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ARTICLE

Investigation of Natural Phosphatidylcholine Sources: Separation and Identification by Liquid Chromatography–Electrospray Ionization–Tandem Mass Spectrometry (LC–ESI–MS^2) of Molecular Species

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This study is a contribution to the exploration of natural phospholipid (PL) sources rich in long-chain polyunsaturated fatty acids (LC-PUFAs) with nutritional interest. Phosphatidylcholines (PCs) were purified from total lipid extracts of different food matrices, and their molecular species were separated and identified by liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI–MS^2). Fragmentation of lithiated adducts allowed for the identification of fatty acids linked to the glycerol backbone. Soy PC was particularly rich in species containing essential fatty acids, such as (18:2–18:2)PC (34.0%), (16:0–18:2)PC (20.8%), and (18:1–18:2)PC (16.3%). PC from animal sources (ox liver and egg yolk) contained major molecular species, such as (16:0–18:2) PC, (16:0–18:1)PC, (18:0–18:2)PC, or (18:0–18:1)PC. Finally, marine source (krill oil), which was particularly rich in (16:0–20:5)PC and (16:0–22:6)PC, appeared to be an interesting potential source for food supplementation with LC-PUFA–PLs, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

KEYWORDS: Phosphatidylcholine; PUFA; supplementation; molecular species; lithium; LC–ESI–MS

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) play very important roles in many aspects of human health, in particular in reducing risks of cardiovascular diseases, inflammation, hypertension, allergies, and immune and renal disorders (*1*, *2*). Among these, linoleic acid (18:2) and α-linolenic acid (18:3) are considered as essential fatty acids (FAs), because they have to be necessarily supplied by the diet and cannot be synthesized by the human organism. Moreover, they are the precursors of long-chain PUFAs (LC-PUFAs), in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential for brain and retina development (*3*–*5*). It is still unclear though whether the consumption of the precursors (18:3 and 18:2) is sufficient to synthesize the necessary amounts of LC-PUFAs or if diet supplementation with EPA and DHA may be required. In fact, while some reports showed infant formulas supplemented with 18:3 giving retina development comparable to human breast milk (containing DHA) (*6*, *7*), others showed reduced visual maturity with infant formulas supplemented with 18:3 compared to formulas that were supplemented with DHA (*4*, *8*). However, there is agreement among investigators on the crucial importance of PUFAs in the diet and their essential role in human nutrition.

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The natural molecular forms of PUFAs are typically triacylglycerols (TAGs) and phospholipids (PLs). While TAGs are quite a homogeneous group of lipids, PLs can be divided into three classes: glycerophospholipids, ether glycerolipids, and sphingophospholipids. Glycerophospholipids represent the most widespread PL class and can be divided into subclasses according to their polar head, with phosphatidylcholine (PC) being the predominant one. In the human diet, TAGs are the major carriers of FAs, with 50–100 g/day for an adult, followed by PLs, with 2–10 g/day (*9*). Studies showed however that, when it comes to FA biodisponibility, PLs are much more efficient carriers than TAGs (*3*, *10*, *11*).

Supplementation of food products with PUFA-rich phospholipids (PUFA–PLs) has recently emerged as an interesting way of increasing the assimilation and the health benefits of LC-PUFAs in the human body. The preparation of food supplements containing pure molecular species (rich in 18:2, 18:3, DHA, and EPA) is not only technically challenging but may turn out to be costly and industrially unrealistic. The solution may however lie in the exploration and tapping of natural PL sources rich in molecular species with PUFAs of nutritional interest.

This study describes the development of an analytical method intended for the determination of molecular species from glycerophospholipids. PC was picked as the model and was extracted and purified from various food matrices: soy as a plant source, egg yolk and ox liver as animal sources, and krill oil as a marine

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animal source. Molecular species profiles were determined using liquid chromatography−electrospray ionization−tandem mass spectrometry (LC−ESI−MS^2) by fragmenting lithiated adducts of the molecular species of PC.

This analytical method can be advantageously used by the food industry to screen both new potential sources of bioactive ingredients and the quality of their raw materials.

MATERIALS AND METHODS

Materials. All solvents used for high-performance liquid chromatography (HPLC) analyses were HPLC-grade. Methanol was purchased from VWR (Strasbourg, France), and acetonitrile was purchased from Sigma-Aldrich (Steinheim, Germany). Chloroform (Sigma-Aldrich, Steinheim, Germany), methanol, and *n*-heptane (Carlo Erba, Val de Reuil, France) used for lipid extraction and PC purification were of analytical grade. Wash solution was prepared from sodium chloride of analytical grade and Ultrapure water (Millipore, Molsheim, France). Soy PC standard was purchased from Avanti Polar Lipids (Alabaster, AL). Hens' eggs (6.5% PC), soybeans (*Glycine max*) (0.7% PC), and ox liver (1.7% PC) were purchased from a local retailer. Krill oil (29.0% PC) has been kindly provided by Nestec SA (Lausanne, Switzerland).

