13.7 ± 0.0	4.3 ± 0.1	6.1 ± 0.6
Purity (%)	Heated	34.9 ± 1.5	55.0 ± 2.2	32.3 ± 2.6	22.2 ± 1.9
	Unheated	36.4 ± 7.2	51.6 ± 0.2	28.4 ± 0.8	18.6 ± 1.5
Recovery (%)	Heated	60.9 ± 3.6	52.3 ± 3.5	9.2 ± 0.9	8.9 ± 0.1
	Unheated	59.1 ± 11.9	48.8 ± 1.3	8.4 ± 0.0	7.9 ± 0.1
Cholesterol (%)	Heated	3.8 ± 0.2	0.4 ± 0.0	0.2 ± 0.1	0.3 ± 0.0
	Unheated	3.7 ± 0.3	0.4 ± 0.1	0.1 ± 0.1	0.3 ± 0.0

[a]Yield = 100 × quantity of PL fraction/quantity of initial yolk material; purity = 100 × total PLs as quantified by HPLC/quantity of PL fraction; recovery = 100 × total PL as quantified by HPLC/total PL in the initial yolk material; n = 2, two replicates of egg yolk extraction.

TABLE 2
Phospholipid Class Composition (%) of the Fractionated Egg-Yolk Lecithin

	PC-enriched fraction			
	PC		PE	
Yolk treatment	Undeoiled	Deoiled	Undeoiled	Deoiled
Heated	72.3 ± 0.2	69.9 ± 0.9	27.7 ± 0.2	30.1 ± 0.9
Unheated	72.0 ± 0.8	66.5 ± 0.2	28.0 ± 0.8	33.5 ± 0.2
	Remaining PL fraction			
	PC		PE	
Yolk treatment	Undeoiled	Deoiled	Undeoiled	Deoiled
Heated	52.8 ± 0.5	37.0 ± 2.2	47.2 ± 0.5	63.1 ± 2.2
Unheated	52.5 ± 3.2	45.0 ± 2.4	47.5 ± 3.2	55.0 ± 2.4

recovery to be much higher in the PC-enriched fraction from the undeoiled yolks than from the deoiled yolks (60.0 vs. 50.6%). This may have been caused by the loss of PL during acetone deoiling before ethanol extraction. The water contained in the fresh yolks may have been partially dissolved in the acetone, making the acetone relatively more polar, and subsequently, the solubility of the PL in the acetone–water mixture might have increased. Wu and Wang (13) also observed this phenomenon. A total of 30–40% PL were lost during the fractionation process in our research.

The highest PL purity obtained in our experiment was 55% from the deoiled and heated egg yolks. However, Sim (3) reported a PL product with 89% purity prepared from spray-dried yolks when the material was extracted with aqueous ethanol. Sim's report offers no explanation that may account for such different results between the two studies. It is unclear whether there are considerable differences in the physicochemical properties of the spray-dried and heated or boiled yolks that could have resulted in the differences in PL extraction.

Cholesterol content in the PL fractions. There were significant differences in the cholesterol content of the two PL fractions obtained from the undeoiled and deoiled yolks (Tables 1, 3). Deoiling the egg yolks before ethanol extraction was beneficial in removing cholesterol from the PL products. Evidently, cholesterol is very extractable with ethanol. Deoiling the yolks with acetone can remove most of the TAG and cholesterol as well as 15% of the PL, according to Nielsen (10). The PC-enriched fraction from undeoiled egg yolks had an increased concentration of cholesterol (3.8%; Table 1) compared with the 1.9% cholesterol in total egg yolk lipids.

PL class composition. Heating significantly affected the PL class composition of the PC-enriched fraction obtained from the deoiled material but not from the undeoiled material (Tables 2, 3).

In the presence of oil, the extraction of PC with ethanol was more effective than extraction from the deoiled material, as shown by the higher percentage of PC (72 vs. 68%, mean values of the heated and unheated yolks). Although these differences were not great, they were statistically significant. This observation could be the result of the oil acting as a solvent for PE, making PC relatively more extractable with ethanol.

For the remaining PL fraction, heating did not affect the PL class composition of the fractions obtained from the undeoiled yolks. However, from the deoiled yolks, the percentage of PC was significantly lower in the heated yolks than in the unheated yolks. Possibly, the PC was already preferentially extracted by ethanol from the deoiled yolks. The remaining PL fraction from the undeoiled material had a higher percentage of PC than that from the deoiled material (Table 2). Schneider (18) reported that alcohol-fractionated egg-yolk PL contained 69% PC and 24% PE, which is in general agreement with our results for the PC-enriched fractions, as shown in Table 2.

TABLE 3
Probability (*P*) and $LSD_{0.05}$ Values for the Effect of Heating and the Presence of Oil on the Extraction of Egg-Yolk Lecithin

	PC-enriched fraction					
	Yield	Purity	Recovery	PC	PE	Cholesterol
P (heat)	0.3009	0.7440	0.6046	0.0113	0.0113	0.9177
P (oil)	<0.0001	0.0029	0.1094	0.0007	0.0007	<0.0001
P (heat × oil)[a]	0.0321	0.4116	0.8607	0.0201	0.0201	0.6347
$LSD_{0.05}$	0.58	7.56	12.76	1.16	1.16	0.38
	Remaining PL fraction					
	Yield	Purity	Recovery	PC	PE	Cholesterol
P (heat)	0.1761	0.0433	0.0399	0.0745	0.0745	0.5582
P (oil)	0.0023	0.0016	0.2547	0.0019	0.0019	0.0156
P (heat × oil)	0.6343	0.9115	0.8060	0.0623	0.0623	0.0929
$LSD_{0.05}$	0.70	3.58	0.85	4.48	4.48	0.10

[a]An × indicates interaction.

Overall, extraction of PL from undeoiled egg yolks resulted in relatively higher PL recovery and higher percentages of PC in both the PC-enriched fraction and the remaining PL fractions than from the deoiled yolks.

Calculated oxidizability of the PL products. As mentioned in the introduction, egg-yolk PL are more saturated, and they should be more oxidatively stable than soybean lecithin. This can be a significant advantage of egg-yolk lecithin over soy lecithin, because in certain food preparations, particularly in emulsions, off-flavor generation has been attributed to lecithin oxidation. Based on the FA composition, oxidizability was calculated (19) for both soybean and egg-yolk lecithin using the soybean PL classes and the FA compositions reported by Wang *et al*. (20) and our GC and HPLC data for yolk lecithin. The calculated oxidizability of soy lecithin is 0.76, which is much higher than the 0.49 value obtained for egg lecithin (a higher value suggests less stability to oxidation). Therefore, egg-yolk lecithin may have higher oxidative stability than soybean lecithin.

This research showed that extraction of PL from fresh egg yolks with ethanol is feasible. Although oil contained in the yolks will be partially extracted with ethanol, decreasing the PL purity of the PC-enriched fraction, it facilitates PC enrichment. This comparative study provides the basis for developing a more efficient PL extraction procedure from egg yolks.

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