J Am Oil Chem Soc (2012) 89:179–182
DOI 10.1007/s11746-011-1893-x

LETTER TO THE EDITOR

Isolation of Pure Phospholipid Fraction from Egg Yolk

Witold Gładkowski · Anna Chojnacka · Grzegorz Kiełbowicz · Tadeusz Trziszka · Czesław Wawrzeńczyk

Received: 5 March 2011 / Revised: 14 June 2011 / Accepted: 18 June 2011 / Published online: 17 July 2011
© AOCS 2011

Abstract The phospholipid (PL) fraction from egg yolk was isolated and purified. In the procedure applied (method 2) the egg yolk was extracted with ethanol, precipitated using acetone chilled to −20 °C and washed using acetone. The purity of the samples was checked by HPLC analysis using a Charged Aerosol Detector (CAD). The results were compared with those obtained for the phospholipid fraction isolated and purified by deoiling yolk before extraction and the precipitation of PL with acetone chilled to 4 °C (method 1). The use of acetone chilled to −20 °C to precipitate and wash the phospholipids yielded the phospholipid fraction with 100% purity (78.7 ± 0.2 of phosphatidylcholine and 21.3 ± 0.2 of phosphatidylethanolamine). When deoiling and the 4 °C purification process was used (method 1) 0.4 ± 0.1% cholesterol and some traces of triacylglycerols remained in the PL fraction.

Keywords Phospholipids · Lecithin · Extraction · Precipitation · Charged Aerosol Detector

Dear Editor:

Among the biologically active lipids, the phospholipids (PL) have attracted great attention due to their unique nutritional and functional properties. Although PL are mainly used in pharmaceuticals [1–8] to a lesser degree, PL are also used in the cosmetics [9] and food [10] industries. Because of their amphiphilic character, phospholipids form microscopic vesicles consisting of an aqueous core enclosed in phospholipid layers. These vesicles or liposomes may be used in the encapsulation of drugs, protein or peptide antigens. Encapsulation improves delivery of these health-promoting agents to targeted tissues [11, 12] in the body. The main PL component in egg yolk is phosphatidylcholine (PC). PC is particularly valuable nutritionally because it is the source of two important nutrients—choline and polyunsaturated fatty acids (PUFA) from the *n*-3 and *n*-6 PL family.

The main commercial source of PL is soyabean lecithin. However, commercial soya lecithin, which is a by-product of oil refining, is a mixture of various lipid fractions and carbohydrates and the content of PC is only 20% [13]. PC content may be increased to about 50% by fractionation with ethanol however 50% is not pure enough for use in pharmaceutical applications. Therefore, interest in egg yolk PC or lecithin production has increased, especially if the production of PUFA enriched phospholipids may be increased through feeding modifications of laying hens [14, 15]. PC isolation and purification methods, particularly the removal of cholesterol, need to be developed and evaluated.

The methods for PC isolation and purification in the literature are rather complicated and the purity of PL fraction is not satisfactory. Generally, egg yolk PL are extracted with organic solvents, especially ethanol, and then purified by removing triacylglycerols (TAG) and cholesterol. Several techniques of purifying the PL fraction have been investigated, including extraction with various solvents [16], low temperature crystallization to remove the

W. Gładkowski (✉) · A. Chojnacka · G. Kiełbowicz · C. Wawrzeńczyk
Department of Chemistry,
Wroclaw University of Environmental and Life Sciences,
50-375 Wrocław, Poland
e-mail: glado@poczta.fm

T. Trziszka
Department of Animal Products Technology and Quality Management, Wroclaw University of Environmental and Life Sciences, 50-375 Wrocław, Poland

Table 1 The comparison of two methods of isolation and purification of egg yolk lecithin

Method of isolation	Yield[a] (%)	PL (%)	PC (%)	PE (%)	Cholesterol (%)
Method 1	6.7 ± 0.3[a]	86.3 ± 0.5[a]	70.4 ± 0.2[a]	15.9 ± 0.7[a]	0.4 ± 0.12
Method 2	9.5b ± 0.4[b]	100 ± 0.0[b]	78.7 ± 0.2[b]	21.3 ± 0.2[b]	–