Sample Preparation and Lipid Extraction. Preparation was different for each sample depending upon its physical state. Both soy beans and ox liver were grinded, but only ox liver was lyophilized after grinding. Egg yolk was lyophilized but without grinding. Krill oil was fractionated and analyzed as such without further preparation. Grinding was achieved under cryogenic conditions (liquid nitrogen, 3 steps of 5 min) using a 6870 Freezer/Mill (Spex CertiPrep, Stanmore, U.K.). Total lipids were extracted according to Folch et al. (*12*), with minor modifications. A total of 1 g of the treated food matrix was suspended in 30 mL of a $CHCl_3/CH_3OH$ (2:1, v/v) mixture and shaken mechanically for 20 min. The suspension was centrifuged at 8500*g* for 10 min, and the supernatant was removed and washed with 5 mL of sodium chloride aqueous solution (1%, w/v). The organic phase containing the lipid fraction was removed, evaporated under vacuum, and dried under a gentle stream of N_2. The total lipid extract was weighed and dissolved in 1 mL of $CHCl_3$.

Liquid Chromatography. *Flash Chromatography.* A 150 mg aliquot of the total lipid extract (from krill oil, soy, or egg) was subjected to preparative flash chromatography (CombiFlash retrieve, Serlabo, Entraigues sur la Sorgue, France) onto a 15 g silica cartridge preconditioned with *n*-heptane. Elution was performed at a flow rate of 10 mL/min using 60 mL of $CHCl_3$, then 120 mL of $CHCl_3/CH_3OH/H_2O$ (35:65:4, v/v/v) mixture, and finally 120 mL of ethanol for a column wash. Fractions (10 mL) were collected and tested by thin-layer chromatography (TLC) using silica plates, $CHCl_3/CH_3OH/H_2O$ (35:65:4, v/v/v) as an eluent, and phosphomolybdic acid in ethanol (20:80, v/v) to visualize compounds. PC was identified in comparison of its retention factor (R_f) to the one of soy PC (Avanti Polar Lipids, Alabaster, AL) as a reference. Fractions containing pure PC were pooled and evaporated under vacuum.

HPLC. A chromatographic system made of a 616 controller, a 2424 ELS detector, and a 717Plus autosampler (Waters, Saint Quentin Fallavier, France) was used to perform the chromatographic analyses. High-purity nitrogen from a nitrogen generator (Domnik Hunter, Villefranche-sur-Saône, France) was used as a nebulizing gas at a pressure of 45 psi. The drift tube temperature was set at 45 °C. Separations were performed at room temperature using a 20 min linear gradient ranging from $CHCl_3/CH_3OH$ (88:12, v/v) to $CHCl_3/CH_3OH$/1 M formic acid adjusted to pH 3 with triethylamine (28:60:12, v/v/v) (*13*). This chromatographic system and this gradient were both used for PC purification and to check purity of the isolated PC.

To isolate PC from ox liver, a 100 mg aliquot of the ox liver lipidic extract was injected on a 250 × 10 mm, 10 μm normal-phase Lichrospher column (Interchim, Montluçon, France). A total of $^2/_3$ of the mobile-phase flow, which was set at a rate of 2 mL/min, was diverted, ahead of the ELSD inlet, to a fraction collector. The PC peak was identified in comparison of its retention time to the standard one, and time windows were set to collect the PC peak.

The purity of PC isolated from each matrix was checked by injecting a fraction of the purified samples on a 150 × 3 mm, 3 μm Luna normal phase (Phenomenex, Le Pecq, France) using a flow rate of 0.5 mL/min. Purity (*P*) of the isolated PC was calculated as follows:

$$P = \frac{A_{PC}}{A_t} \times 100$$

where A_{PC} is the PC peak area and A_t is the total of peak areas on the chromatogram.

Separation and Identification of Molecular Species by LC−ESI−MS. Molecular species of PC were determined using a Prostar HPLC system made of two 210 solvent delivery modules, a 410 autosampler, and a 1200 L triple quadrupole mass spectrometer fitted with an ESI source (Varian, Les Ulis, France). High-purity nitrogen (Domnik Hunter) was used as a nebulizing gas, set at 46 psi, and as a drying gas, set at 300 °C. Separation was performed on a C18 reverse-phase 250 × 3 mm, 3 μm Isis Nucleodur column (Macherey-Nagel) using an isocratic flow of CH_3CN/CH_3OH (40:60, v/v) containing 0.1% NH_4OH at a rate of 1 mL/min. A split system however allowed the HPLC effluent to enter the mass spectrometer at a flow rate of 0.2 mL/min. To help the identification of the molecular species, 3 mL of 10 mM lithium formate was added to 1 L of mobile phase. Major detected ions were $[M + Li]^+$ referring to the molecular ion with a lithium adduct. Acquisition was performed in positive mode in a mass range between *m/z* 450 and 900. *m/z* ($[M + Li]^+$) were fragmented in positive mode (MS^2), which was used to identify FA linked to the PC glycerol backbone. The mass range was set between *m/z* 200 and 600 for fragmentation products. The dwell time was set to 0.2 s for each *m/z*. Argon was used as a collision gas, and collision energy was set to 30 V. The percentage of each molecular species was calculated as follows:

