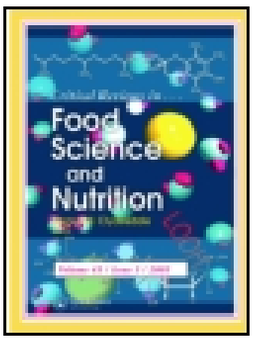

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Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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To cite this article: Abdelmoneim H. Ali , Xiaoqiang Zou, Sherif M. Abed, Sameh A. Korma, Qingzhe Jin & Xingguo Wang (2017): Natural phospholipids: occurrence, biosynthesis, separation, identification, and beneficial health aspects, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2017.1363714

To link to this article: http://dx.doi.org/10.1080/10408398.2017.1363714

Accepted author version posted online: 18 Aug 2017.

Natural phospholipids: occurrence, biosynthesis, separation, identification, and beneficial health aspects

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Abstract

During the last years, phospholipids (PLs) have attracted great attention because of their crucial roles in providing nutritional values, technological and medical applications. There are considerable proofs that PLs have unique nutritional benefits on human health, such as reducing cholesterol absorption, improving liver functions, and decreasing the risk of cardiovascular

diseases. PLs are the main structural lipid components of cell and organelle membranes in all living organisms, and therefore, they occur in all organisms and the derived food products. PLs are distinguished by the presence of a hydrophilic head and a hydrophobic tail, consequently they possess amphiphilic features. Due to their unique characteristics, the extraction, separation, and identification of PLs are critical issues to be concerned. This review is focused on the content of PLs classes in several sources (including milk, vegetable oils, egg yolk, and mitochondria). As well, it highlights PLs biosynthesis, and the methodologies applied for PLs extraction and separation, such as solvent extraction and solid-phase extraction. In addition, the determination and quantification of PLs classes by using thin layer chromatography, high-performance liquid chromatography coupled with different detectors, and nuclear magnetic resonance spectroscopy techniques.

Keywords

phospholipids, biosynthesis, separation, identification, beneficial health aspects

Abbreviations

PLs	Phospholipids
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PS	phosphatidylserine
SM	sphingomyelin
PA	phosphatidic acid
MFGM	milk fat globule membrane
LPC	lysophosphatidylcholine
CL	cardiolipins
LPE	lysophosphatidylethanolamine
CDP	cytidine diphosphate
DAG	diacylglycerol
SPE	solid-phase extraction
TLC	thin layer chromatography

SC-CO_2 supercritical carbon dioxide

HPLC high-performance liquid chromatography

ELSD evaporative light scattering detector

GC gas chromatography

DAD diode-array detection

UV ultraviolet

MS mass spectrometry

CAD charged aerosol detector

LC-MS liquid chromatograph-mass spectrometry

MS-MS mass spectrometry-mass spectrometry

ESI electrospray ionization

HPLC-ESI-Q-TOF-MS high-performance liquid chromatography-electrospray ionization-quadrupole time-of-flight-mass spectrometry

HILIC hydrophilic interaction liquid chromatography

LPCC low-pressure column chromatography

NMR nuclear magnetic resonance

1. Introduction

Phospholipids (PLs) are one of the basic components of natural membranes. They can be defined as a class of lipids containing phosphorus in their structure. In most cases, they are esters of phosphoric acid (H_3PO_4), and sometimes esters of phosphonic acid (H_3PO_3). PLs exist in several sources among them, foods with the naturally high content of PLs are vegetable oils, egg yolk, brain, milk, some guts, meat, and fish (Weihrauch and Son, 1983). PLs are distinguished by the presence of a hydrophobic tail and a hydrophilic head, and therefore they demonstrate amphiphilic characteristics. The roles, attitudes, applications, and functions of PLs would be affected by these characteristics. PLs belong to the group of polar lipids and, literally, they can be characterized as "phosphorus lipids" (Contarini and Povolo, 2013).

Concerning PLs classification, there are numerous classification methods, and there is no common harmony on the ideal manner for PLs classification, but most of these classifications include two major categories or groups based on the type of alcohol backbone. The first group is glycerol-containing PLs (glycerophospholipids), and the second group is sphingolipids (sphingophospholipids) (Erickson, 2008;Sikorski and Kolakowska, 2003). Glycerophospholipids are composed of one molecule of glycerol with a polar headgroup, and two fatty acids esterified at the *sn*-1 and *sn*-2 positions of the glycerol backbone. This group includes phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) (Huang, 2001). On the other hand, sphingolipids are a class of lipids containing a backbone of sphingoid bases, a set of aliphatic amino alcohols that includes sphingosine. Sphingomyelin (SM) is the most important species, which composed of a phosphorylcholine head-group and a

fatty acid linked to the amide nitrogen of the long chain sphingoid base (Cevc and Paltauf, 1995). From the technological point of view, PLs are classified into hydratable and non-hydratable. The classes of PC, PI, and lyso-PLs are simply hydratable, and PE is relatively hydratable. Phosphatidic acid (PA) is classified as a non-hydratable species (Subramanian et al., 1999; Zufarov et al., 2009). The chemical structure of the major classes of PLs is shown in **Fig. 1**.