Yield = 100 × quantity of PL fraction/quantity of initial yolk material

The results represent mean values of four independent replicates ± SD

The different superscripts in the same column indicate significant differences by Duncan's multiple range test

solidified neutral oil from PL fraction [17], ultrafiltration [18] or precipitation of PL using acetone [19]. Among the methods of isolation, the best results have been achieved by Palacios and Wang [18] who isolated phospholipids with 95.9% purity. In their research, they used multistep extraction with ethanol and hexane, and, in the final purification step, the precipitation with chilled acetone was applied to give the final product. The content of cholesterol in the PL fraction was 0.5%. In another paper, the same authors found that deoiling the egg yolk before the extraction with ethanol improves the purity of PL fraction [20].

As a part of our efforts to obtain egg yolk PL, we found some useful methods [21, 22] but we needed to simplify and improve on PL purity. In these studies, we used both the deoiling step and the precipitation of the crude phospholipid fraction (method 1). Fresh egg yolk (20 g) was placed in a 100-ml flask on a magnetic stirrer and deoiled with acetone (2 × 50 ml, 10 min). The acetone-extracted material was centrifuged (12,000*g*, 10 min) and the pellet was extracted with ethanol (3 × 50 ml, 15 min). After centrifugation (12,000*g*, 10 min), the ethanol extracts were pooled and the solvent was evaporated in vacuo. The residue, which was a crude phospholipid fraction, was dissolved in hexane (30 ml) and transferred to a flask placed in the ice bath (0 °C). Next, 60 ml of chilled acetone (4 °C) was carefully poured into the stirring mixture. The precipitate was centrifuged (10,000*g*, 5 min) and 1.35 g of the phospholipid fraction was obtained.

The results of HPLC analysis of the phospholipid fraction are shown in Table 1. Although the cholesterol content was low, the purity of the PL fraction was also relatively low. In addition to PC, phosphatidylethanolamine (PE) and cholesterol (Fig. 1a), the HPLC analysis showed the presence of a complex mixture of TAG. The TAG were not determined quantitatively, however, we could identify them by comparing the retention time with that of tripalmitin standard. These results were not satisfactory especially when compared with the purity of phospholipids reported by Palacios and Wang [18].

Because the crucial factor in the purification process might be the temperature of acetone, we decided to apply the solvent chilled to −20 °C, both to precipitate and to wash the PL. We hoped that the reduced temperature would increase the purity of the resulting PL because of the decreased solubility of PL in cold acetone. Deoiling prior to extraction of PL was also omitted. In method 2, egg yolk (20 g) was placed in a 100-ml flask with a magnetic stirrer, 60 ml of ethanol was added and the mixture was stirred for 10 min. The supernatant was then removed and filtered into a round-bottom flask. The extraction of egg yolk with ethanol was repeated twice. The extracts were pooled and the ethanol was evaporated under reduced pressure. The residue was dissolved in hexane (30 ml) and transferred to a flask placed in an ice bath (0 °C). Next, 60 ml of cold acetone (−20 °C) was carefully poured into the stirring mixture. Following precipitation of PL, the stirring was stopped and the supernatant was decanted off. The precipitate was carefully washed 5 times with 20-ml portions of cold (−20 °C) acetone and the solvent was removed by decantation each time. As a result, 1.9 g of PL was obtained.

The HPLC chromatogram analysis (Fig. 1b) as well as the data (Table 1) show complete removal of cholesterol and TAG. All the results were submitted to analysis of variance, at a significance level of 5%, using Statistica (version 8). The Duncan test determined that the results from methods 1 and 2 differed significantly. Only the PL fraction was present in the extract obtained in method 2 and the amount of PC and PE was significantly higher than that obtained in method 1. The yield of the isolated fraction was also significantly higher in method 2.