$$\% = \frac{A(\text{peak}(m/z))}{\sum A(\text{peaks})} \times 100$$

where $A(\text{peak}(m/z))$ refers to the peak area of the selected *m/z* and $\sum A(\text{peaks})$ refers to the sum of all peak areas. Analyses were performed in triplicate, with results expressed as mean ± standard deviation (SD).

Gas Chromatographic Analysis of FA. A 3400 Varian gas chromatograph equipped with a flame ionization detector was used for the analysis of FA methyl esters (FAMEs) after transesterification of

Table 1. FA Profiles of Purified PC from Soybeans, Egg Yolk, Krill Oil, and Ox Liver[a]

FAs	soy PC (%)[b]	egg yolk PC (%)[b]	krill oil PC (%)[b]	ox liver PC (%)[b]
14:0	nd[c]	nd	2.2 ± 0.09	nd
15:0	nd	0.3 ± 0.01	0.4 ± 0.02	nd
16:0	19.9 ± 0.03	34.1 ± 0.2	26.8 ± 0.1	20.3 ± 0.6
16:1 (n-7)	nd	1.6 ± 0.03	1.8 ± 0.08	nd
17:0	0.2 ± 0.01	nd	0.2 ± 0.06	0.5 ± 0.4
18:0	4.8 ± 0.02	13.6 ± 0.2	1.6 ± 0.05	36.4 ± 0.02
18:1 (n-9)	9.4 ± 0.08	29.3 ± 0.4	5.7 ± 0.03	11.7 ± 0.5
18:2 (n-6)	57.6 ± 0.10	16.0 ± 0.2	3.0 ± 0.02	21.2 ± 0.7
18:3 (n-3)	6.6 ± 0.01	nd	1.7 ± 0.01	nd
20:0	nd	nd	0.1 ± 0.05	nd
20:1 (n-11)	nd	nd	0.6 ± 0.01	1.5 ± 0.08
20:2 (n-6)	nd	nd	2.4 ± 0.01	nd
20:3 (n-3)	nd	nd	nd	2.5 ± 0.07
20:4 (n-6)	nd	4.5 ± 0.02	0.6 ± 0.01	5.8 ± 0.2
22:0	0.8 ± 0.01	nd	nd	nd
22:1 (n-13)	nd	nd	1.3 ± 0.02	nd
22:2 (n-6)	nd	nd	0.8 ± 0.01	nd
24:0	0.5 ± 0.01	nd	nd	nd
24:1 (n-9)	nd	nd	0.5 ± 0.01	nd
20:5 (n-3)	nd	nd	34.1 ± 0.07	nd
22:6 (n-3)	0.3 ± 0.01	0.8 ± 0.7	16.4 ± 0.1	nd
∑ saturated FAs	26.1	48.0	31.2	57.2
∑ monounsaturated FAs	9.4	30.9	9.9	13.3
∑ PUFAs	64.5	21.1	59.0	29.5

[a] Results ($n = 3$) are expressed as mean ± SD. [b] Percentage of the total peak area of FAs. [c] nd = not detected.

purified PC. Separation was made by a CP Sil 88 column [(88% cyanopropyl)-arylpolysiloxane, 100 m × 0.25 mm, 0.2 μm, Varian]. Samples (1 μL) were injected. Carrier gas was helium of high purity (99.9995%). The injector and detector were set to 230 °C, and the column was set at 60 °C, held for 5 min, and raised to 165 °C (rate of 15 °C/min). The temperature of 165 °C was held for 1 min and then raised to 225 °C (rate of 2 °C/min). The final temperature was maintained for 30 min. Peaks were identified by a comparison to standards (FAME mix C4−C24, Sigma-Aldrich, Saint-Quentin-Fallavier, France). Purified PC were transesterified using KOH (0.5 M) in CH_3OH by vortexing for 2 min at room temperature. After decantation, FAME were extracted using *n*-heptane. Gas chromatography (GC) data were normalized, and the percentage of each FA was calculated as follows:

$$\% = \frac{A(\text{peak}(m/z))}{\sum A(\text{peaks})} \times 100$$

where A(peak) refers to the area of each identified peak and $\sum A$(peaks) refers to the sum of areas of each identified peak. Each analysis was performed in triplicate. All results are expressed as mean ± SD.