2. Sources of Phospholipids

2.1. Milk and dairy products

Polar lipids have significant functional roles in milk and dairy products for the emulsification and diffusion of milk fat in the aqueous phase, since together with milk proteins, they are the major components of the milk fat globule membrane (MFGM), which surrounds the lipid droplets secreted by the mammary gland cells, and protects fat droplets from separation on the surface of milk forming the creamy layer. MFGM lipids are composed of triglycerides, PLs, and cholesterol in different concentrations (Fox and McSweeney, 1998; Keenan et al., 1983).

Approximately 60−65% of milk PLs are linked with the whole intact MFGM. The other 35−40% are existed in the aqueous phase and correlated with fragment material of protein membranes in the solution instead of still attached to the membrane (Huang and Kuksis, 1967; Patton and Keenan, 1971). MFGM is a trilaminar surrounding the intracellular neutral lipids, and mainly composed of PLs and lipoproteins. The internal layer is surrounded by a bilayer membrane derived from the secretory cell apical plasma membrane (Evers et al., 2008). Concerning the structure of lipids, they considered asymmetrically arranged. PLs are mostly concentrated in the external leaflet, and organized as a liquid-disordered phase synchronized

with a liquid-ordered phase (called lipid rafts), the latter contains higher quantities of SM and cholesterol (Gallier et al., 2010; Lopez et al., 2010). PLs containing choline head-group in their structure, such as PC, SM, and the glycolipids, gangliosides, and cerebrosides are mainly positioned on the external surface of fat globules membrane. Conversely, PE, PS, and PI are mainly located on the internal surface of the membranes (Deeth, 1997).

The percentage of PLs in milk fat typically ranged between 0.5 and 1.0% of the total lipids (Patton and Jensen, 1976). The contents of the total polar lipids and the detailed classes of PLs in different types of milk and a variety of dairy products are presented in **Tables 1, 2**. Expressed as a percentage of the total PLs in milk, PE (26.4−72.3%), PC (8.0−45.5%) and SM (4.1−29.2%) followed by PS (2.0−16.1%) and PI (1.4−14.1%) are the predominant classes (Graves et al., 2007). This variation can be attributed to the differences in extraction and analysis methods, animal diet, and lactation season (Lopez et al., 2008). Bitman and Wood (1990) reported that the content of milk PLs decreased gradually with the progress in lactation period. Kinsella and Houghton (1975) detected a slight change in the profile of PLs, which was ascribed to the differences in animal feed, lactation season or other factors. Since there was a variation in the concentration of the total PLs, the ratio of the major classes remained relatively constant, proposing a constant percentage of PLs in the globule membranes. The profile of PLs in milk and dairy products strongly influenced by the properties of the raw material and the technological processes applied during the manufacturing process. PLs are mostly positioned on the MFGM, as a result, any process causes a disruption of the membrane and/or a separation or fractionation of fat globules, such as homogenization or centrifugation and/or of neutral and polar materials of milk fat could affect the profile and the distribution of PLs in the final product.

Consequently, due to the influence of technological processes, expressed as a percentage of milk total lipids, the content of polar lipids in cream is lower than their content in the skim milk. As well, cheese and butter have lower polar lipid contents than whey and buttermilk, respectively (Gallier et al., 2010; Rombaut et al., 2006).

2.2. Vegetable oils

PLs have an important role in the stability, quality and characteristics of fats, edible oils, and fatty foods through their anti-oxidative activity and/or contribution to the smoothness, the consistency, and the mouth-feel of foods (Carelli et al., 1997; Ohshima et al., 1993; Singleton, 1993). In addition, PLs can collaborate with tocopherols to delay the beginning of rancidity or lipids oxidation (Arranz et al., 2008; Carelli et al., 1997; Singleton et al., 1999). PLs also take part in the Maillard reaction, increasing the stability of oils at high temperatures, as well as contributing to the evolution of flavors and colors (Alaiz et al., 1995; Anjum et al., 2006). The concentration of PLs in the crude oils represents approximately 0.1−1.8%. The removing of PLs or gums increased the oxidative and flavor stability of vegetable oils through the elimination of iron and copper (Flider and Orthoefer, 1981; Goh et al., 1982). Consequently, determining phosphatides concentration throughout oil processing is needed by oil producers in order to investigate the effect of PLs on the properties of oils. Furthermore, PLs are responsible for the discoloration of oils during the processes of deodorization and steam distillation, and therefore the analysis of PLs has a significant role in the assessment of degumming process efficiency (Panagiotopoulou and Tsimidou, 2002). Several oils contain fundamental amounts of PLs, for instance peanut oil and sunflower oil. However, these compounds are presented with an average

of 1−2% of oils. The classes of PE, PI, PS, and PC were the most important compounds of lipids detected in the edible oils.