In our research, we used a new type of HPLC detector with non-linear response—CORONA™ Charged Aerosol Detector (CAD, ESA Biosciences, Chelmsford, MA) operated with nitrogen as a nebulizing gas. HPLC analysis of PL was performed on a DIONEX UltiMate 3000 LC system equipped with a pump (model DGP-3600A), and at a range of 100 pA and autosampler (model WPS-3000TSL). A Waters Spherisorb® S5 W column (150 × 4.6 mm) was used for analysis. HPLC gradient program (flow rate 0.6 ml x min^{-1}): 0 min 0/90/10 (%A/%B/%C) at 2 min, 0/40/60 at 20 min, 1/40/59 at 22 min, 10/40/50 at 38 min, 8/40/52 at 44 min, 1/40/59 at 55 min, 0/90/10 at 56 min, 0/90/10 hold 10 min (A/B/C, water/0.1% solution of formic acid in hexane/isopropanol). The lipid

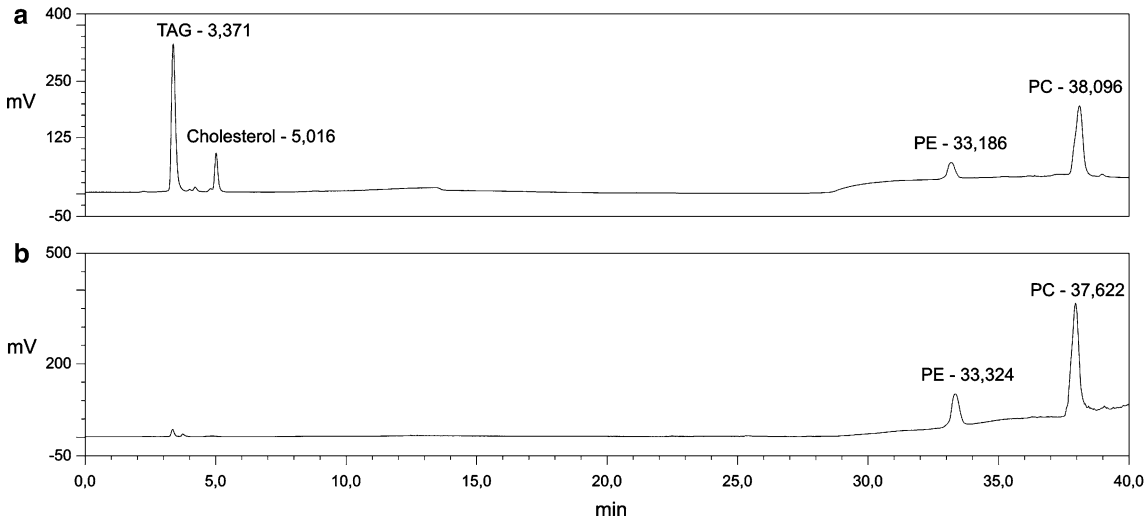

Fig. 1 The HPLC chromatogram. **a** Phospholipid fraction obtained by method 1, **b** phospholipid fraction obtained by method 2

components were identified by comparison of their retention times to those of commercial standards. Calibration standards were diluted with propan-2-ol and six different concentration of standards were injected in three replicates.

CAD was successfully used in the analysis of PL by Moreau [23]. He used normal phase isocratic as well as a gradient system. In our studies, we used the gradient system because of the better resolution of the compounds. We applied the program in which we could detect and resolve cholesterol and TAG in addition to PC and PE.

Using acetone at −20 °C to precipitate and wash crude PL from egg yolk removes the cholesterol and TAG completely affording a pure phospholipid fraction in 9.5% yield (Table 1). No deoiling or column chromatography were necessary in the method of choice. The method is relatively fast, simple and easy to scale-up.