RESULTS AND DISCUSSION

Purification of PC. PC was separated from non-polar lipids and other PLs using either flash chromatography or preparative HPLC. While flash chromatography was fast and easy, it was ineffective with food samples containing phosphatidylserine or sphingomyeline. Preparative HPLC on the other hand allowed for the isolation of PC from such samples as ox liver for instance, which contained both of these PLs. After each chromatographic step, fractions containing PC were pooled and purity was checked by HPLC−evaporative light-scattering detector (ELSD). Purity levels for PC isolated from each food sample were as follows: 98.7% for egg yolk, 98.0% for krill oil, 95.0% for soy, and 90.1% for ox liver. These levels were sufficient for the subsequent determinations of FA profiles and molecular species in PC samples.

FA Profiles of Purified PC. Table 1 shows FA profiles of PC purified from the studied food matrices as determined from the normalized data of GC−FID analysis. Soy PC was naturally shown to contain higher proportions of unsaturated FA, as compared to PC from animal sources. For instance, 18:2 (57.6%) and 18:3 (6.6%) were particularly abundant in soy PC. Some saturated FA, such as 22:0 (0.8%) and 24:0 (0.5%), were also present in soy PC, while they were not found in PC from egg yolk, ox liver, and krill oil. Conversely, 20:4 was identified in egg yolk PC (4.5%) and ox liver PC (5.8%), while it was not detected in soy PC.

As far as egg yolk PC is concerned, 16:0 (34.1%) and 18:1 (29.3%) were the two main FA identified, followed by 18:2 (16.0%) and 18:0 (13.6%) (**Table 1**), which is in agreement with a previous report (*14*). The same FAs were predominant in ox liver but at different levels: 18:0 (36.4%), 18:2 (21.2%), 16:0 (20.3%), and 18:1 (11.7%). Minor FA were 20:1 (1.5%) and 20:3 (2.5%) in ox liver and 16:1 (1.6%) and DHA (0.8%) in egg yolk.

Krill oil PC contained the largest variety of FAs in comparison to other matrices but with EPA (34.1%), 16:0 (26.8%), and DHA (16.4%) being the three major FAs. This result is in accordance