The content of PLs species in a variety of vegetable oils is shown in **Table 3**. PC, PE, PI, and PA are the most abundant fractions of sunflower oil PLs, with a total concentration less than 1.2% (Padley et al., 1986). PLs are natural components of oilseeds that pass to oil during the process of extraction. However, they are eliminated at the crushing plant by degumming with water, and almost completely removed from oils throughout refining. Researchers have reported that oils obtained by solvent extraction contained higher concentrations of PLs than those obtained by pressing, with particular higher contents of PE and PC (Brevedan et al., 2000; Carelli et al., 1997). Nevertheless, no considerable differences in the profile of PLs were observed. In addition, the water degumming process caused a decrease in the concentration of PLs, independently of the extraction methods. Degummed oils had higher concentrations of PA and lower concentrations of PC as compared to the original crude oils.

2.3. Egg yolk

During the last few years, researchers paid more attention for the separation and identification of egg yolk PLs, which was vast and not well-developed. Perfect isolation of PLs became a critical target for nutrition researchers. It was reported that PLs content of egg yolk composition ranged between 20 and 80%, and the percentage of glucose to PLs contained in the composition was 0.10% by weight or less (Narabe et al., 1999). Egg is a major source of PLs in the consumer diet. PLs represent about 10% of the wet weight of the egg yolk, equivalent to approximately 22% of egg yolk total solids. Therefore, the high-efficiency extraction and

purification of egg PLs are vital issues due to the economic importance and availability of these high-valued lipid products (Wang et al., 2014).

Generally, egg consists of three constituents; shell, albumen, and yolk. Lin and Lee (1996) reported that the main components of the whole egg are shell (11.0−11.1%), albumen (59.7−60.6%), and yolk (28.4−29.3%). Yolk solids, 59.0−64.4% of the total solids are constituted of 62.4−65.2% lipids, and 34.8−37.8% proteins. PLs represent about 33% of egg lipid fractions, 70% of these fractions are PC, 15.5% PE, 5.8% lysophosphatidylcholine (LPC), and about 2% each of SM and LPC (Ternes et al., 1994). **Table 4** presents the contents of PLs classes in egg products. PC contributes significantly in the absorption of intestinal lipid by promoting micelle lipid solubility and formation of chylomicrons. Regarding the other classes of PLs, egg yolk contained lower concentrations of PI, PS, cardiolipins (CL) and lysophosphatidylethanolamine (LPE) (Kuksis, 1992).

The results obtained by Sunwoo and Gujral (2015) revealed that PC and PE were the major classes of egg yolk PLs, which approximately represent 81% and 12% of egg yolk lecithin. In addition, LPC, LPE, and SM were detected in trace amounts. The major fatty acids associated with egg yolk PC were palmitic, stearic, oleic, and linoleic, representing 32, 26, 16, and 13%, respectively. Arachidonic acid and docosahexaenoic acid (4.8% and 4%, respectively) were also found in significant amounts.

2.4. Mitochondria

Mitochondria have an exceptional pattern of PLs (Fleischer et al., 1967). They are encircled by a double membrane with a specific combination of lipids and proteins. PLs have several

functional roles and considered one of the most vital constituents of mitochondria for the maintenance of membrane construction and appropriate enzyme activity (Kiebish et al., 2009; Paradies et al., 1993). Mitochondrial membranes PLs have an important role in the mitochondrial structure, the respiratory proteins activity, and the transportation of proteins into the mitochondria. Many researches have focused on the contribution of dietary fatty acids to the reordering of PLs and mitochondrial membrane homeostasis. Understanding the influence of PLs on the mitochondrial membranes and their metabolism would interpret the molecular mechanisms related to the organization of mitochondria functions and mitochondrial-correlated diseases (Schenkel and Bakovic, 2014).

Mitochondrial membranes are enriched in PLs and proteins that are required for mitochondrial biogenesis, maintenance of mitochondrial morphology, and the tubular network (Gohil and Greenberg, 2009). CL and PE are non-bilayer forming PLs in the mitochondrial membranes (Gonzalvez and Gottlieb, 2007) that play critical roles in the properties and functions of mitochondria. The content of PLs classes in mitochondria extracted from different sources is shown in **Table 5**. The variation of mitochondrial membranes PLs affected by the differences in the length and degree of unsaturation of fatty acyl residues presented within each class of PLs. Though, the effects of acyl chain composition of PLs on the functions of mitochondria need further investigations (Schenkel and Bakovic, 2014).

3. Phospholipid Biosynthesis

Among the different species of PLs in various matrices, PC is considered to be the most plentiful species. A variety of the other classes including PE, PS, SM, CL, in addition to PI and

their phosphorylated derivatives are also significant components of the cell membranes. PLs are unequally distributed in the different organelles, and thus raise the query about the arising of this distribution. There are certain mechanisms might be involved in creating the distinctive PLs compositions of organelle membranes, such as the location and the activity of the enzymes that synthesize and degrade PLs enriched in the organelle. In addition, distinguished pathways might exist for the transportation of specific PLs between the organelle that synthesizes PLs and the membrane in which the PLs are enriched (Vance, 2015).