Acknowledgments This work was supported by the Ministry of Science and Higher Education, Grant no N N209 275035

References

1. Meck WH (1998) Choline and development of brain memory functions across the life span. In: Zeisel SH, Szuhaj BF (eds) Choline, phospholipids, health, and disease. 7th international congress on phospholipids. AOCS Press, Brussels p.xi
2. Orthoefer FT, List GR (2006) Phospholipids/lecithin: a class of nutraceutical lipids. In: Shahidi F (ed) Nutraceutical and specialty lipids and their co-products. CRC Press, pp 509–530
3. Innis SM, Davidson A, George F, Melynk S, James SJ (2007) Choline-related supplements improve abnormal plasma methionine-homocysteine metabolites and glutathione status in children with cystic fibrosis. Am J Clin Nutr 85:702–708
4. Da Costa K, Gaffney CE, Fischer LM, Zeisel SH (2005) Choline deficiency in mice and humans is associated with increased plasma homocysteine concentration after a methionine load. Am J Clin Nutr 81:440–444
5. Wilson TA, Meservey CM, Nicolosi RJ (1998) Soy lecithin reduces plasma lipoprotein cholesterol and early atherogenesis in hypercholesterolemic monkeys and hamsters: beyond linoleate. Atherosclerosis 140:147–153
6. Bigger JT, El-Sherif T (2001) Polyunsaturated fatty acids and cardiovascular events: a fish tale. Circulation 103:623–625
7. Uauy R, Dangour AD (2006) Nutrition in brain development and aging: role of essential fatty acids. Nutr Rev 64:24–33
8. Innis SM (2008) Dietary omega 3 fatty acids and the developing brain. Brain Res 1237:35–43
9. Van Gool CJ, Zeegers MP, Thijs C (2004) Epidemiology and health services research. Oral essential fatty acid supplementation in atopic dermatitis–a meta-analysis of placebo-controlled trials. Brit J Dermatol 150:728–740
10. Hernandez E, Quezada N (2008) Uses of phospholipids as functional ingredients. In: Gunstone FD (ed) Phospholipid technology and applications. The Oily Press, Bridgewater, pp 83–94
11. Copland MJ, Baird MA, Rades T, McKenzie JL, Becker B, Reck F, Tyler PC, Davies NM (2003) Liposomal delivery of antigen to human dendritic cells. Vaccine 21:883–890
12. Mamot C, Drummond DC, Hong K, Kirpotin DB, Park JW (2003) Liposome-based approaches to overcome anticancer drug resistance. Drug Resist Update 6:271–279
13. Schneider M (2008) Major sources, composition and processing. In: Gunstone FD (ed) Phospholipid technology and applications. The Oily Press, Bridgewater, pp 21–40
14. Fredriksson S, Elwinger K, Pickova J (2006) Fatty acid composition of egg yolk as an effect of microalgae addition to feed formula for laying hens. Food Chem 99:530–537
15. Gładkowski W, Kiełbowicz G, Chojnacka A, Gil M, Trziszka T, Dobrzański Z, Wawrzeńczyk C (2011) Fatty acid composition of egg yolk phospholipid fractions following feed supplementation of Lohmann Brown hens with humic-fat preparations. Food Chem 126:1013–1018
16. Tokarska B, Clandinin MT (1985) Extraction of egg yolk oil of reduced cholesterol content. Can Inst Food Sci Tech J 18:256–258
17. Sim JS (1995) Extraction of fresh liquid egg yolk. Canadian Patent 1,335,054
18. Miyata K, Matsumoto H (2001) Manufacture of purified egg-yolk lecithins. Japanese Patent 2,001,072,693
19. Palacios LE, Wang T (2005) Egg-yolk lipid fractionation and lecithin characterization. J Am Oil Chem Soc 82:571–578

20. Palacios LE, Wang T (2005) Extraction of egg-yolk lecithin. J Am Oil Chem Soc 82:565–569
21. Gładkowski W, Kiełbowicz G, Chojnacka A, Pisarski B, Trziszka T, Wawrzeńczyk C (2009) Sposób otrzymywania fosfolipidów. Pol Pat Appl 387:383
22. Gładkowski W, Kiełbowicz G, Chojnacka A, Pisarski B, Wawrzeńczyk C (2010) Izolowanie fosfolipidów z liofilizowanych żółtek jaj kurzych. Przem Chem 89:352–355
23. Moreau RA (2006) The analysis of lipids via hplc with a charged aerosol detector. Lipids 41:727–734