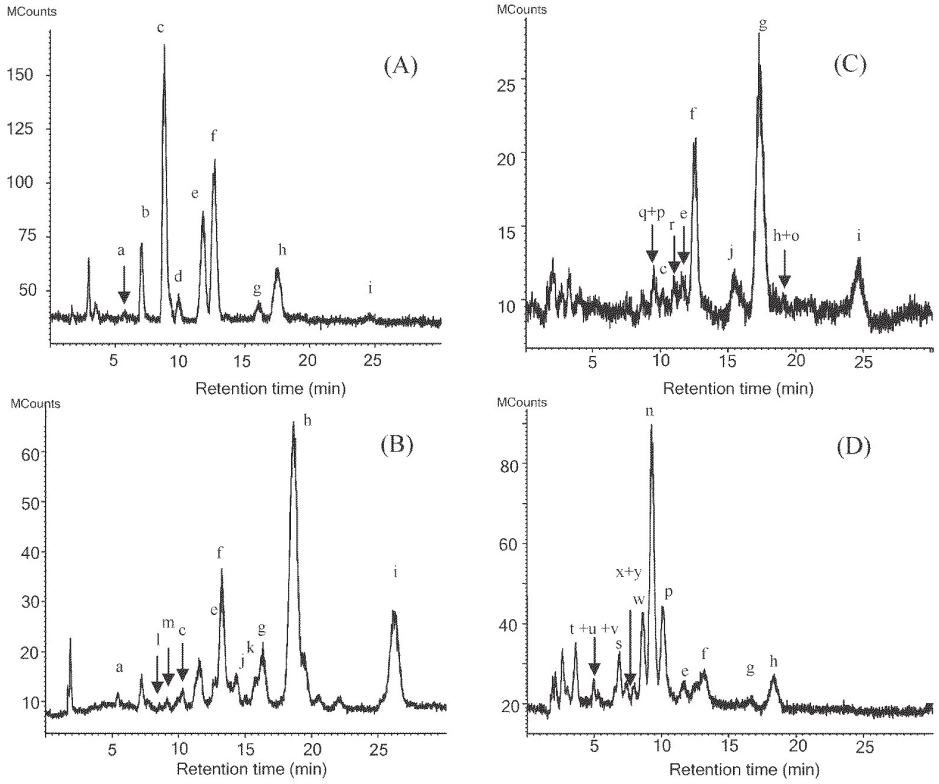

Figure 1. Chromatograms of purified PC: (A) soy, (B) ox liver, (C) egg yolk, and (D) krill oil using LC−ESI−MS. Separation was performed using isocratic conditions: CH_3CN/CH_3OH (40:60) containing 0.1% NH_4OH at a flow rate of 1 mL/min onto a reverse-phase column (250 × 3 mm, 3 μm). Letters are assigned to major identified peaks: a, 18:3−18:3-PC; b, 18:3−18:2-PC; c, 18:2−18:2-PC; d, 16:0−18:3-PC; e, 18:1−18:2-PC; f, 16:0−18:2-PC; g, 16:0−18:1-PC; h, 18:0−18:2-PC + 18:1−18:1-PC; i, 18:0−18:1-PC; j, 18:0−20:4-PC; k, 18:0−20:3-PC; l, 18:1−20:4-PC; m, 18:2−20:3-PC; n, 16:0−20:5-PC; o, 16:0−18:0-PC; p, 16:0−22:6-PC; q, 18:1−22:6-PC; r, 18:0−22:6-PC; s, 14:0−20:5-PC; t, 20:5−20:5-PC; u, 20:5−22:6-PC; v, 22:6−22:6-PC; w, 18:1−20:5-PC; x, 18:0−20:5-PC; and y, 18:1−20:4-PC.

with a previous study showing that these three FAs were very abundant in krill oil (*15*), including krill PC (*14*). Saito et al. showed that, even if krill FA composition varied according to the period of capture, its PC was always rich in EPA, DHA, and 16:0 (*16*).

The relative rates of saturated, monounsaturated, and PUFAs were very different among food matrices (**Table 1**). PUFAs were most abundant in soy PC (64.5%) and in krill oil PC (59.0%) but with the latter containing far higher amounts of LC-PUFAs, namely, EPA and DHA, which is in line with the usual composition of food products from marine sources (*1*). Saturated FAs on the other hand were predominant in animal source foods: 31.2% in krill oil PC, 48.0% in egg yolk PC, and 57.2% in ox liver PC. These data on FA distribution profiles in purified PC were later used in a comparative analysis of the molecular species determinations to check if the identified structures in each PC matched its determined FA composition.

PC Molecular Species. *Chromatographic Separation.* Separation was achieved using a 3 μm column, and MS chromatograms are given in **Figure 1**. With less than 10 chromatographic peaks, soy PC (**Figure 1A**) and egg yolk PC (**Figure 1C**) had a simple composition compared to ox liver PC (**Figure 1B**) and krill PC (**Figure 1D**), which showed at least 15 peaks. Judging by the retention times, the same molecular species could be present in different foods. It is particularly true for the animal source ones: egg yolk and ox liver, where several peaks have the same retention times.

Identification of PC Molecular Species. Molecular species of PC were detected as m/z of lithium adducts $[M + Li]^+$. Lithium was added to the chromatographic mobile phase and was shown not to interfere with the separation, which allowed for the combination of an effective chromatographic separation and an improved structural identification. Lithium has already been used in previous studies to identify molecular species of PC but with no chromatographic separation beforehand (*17*–*19*). To identify PC molecular species, lithium adducts were fragmented in MS^2, which was previously shown to yield three main fragments: $[MLi - FA]^+$ corresponding to the loss of a FA group, $[MLi - FALi]^+$ corresponding to the loss of a lithium salt of an FA group, and $[MLi - TMA - FA]^+$ corresponding to the loss of a trimethylamine group (TMA, 59 Da) and a FA group (*17*). The obtained fragmentation patterns of lithium adducts proved in fact to be effective in identifying various PC species, including symmetrical, asymmetrical, saturated, and unsaturated ones (*17*). The identification performed in this study did not take into account the position of the FA in the glycerol moiety and was in most cases tentative.

Representative MS^2 fragmentation spectra of the molecular species of PC are presented in **Figure 2**.

Figure 2a shows the MS^2 spectrum of the species m/z 764 (RT = 13.2 min). One of its two FAs was identified as 16:0 based on m/z 449 and 502, which correspond respectively to fragments $[MLi - (16:0) - TMA]^+$ and $[MLi - (16:0)Li]^+$. The second FA was identified as 18:2 based on m/z 484, which corresponds to fragment $[MLi-(18:2)]^+$. An additional fragment, m/z 575, matched the mass of the PC minus its phosphocholine head with a lithium adduct (*17*). Consequently, the molecule detected as a lithium adduct with m/z 764 (RT = 13.2 min) was identified as $[(16:0-18:2)PC + Li]^+$.

Figure 2b shows the MS^2 fragmentation spectrum of the molecular species m/z 812 (RT = 8.6 min), which was identified as $[(16:0-22:6)PC + Li]^+$. The two ions obtained, m/z 478 and 556, corresponded in fact respectively to fragments $[MLi - (22:6)-Li]^+$ and $[MLi - (16:0)]^+$, showing DHA and 16:0 as the two FAs of the molecule.