The biosynthetic pathways of PLs classes are summarized in **Fig. 2**. PLs synthesis occurs in the endoplasmic reticulum and started by glycerol-3-phosphate acylation (or acylation and following dihydroxyacetone-phosphate reduction) to produce PA, from which all other PLs species are formed through the addition of a polar headgroup. The biosynthesis of PLs is controlled by numerous factors, among them water-soluble PLs precursors, lipids, nucleotides, phosphorylation, growth phase, and the availability of zinc (Carman and Henry, 1999; Greenberg and Lopes, 1996). The regulation of PLs synthesis by these factors is mediated by two mechanisms. Firstly, the biochemical mechanism, which controls enzyme activity directly, and the second is the genetic mechanism, which regulates gene expression and protein synthesis (Carman and Henry, 1999; Greenberg and Lopes, 1996).

PLs can be synthesized through two mechanisms. The first mechanism employs a cytidine diphosphate (CDP)-activated polar headgroup for attachment to the phosphate of PA. In the second mechanism, CDP-activated 1,2-diacylglycerol and an inactivated polar headgroup are utilized. The synthesis of mammalian PLs involves the gaining of a diacylglycerol (DAG) unit

which participated by either CDP-diacylglycerol or diacylglycerol *per se*. PA is synthesized through the consecutive addition of two fatty acyl-CoAs to glycerol-3-phosphate, a product of glycolysis. PA is the forerunner for CDP-DAG that is used in PI and CL synthesis, and dephosphorylated to DAG for PC and PE synthesis as well as triacylglycerol. Therefore, PA dephosphorylation to DAG is a key step that controls PLs biosynthesis and energy storage in the form of triacylglycerol (Ridgway and McLeod, 2015).

PS is produced by the PS synthase, which replaces cytidine monophosphate with serine. In mammalian cells, PS is synthesized by a calcium-dependent reaction in which the head group of PC or PE is replaced by serine. The synthesis pathway of PS includes an exchange reaction of serine for ethanolamine in PE. This exchange occurs at the time that PE located in the lipid bilayer of the membranes (Choi, 2007; Nikawa et al., 1987). The synthesis of PI comprises the condensation of CDP-activated 1,2-diacylglycerol with myo-inositol. PI afterward undergoes a sequence of phosphorylations of the hydroxyls of inositol resulted in polyphosphoinositides production. One polyphosphoinositide (PI 4,5-bisphosphate, PIP2) considered a critically significant membrane phospholipid related to signals transmission for cell growth and comparison from outside the cell to inside.

The synthesis of PE involves the activation of ethanolamine through phosphorylation, and then by conjugation with CDP. The ethanolamine is then transported from CDP-ethanolamine to PA to produce PE. The synthesis of PE can also be attained through PS decarboxylation with the contribution of the CDP-ethanolamine versus PS decarboxylation pathways being cell type dependent. PC is almost exclusively synthesized through the CDP-choline pathway in all

mammals' cell kinds (Walkey et al., 1998). Firstly, choline is activated by phosphorylation and then by coupling to CDP before attachment to PA. PC can likewise synthesized through choline addition to CDP-activated 1,2-diacylglycerol. Another pathway for PC synthesis requires PS or PE conversion to PC. The conversion of PS to PC first involves the decarboxylation of PS to produce PE, which then undergoes a sequence of three methylation reactions (McMaster and Bell, 1994a, b).

4. Separation of Phospholipids

There are significant differences in the methodologies applied for the extraction and separation of total lipids, including PLs, regarding the mixtures and the concentrations of solvents. The process of PLs separation includes the removing of proteins, sugars, and other components that might affect the determination of PLs. There are two major methods; a liquid-liquid extraction method, which often used to separate the total lipids from the rest of biological samples. The other method is the solid-phase extraction (SPE), which regularly used for additional purification of the concerned lipids. The most widespread procedure of liquid-liquid extraction of lipids was investigated by Folch et al. (1957). Numerous researchers have analyzed the composition of PLs in several foods (including dairy products, oils, and bakery products) following the Folch procedure by using chloroform/methanol (2:1, v/v), and a solvent-to-sample ratio of 20:1 (Donato et al., 2011; Dugo et al., 2013; Gallier et al., 2010; García-Márquez et al., 2013; Kiełbowicz et al., 2013; Narváez-Rivas et al., 2011; Pérez-Palacios et al., 2010a; Pérez-Palacios et al., 2010b). In this respect, the data reported by Pérez-Palacios et al. (2012) showed that when using the solvent-to-sample ratio of 20:1, a certain amount of PLs still non-extracted.

4.1. Separation of milk phospholipids

The Folch procedure is the most applied method for the extraction and purification of lipids. Although this method is highly effective for polar lipids extraction, it is also suitable for low sample quantity, consuming large solvents and long time. According to Avalli and Contarini (2005), lipids fractions were extracted from milk samples as described by Folch et al. (1957) to ensure the complete extraction of the total lipids content. Then, the extracted total lipids were evaporated under vacuum, and the dry residues were re-dissolved in a mixture of chloroform/methanol (2:1, v/v). SPE cartridges were used for the purification of PLs. The cartridges were firstly conditioned using 3 mL of hexane, and then the neutral lipids were eluted by adding 3 mL of hexane/diethyl-ether (8:2, v/v) and 3 mL of hexane/diethyl-ether (1:1, v/v). The recovery of PLs was attained in two-steps elution. First, adding 4 mL of methanol, and then 2 mL of methanol followed by 2 mL of chloroform/methanol/water (3:5:2, v/v/v). The recovered fractions were evaporated to dryness under nitrogen, and the residue was finally re-dissolved in chloroform/methanol (2:1, v/v).