A third example is given in **Figure 2c** with the MS^2 spectrum of m/z 784 (RT = 6.0 min) containing three fragments that allowed

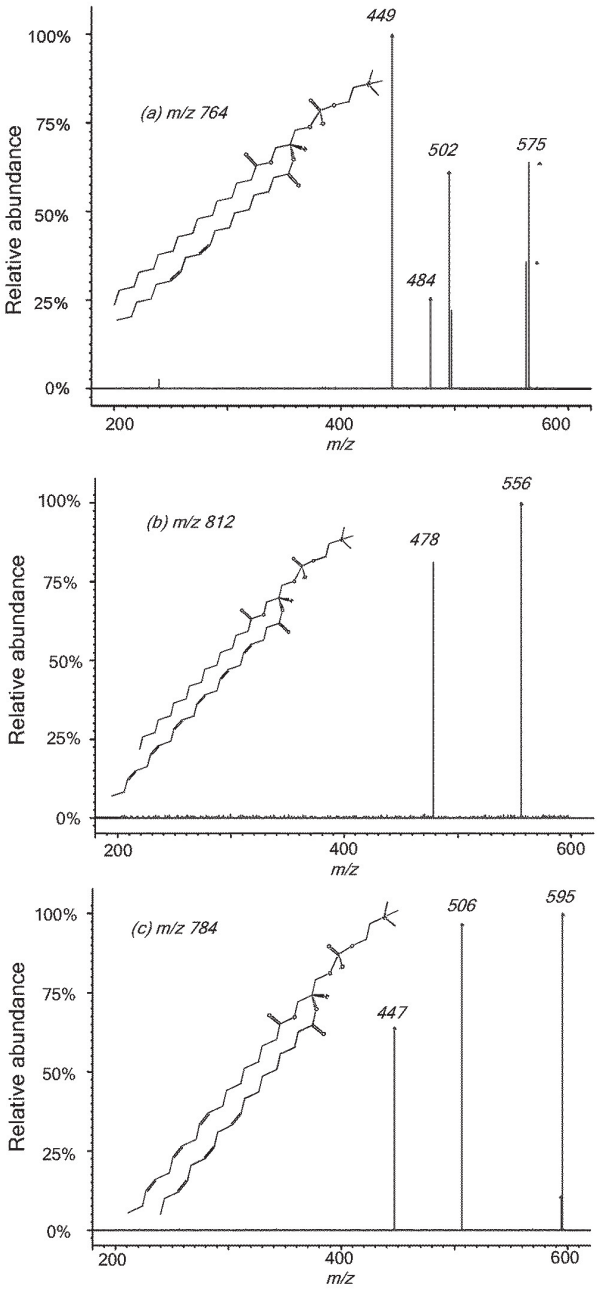

Figure 2. Ions obtained in positive mode in ESI–MS^2 between 200 and 600 from fragmentation of lithiated adducts: (a) fragmentation of m/z 764, (b) fragmentation of m/z 812, and (c) fragmentation of m/z 784 and their corresponding structures.

for its identification as $[(18:3-18:3)PC + Li]^+$. Two of these fragments, m/z 506 and 447, were identified as $[MLi-(18:3)]^+$ and $[MLi - TMA - (18:3)]^+$, which pointed to 18:3 as the sole FA. The third fragment, m/z 595, corresponded, as above for m/z 575, to the loss of the phosphocholine head with a lithium adduct. This approach was applied to each m/z detected with purified PC samples and allowed for the identification of the constitutive molecular species for each food investigated.

PC Molecular Species Profiles. **Table 2** gives the relative distributions of the various PC molecular species in the foods investigated. As far as soy PC is concerned, the major species was

Table 2. Molecular Species Profiles of Each Purified PC[a]