Giuffrida et al. (2013) extracted milk PLs according to the modified Folch procedure. Briefly, 250 mg of milk samples were precisely weighed and mixed with 250 mg of deionized water and 9.5 mL of chloroform/methanol (2:1, v/v). The sample solution was transported to an ultrasonic bath for 15 min at 40°C after the exact addition of 10 μL of a propylene glycol internal standard solution (5 mg/mL). Centrifugation was carried out at 1000 relative centrifugal force for 10 min, and the samples solutions were filtered through 0.2 μm polytetrafluoroethylene filters into glass tubes by using a vacuum manifold and elute reservoirs. The filtrate was then mixed

with 2 mL of potassium chloride solution (8.8 g/L), and centrifuged for 10 min at 1000 relative centrifugal force. The solvent layer was quantitatively transported into Extrelut vials, and the solvent was evaporated to dryness at 40°C under a gentle stream of nitrogen. The remaining lipids were redissolved in 150 μL of chloroform/methanol (9:1, v/v) and filtered through 4-mm polyvinylidene difluoride membrane filters into conical auto sampler flask.

Maswadeh et al. (2015) extracted the total lipids fractions from camel milk. Firstly, milk samples were freeze-dried in order to prevent milk and water interference, as well as the interaction of PLs with both lipids and proteins because of their amphiphilic characteristics (Contarini and Povolo, 2013). The freeze-dried milk samples were extracted three times with methylene chloride. The residue was then extracted by using a mixture of methylene chloride/methanol (1:1, v/v) and finally with 100% methanol. The solvent mixture was evaporated, and the lipids content was estimated by using the thin layer chromatography (TLC) technique with methylene chloride/methanol/acetic acid/water (65:30:6:2) as a developing mobile phase system. The combined methylene chloride portion was evaporated under reduced pressure using a rotary evaporator, and the dried residue was injected in the vacuum liquid chromatography by using methanol for PLs, and methylene chloride for neutral lipids analysis.

4.2. Separation of oil phospholipids

In order to achieve a complete and sufficient extraction of PLs fractions from plant sources, generally, high polarity organic solvents-mixtures containing alcohols are used, which in complex lipids break down the bonds with other fractions of natural compounds in the plant cell

(Kates, 1972). The most common solvent used for the extraction of PLs is chloroform/methanol (2: l, v/v) (Folch et al., 1957).

Totani et al. (1982) used glacial acetic acid to extract PLs from vegetable oil. 1 mL of acetic acid was added to 1 g of oil in 10-mL glass test tube equipped with a ground-glass stopper. The mixture of oil and acid was strongly stirred for 1 min and centrifuged at 1200 g for 1 min. The ratio of oil to acetic acid can be changed based on the content of PLs in the vegetable oil. In the crude vegetable oil, the appropriate percentage of oil to acetic acid was 1:2. Finally, aliquots of the acetic acid layer containing 1−12 μg of PLs were removed by using a microsyringe for colorimetric analysis.

Before the extraction of sunflower oil PLs, diol phase cartridges were firstly conditioned by 2 mL of methanol, 2 mL of chloroform, and 4 mL of hexane. Then 50−150 mg of oil dissolved in chloroform was loaded onto the cartridges. The triacylglycerols were removed from the sorbent bed by adding 2.5 mL of chloroform to the cartridge. PLs fractions were not eluted in this step as confirmed by recovery studies. 7 mL of methanol containing 0.5 mL/100 mL of a 25% ammonia solution was used in order to elute PLs fractions. The recovered fractions were finally transferred to a conical flask and dried under a gentle stream of nitrogen (Carelli et al., 1997).

Nzai and Proctor (1998) investigated an experimental scheme for PLs extraction from crude soybean oil. 2% of distilled water was added to 0.5 kg of the crude soybean oil, and the mixture was then heated at 90°C for 30 min with stirring. Then, the oil was centrifuged at 5000 rpm for 20 min, decanted, and the rich layer of PLs was washed three times by using 100 mL of acetone.

Finally, the crude PLs extract was dried under vacuum in order to remove the residual acetone left after the process of washing.

Hatzakis et al. (2008) extracted PLs fractions from olive oil according to the procedure established by Galanos and Kapoulas (1962). 400 mL of hexane was used to dissolve 100 g of olive oil, and the solution was then extracted three times with 100 mL of ethanol/water (87:13, v/v). The ethanolic extract was washed by using 100 mL of hexane, and PLs were obtained after removing the solvent under vacuum. Furthermore, olive oil PLs were extracted by dissolving 40 g of oil in 40 mL *n*-hexane, and then PLs fractions were eluted by using SPE cartridges under negative pressure. The cartridges were conditioned with *n*-hexane and the neutral lipids were eluted with 6 mL of *n*-hexane, 6 mL of *n*-hexane/diethyl ether (8:2, v/v), and then with 6 mL of *n*-hexane/diethyl ether (1/1, v/v). PLs fractions were recovered by adding 4 mL of methanol and 6 mL of chloroform/methanol/water (3:5:2, v/v/v). Finally, the solvent was evaporated to dryness under vacuum at 35°C, and the obtained fractions were dissolved in 1 mL of chloroform/methanol (88:12, v/v) (Verardo et al., 2013a).

4.3. Separation of egg yolk phospholipids

The procedures of PLs isolation and purification in the literature are relatively complicated, and the purity of the extracted PLs fractions is not good enough. Generally, egg yolk PLs can be extracted by organic solvents, particularly ethanol and then purified by removing the triacylglycerols and cholesterol. Numerous procedures for PLs fractions purification have been investigated, including the extraction with different solvents (Tokarska and Clandinin, 1985).

Since egg yolk can be separated easily from liquid egg white, and the lipids mainly exist in the yolk, egg yolk is considered the appropriate starting material for PLs extraction. In the different methods of lipids extraction from liquid egg yolk, various organic solvents, such as ethanol, acetone, hexane, and ether were used (Sim, 1994), and two to three solvents with diverse polarity were usually used as well. Lipids from egg yolk were extracted as described by Folch et al. (1957), and the content of PLs in fresh egg, salted egg, tea egg, and iron egg was determined according to the procedure reported by Murphy and Riley (1962).

Boselli and Caboni (2000) used the neat supercritical carbon dioxide (SC-CO_2) method for the extraction of PLs from dried egg yolk. Spray-dried egg yolk was extracted with SC-CO_2 at a density of 1.06 g/mL at 517 bars and 40°C (the maximum density reachable with the machine). The extraction yield attained by this method was 67 g/100 g sample compared to the conventional extraction method (Bligh and Dyer, 1959) (63 g/100 g of sample), which involves the using of chloroform: methanol: water mixture for PLs extraction. They reported that the solvent extract composed of 29% of PLs, while the extract obtained from the neat SC-CO_2 contained 26% PLs. High-performance liquid chromatography (HPLC) analysis coupled with an evaporative light scattering detector (ELSD) indicated insignificant selectivity in the extraction of PLs constituents by using SC-CO_2. The extraction with neat SC-CO_2 seems to be an effective alternative method for PLs extraction for analytical studies.

Nielsen and Shukla (2004) extracted 48% of yolk total lipids from spray-dried egg yolk powder with the continuous elution by using 96% ethanol at room temperature. The content of PLs in the extracted lipids was approximately 73%. PLs in the ethanol-lipid mixture were more

concentrated by chilling overnight at 0ºC to 83%, and by chilling overnight at −20ºC to 86%. The further concentration by chilling treatment was a result of that the neutral lipids were crystallized and then centrifuged, thus leading to increase PLs concentration in the supernatant.

As described by Kivini et al. (2004), egg yolk PLs were extracted by using glass-distilled grade acetone and 99.5% ethanol following the procedure adapted from Juneja et al. (1997). The addition of acetone (1×20 mL, 2×30 mL) resulted in the removing of water from yolk samples and dissolving the nonpolar lipids, for instance triacylglycerols and carotenoids. More polar lipids were recovered from the residue by adding 25 mL of ethanol twice. The insoluble material was filtered through glass microfiber filters, and the solvent solution was finally evaporated under vacuum by using a rotating evaporator. The results showed that the proportion of PC, PE, and SM represented approximately 70, 28, and 3% of the total PLs, respectively.

Palacios and Wang (2005) isolated PLs fractions with 94.9−95.9% purity, and a yield of 11.2−11.9% from fresh egg yolk. Ethanol and hexane were used to successively extract egg yolk total lipids, and then the final PLs were isolated by chilled acetone. The purification of PLs fraction by hexane and acetone is required to remove not only neutral oil but also cholesterol that is co-extracted with the ethanol. They concluded that this procedure might be conveniently adopted by the industry in order to produce a high-purity egg yolk lecithin.

Hruschka et al. (2009) extracted 47% purity egg yolk PLs by using low and high concentrations of ethanol in numerous steps. In the study reported by Aro et al. (2009), yolk lipids were extracted by the supercritical carbon dioxide method, followed by a supercritical anti-solvent procedure, in which the purified PLs were precipitated on the wall of the pressurized

chamber. Though, this method was perhaps still years away from commercial implementation because of the small production scale, and the high cost of the operation and the equipment.

Gładkowski et al. (2012) obtained high purity PLs by adding ethanol to egg yolk and stirring for 10 min. The supernatant was afterward removed and filtered, and the yolk was extracted with ethanol twice. The extracts were combined together and the rotary evaporator was used to remove the solvent. The residue was then dissolved in hexane and transferred to a flask placed in an ice bath (0ºC). Chilled acetone (−20ºC) was carefully added to the stirring mixture. After PLs precipitation, the stirring was stopped and the supernatant was poured outside. The remaining precipitate was carefully washed five times using chilled acetone (−20ºC), and acetone was removed by decantation each time. The final PLs were dried under nitrogen and stored at (−20ºC). The use of chilled acetone for PLs precipitation and washing yielded PLs fractions with 100% purity (78.7% PC and 21.3% PE).