m/z [M + Li]	structure[b]	retention time (min)	soy PC (%)[c]	egg yolk PC (%)[c]	krill oil PC (%)[c]	ox liver PC (%)[c]
738.6	(16:0−16:1)PC	11.5	nd[d]	nd	2.7 ± 0.1	nd
740.6	(16:0−16:0)PC	17.4	0.9 ± 0.2	nd	nd	0.7 ± 0.0
758.6	(14:0−20:5)PC	6.5	nd	nd	3.2 ± 0.0	nd
	(14:1−20:4)PC	6.0	nd	nd	nd	0.2 ± 0.0
762.6	(16:0−18:3)PC	10.4	2.8 ± 0.4	nd	nd	nd
764.6	(16:0−18:2)PC	13.2	20.8 ± 10	20.2 ± 0.7	2.6 ± 0.0	10.9 ± 0.2
	(16:1−18:1)PC	13.6	nd	nd	1.1 ± 0.1	nd
766.6	(16:0−18:1)PC	15.6	5.5 ± 1.5	39.7 ± 1.9	5.9 ± 0.1	9.1 ± 0.2
768.6	(16:0−18:0)PC	17.5	nd	4.5 ± 0.1	nd	nd
778.6	(17:0−18:2)PC	15.5	nd	nd	nd	0.7 ± 0.3
780.6	(17:0−18:1)PC	20.9	nd	nd	nd	0.9 ± 0.0
784.6	(18:3−18:3)PC	6.0	0.7 ± 0.2	nd	nd	0.2 ± 0.0
	(14:0−22:6)PC	5.8	nd	nd	1.4 ± 0.0	nd
	(16:1−20:5)PC	6.5	nd	nd	1.3 ± 0.3	nd
786.6	(18:2−18:3)PC	8.6	7.7 ± 1.2	nd	nd	nd
	(16:0−20:5)PC	8.1	nd	nd	32.4 ± 0.3	0.3 ± 0.0
788.6	(18:2−18:2)PC	11.0	34.0 ± 2.0	3.3 ± 0.2	1.0 ± 0.1	3.3 ± 0.3
	(16:0−20:4)PC	8.7	nd	nd	4.1 ± 0.1	nd
790.6	(18:1−18:2)PC	13.3	16.3 ± 0.2	3.1 ± 0.3	1.0 ± 0.1	3.5 ± 0.4
	(16:0−20:3)PC	12.6	nd	nd	4.1 ± 0.1	2.8 ± 0.4
792.6	(18:0−18:2)PC	18.7	6.3 ± 0.3	8.3 ± 0.7	6.6 ± 0.9	34.3 ± 0.5
	(18:1−18:1)PC	17.8	3.2 ± 0.4	3.3 ± 0.7	nd	nd
794.6	(18:0−18:1)PC	22.4	1.6 ± 0.2	8.9 ± 0.5	nd	15.9 ± 0.5
812.6	(16:0−22:6)PC	8.6	nd	2.8 ± 0.1	11.9 ± 0.0	nd
	(18:2−20:4)PC	8.2	nd	nd	nd	0.4 ± 0.0
	(18:1−20:5)PC	7.5	nd	nd	7.4 ± 0.4	nd
814.6	(18:0−20:5)PC	9.1	nd	nd	1.3 ± 0.4	nd
	(18:1−20:4)PC	9.7	nd	nd	1.9 ± 0.1	1.6 ± 0.6
	(18:2−20:3)PC	10.8	nd	nd	nd	1.2 ± 0.7
816.6	(18:0−20:4)PC	15.5	nd	4.5 ± 0.3	nd	5.7 ± 0.3
	(18:1−20:3)PC	15.7	nd	nd	nd	
818.6	(18:0−20:3)PC	16.0	nd	nd	nd	5.5 ± 0.2
	(18:2−20:1)PC	16.4	nd	nd	nd	
832.6	(20:5−20:5)PC	4.4	nd	nd	1.7 ± 0.0	nd
836.6	(20:4−20:4)PC	9.4	nd	nd	nd	0.1 ± 0.1
838.6	(18:1−22:6)PC	8.9	nd	0.3 ± 0.6	nd	nd
840.6	(18:0−22:6)PC	12.0	nd	1.1 ± 0.3	1.1 ± 0.1	nd
	(20:3−20:3)PC	13.2	nd	nd	nd	0.8 ± 0.0
	(20:1−20:5)PC	8.2	nd	nd	nd	nd
842.6	(20:4−20:1)PC	15.2	nd	nd	nd	2.21 ± 0.2
858.6	(20:5−22:6)PC	4.6	nd	nd	2.3 ± 0.0	nd
884.6	(22:6−22:6)PC	5.0	nd	nd	0.8 ± 0.0	nd

[a] Results ($n = 3$) are expressed as mean ± SD. [b] The position of fatty acids in the glycerol moiety has not been determined. [c] Percentages of all identified molecular species. [d] nd = not detected.

(18:2−18:2)PC (34.0%), followed by (16:0−18:2)PC (20.8%) and (18:1−18:2)PC (16.3%), while minor ones included (18:0−18:2)-PC (3.2%), (16:0−18:3)PC (2.8%), (18:0−18:1)PC (1.6%), and (18:3−18:3)PC (0.7%). Similar patterns have already been reported for soy PC (*20*−*22*). Species determination data for soy PC is in agreement with its FA profile (**Table 1**), with high amounts of 16:0, 18:0, 18:1, 18:2, and 18:3 and 18:2 being by far the predominant one.

The major molecular species in egg yolk PC was identified as (16:0−18:1)PC (39.7%), followed by (16:0−18:2)PC (20.2%), (18:0−18:2)PC (8.9%), and (18:0−18:1)PC (8.3%). Minor species included (18:2−18:2)PC (3.3%), (18:1−18:2)PC (3.1%), (16:0−22:6)PC (2.8%), and (18:0−22:6)PC (1.1%). The data reported here for egg yolk are to a certain extent in agreement with a previous report by Pacetti et al. (*14*), essentially showing a similar pattern and (16:0−18:1)PC as the predominant species. Some quantitative differences that were observed, especially with minor molecular species, may be attributed to differences in hens' diet, which affects FA composition. On the basis of the PC molecular species, 16:0 is the most abundant FA, being part of two species that accounted for ca. 60% of all species. This is in accordance with the FA profile of egg yolk PC, which showed that 16:0 represented 34.1% of the total FA content (**Table 1**). To a lesser extent, a similar observation was made for 18:1.