Su et al. (2015) developed and validated a new method to separate PLs, cholesterol, and triacylglycerols from fresh egg yolk. This procedure involved the extraction with ethanol, low-temperature crystallization, and β-cyclodextrin-based process together in order to isolate these three major fractions. After the preliminary separation of total lipids and protein by ethanol, the ethanol extracts were subjected to low temperature for 10 hours to remove most of the solidified triacylglycerol from the total lipids. β-cyclodextrin/cholesterol molar ratio of 5:1, reacting temperature of 50°C, and water addition of 15 g/g β-cyclodextrin were the optimum conditions for cholesterol removal. The cholesterol removal rate approximately reached 99% and recycling

β-cyclodextrin could be efficiently conducted. The results revealed that the purity of PLs obtained using this method was 51.47%.

4.4. Separation of mitochondrial phospholipids

de Kroon et al. (1997) extracted PLs species from the external membranes of *Neurospora crassa* mitochondria, and rat liver according to the procedure of Bligh and Dyer (1959). The results revealed that PC, PE, and PI were the major PLs constituents, while CL was presented in trace amounts. Furthermore, this method might be generally applicable for obtaining a good yield of mitochondria external membranes. As well, lipids were extracted from caprine heart mitochondria by using a mixture of chloroform/methanol (2:1). This isolation procedure resulted in a large representative population of mitochondria, and the yield of mitochondria based on total mitochondrial protein was about 30−40 mg/g wet weight of tissue (Ashavaid and Kumbhat, 2005).

Kim and Hoppel (2013) reported that heart and skeletal muscle mitochondria isolated from rats were diluted in 50 μL of 100 mM potassium phosphate buffer, and then extracted according to the procedure described by Christiansen (1975). Mitochondria (30 μg) in 50 μL of 100 mM potassium phosphate buffer was mixed with 72 pmol of 1,2-dipalmitoyl-*sn*-glycero-3-phospho-N-methylethanolamine (internal standard) and extracted with 1 mL of chloroform/methanol (2:1, v/v) containing 2 mM of butylated hydroxytoluene. Then, the mixture was strongly shaken for 2 min and centrifuged at 2000 g for 10 min, and the upper layer was decanted and evaporated to dryness under a gentle stream of nitrogen. PLs were separated from the lipids mixture extracted from mitochondria by using pre-packed silica gel cartridges. The cartridges were firstly

conditioned with 1 mL of 10 mM ammonium acetate in methanol and equilibrated with 0.5 mL of isooctyl alcohol/ethyl acetate/acetic acid (75:25:2). The lipid residue was re-dissolved in 0.2 mL of isooctyl alcohol/ethyl acetate/acetic acid (75:25:2) and loaded onto the cartridges. The neutral lipids were eluted by adding 0.2 mL of isooctyl alcohol/ethyl acetate/acetic acid (75:25:2). Lipid-loaded column was additionally washed by adding 0.5 mL of isooctyl alcohol/ethyl acetate/acetic acid (75:25:2), followed by 0.5 mL of isooctyl alcohol/ethyl acetate (75:25). Finally, 1.5 mL of methanol was added to recover PLs fractions, and the obtained fractions were evaporated to dryness under nitrogen.

5. Identification of Phospholipids

In order to clarify the function of PLs, it is necessary to identify not only their classes and subclasses but their molecular species as well. Therefore, great efforts have been made in the past decades to develop quali-quantitative techniques for determining PLs composition from different sources. In the last years, PLs fractionation was mainly accomplished by using the TLC method. However, HPLC has overcome TLC as the preferred technique for the analysis of PLs (Nollet et al., 2004), and the latter was mainly applied for validation purposes, whereas SPE is rarely used recently. After the separation of PLs fractions by TLC, SPE, or HPLC with nondestructive detectors, the individual compounds can be recovered for the identification of their fatty acids composition by using the gas chromatography (GC).

5.1. Identification of phospholipids by using chromatography techniques

In order to improve the resolution of complex mixtures of lipids, HPLC was coupled with different kinds of detectors, such as diode-array detection (DAD) and ELSD. Recently, HPLC

coupled to an ultraviolet (UV) or mass spectrometry (MS) detectors is commonly used in the analysis of PLs classes from different sources. Wang et al. (2009) used the HPLC-UV to separate PC and PE from duck meat. By comparing HPLC-UV with HPLC-ELSD for PLs identification, the obtained results showed that low amounts of PI, PS, SM, and LPC were only detectable by HPLC-ELSD. However, they emphasized that this technique appears to be not a perfect method for detecting the larger quantities of PE and PC existed in duck meat samples. In addition, recent studies have validated the utility of HPLC coupled with mass spectrometer in quantitative and qualitative studies of oil PLs components. By using this method, the molecular species of PLs were identified, but the procedure still required that the fatty acids composition should be analyzed by GC. Off-line coupling of HPLC separation with fast-atom bombardment mass spectrometry has also been utilized for the analysis of PLs in peanuts (Singleton et al., 1999). HPLC-ELSD was applied for the identification of the PLs purified fractions (Avalli and Contarini, 2005; Caboni et al., 1996; Donato et al., 2011), and directly on the total fat extracted from milk and some dairy products (Le et al., 2011; Rodríguez-Alcalá and Fontecha, 2010; Rombaut et al., 2007).