The four main molecular species in ox liver were the same as in the other animal source food, egg yolk, albeit in different proportions: (18:0−18:2)PC (34.3%), (18:0−18:1)PC (15.9%), (16:0−18:2)PC (10.9%), and (16:0−18:1)PC (9.1%). This matched the FA pattern, which showed 18:0 (36.4%), 18:2 (21.2%), 16:0 (20.3%), and 18:1 (11.7%) as the dominant FAs in ox liver (**Table 1**). Bovine liver PC molecular species have been determined by Bang et al. (*20*) and Dobson et al. (*23*). With a content of 21.59%, (18:0−21:3)PC was found by Bang et al. to be the major molecular species. What is striking about this identification is that 21:3, which was not present in our determinations, is an extremely rare FA. Dobson et al. on the other hand showed a pattern much closer to our findings, with (18:0−18:2)PC, (18:0−18:1)PC, and (16:0−18:1)PC being the main species and no (18:0−21:3)PC being detected (*23*). These remarks let us think that the identification performed by Bang et al. is probably

wrong. Minor species found in ox liver included (18:0−20:3)-PC + (18:2−20:1)PC (5.5%), (18:0−20:4)PC + (18:1−20:3)PC (5.7%), (18:1−18:2)PC (3.5%), (16:0−20:4)PC (3.3%), and (16:0−20:3)PC (2.8%) and were essentially the same as those identified in other studies. Some few differences observed with minor molecular species could be ascribed to the animal origin and diet, which affect the FA content (*14*). For molecular species that could not be adequately separated by chromatography, e.g., (18:0−20:3)PC and (18:2−20:1)PC, the calculated content took the two structures into account (**Table 2**).

As far as krill oil is concerned, to our knowledge, this is the first time the identification of PL molecular species is investigated, although FA profiles have already been determined for phosphatidylethanolamine, PC, and TAG in krill (*Euphausia pacifica*) (*16*). The major PC molecular species was determined as (16:0−20:5)-PC and represented 32.4% of all PC. Other species, such as (16:0−22:6)PC (11.9%), (18:1−20:5)PC (7.4%), (18:0−18:2)PC (6.6%), (16:0−18:1)PC (5.9%), and (16:0−20:4)PC (4.1%), were less abundant. Once again, the patterns of molecular species and FA coincided, because both determinations indicated that EPA, DHA, and 16:0 were the predominant FAs. Interestingly, minor molecular species of krill oil PC included two [(14:0−20:5)PC (3.2%) and (14:0−22:6)PC (1.4%)] with 14:0, the presence of which is a common feature of marine oils.

In this study, an effective method of separation and identification of PC molecular species was reported. This method used lithium formate to form lithiated adducts. MS^2 fragmentation of these adducts allowed for the identification of FA linked to the glycerol backbone of PC molecular species. As an example of application, the relative contents of individual molecular species were determined, which allowed for a sound comparison of PC structures from foods of plant, animal, and marine origins, represented by soy, ox liver, egg yolk, and krill oil. Soy lecithin, mainly made of PC and already in use as a food additive and a nutritional complement, was therefore shown to be highly rich in essential FAs but not in their LC-PUFA metabolites. Krill oil PC showed an opposite pattern in comparison to soy PC, with very low amounts of 18:2 or 18:3 detected (< 5%) but with EPA and DHA being the most abundant among the foods tested (> 50%). This study developed is an interesting contribution to the investigation of new sources rich in LC-PUFA−PLs. As an example, krill oil was shown as a potential source for food supplementation with EPA and DHA.

ABBREVIATIONS USED

LC-PUFA, long-chain polyunsaturated fatty acid; EPA or 20:5 (n-3), eicosapentaenoic acid; DHA or 22:6 (n-3), docosahexaenoic acid; PL, phospholipid; PUFA−PL, PUFA-rich PL; PC, phosphatidylcholine; TAG, triacylglycerol; LC, liquid chromatography; ESI, electrospray ionization; MS, mass spectrometry; ELSD, evaporative light-scattering detector; GC, gas chromatography; FID, flame ionization detector; 14:0, myristic acid; 16:0, palmitic acid; 16:1 (n-7), palmitoleic acid; 17:0, heptadecanoic acid; 18:0, stearic acid; 18:1 (n-9), oleic acid; 18:2 (n-6), linoleic acid; 18:3 (n-3), α-linolenic acid; 20:1 (n-11), gadoleic acid; 20:3 (n-3), dihomo-γ-linolenic acid; 20:4 (n-6), arachidonic acid; 22:0, behenic acid; 24:0, lignoceric acid.

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