GC is usually applied for the analysis of lipids, but because many classes of lipid are nonvolatile, it is necessary to derivatize them before GC analysis. This increased the complexity of the analysis, requiring additional sample preparation and the use of internal standards. Due to the structural diversity of the many classes of lipids, HPLC separations could be achieved by using a variety of chromatographic conditions, with reversed-phase and normal-phase being the most extensively used. The use of HPLC allowed a simpler chromatographic method because derivatization is not mandatory, and mass detectors such as ELSD, MS, and charged aerosol

detector (CAD) are available. UV detection is not extensively used, since lipids usually lack a chromophore for the required light absorption (Plante et al., 2011).

The development of PLs quantitative analysis methods became very important to control the quality of non-refined oils and also to estimate the efficiency of degumming process. Although the phosphatide content in oils can be evaluated from the total phosphorus percentage, there is a need for knowledge of the PLs profile. The approved techniques for PLs analysis are using the HPLC, which considered the preferred method that substituted TLC technique (Carelli et al., 1997). Owing to the lower concentration of PLs, mainly after oil refining, concentrating method before HPLC analysis is often required. After the extraction of lipids by using chloroform/methanol mixture according to Folch et al. (1957) and Bligh and Dyer (1959) procedures, polar lipids were isolated by the traditional solid-liquid column chromatography or SPE method (Panagiotopoulou and Tsimidou, 2002). Many researchers have applied the TLC method by using different mobile phases for the initial separation of oil PLs, which were scraped and followed by enzymatic hydrolysis to allow their separation and detection by using GC (Yoshida et al., 2001; Yoshida and Takagi, 1997). Moreover, great efforts have been done in the last few years for the identification of oil PLs using electrospray-tandem mass spectrometry. Boukhchina et al. (2004) described liquid chromatography-mass spectrometry (LC-MS) and mass spectrometry-mass spectrometry (MS-MS) as rapid and precise methods for the separation and identification of the main classes of PLs and their fatty acids composition in rapeseed, olive, almond, and sunflower oils without the need to additional chemically modify the phospholipids.

Although the use of high-performance liquid chromatography-mass spectrometry (HPLC-MS) is mainly focused on determining the molecular species of PLs, this method has also been utilized for the fractionation and identification of PLs individual classes. Thus, Fong et al. (2013), quantified PC, PE, PI, PS, and SM in infants formulas by HPLC-MS-MS. As well, HPLC coupled to an electrospray ionization (ESI) and MS detector has been applied to separate and identify PA, PC, PE, PS, and PI in yeast (Henderson et al., 2011). Verardo et al. (2013a) validated a technique based on high-performance liquid chromatography-electrospray ionization-quadrupole time-of-flight-mass spectrometry (HPLC-ESI-Q-TOF-MS) for the identification of PG, PA, lyso-PA, PI, PC, and LPC in olive oil. A rapid method for PC, lyso-PC, PE, PS, and PI quantitative analysis in milk samples using HPLC coupled to CAD has been recently described. In this method, a diol stationary phase with a mobile phase composed of formic acid (13%, v/v), 2-propanol and hexane were used, and the different classes of PLs were eluted within 29 min (Kiełbowicz et al., 2013).

Authors have established innovative systems based on HPLC and MS detectors for the characterization of PLs molecular species in foods. A reconstructed HPLC-ELSD-MS system was used to identify the molecular species of PC and PE in duck samples by Wang et al. (2009). The direct infusion combined with ESI-MS-MS was confirmed to be an effective method for the analysis of PC, PE, PS, PI, and SM molecular species from fish muscles (Wang and Zhang, 2011). Recently, a novel inclusive two-dimensional liquid chromatographic system, using hydrophilic interaction liquid chromatography (HILIC) in the first dimension and reversed-phase liquid chromatography with a C18 column in the second dimension, coupled with MS detector was developed (Dugo et al., 2013). The ability of the investigated two-dimensional liquid

chromatography approach was demonstrated in the separation of PC, PE, PS, PI, LPC, and SM molecular species in cow milk. The only disadvantage was the long-time of analysis because of the stop-flow mode employed, albeit this is well compensated with the enhanced resolving power and the greater amount of analyses information obtained.

Yoon and Kim (2002) studied the application of HPLC for the identification of PLs from freeze-dried egg yolk, which contains 80% PC, and 15% PE. Column temperature (30, 40 or 45°C), mobile phase composition and its flow-rate (from 1.0 to 2.0 mL/min), and the kinds of stationary phase were varied in order to understand the efficiency of PC separation. In order to study the association between the recovery yield and sample loading amount in HPLC, overloading experiments were performed. In this way, over 99% purity PC was successfully separated with 98% yield through the using of 100% methanol as a mobile phase, adjusting the flow-rate to 2.0 mL/min and feed concentration of 1000 mg/mL with a 45°C column temperature.

Lei et al. (2012) performed a short packed silica gel column chromatography to enhance the separation of PE and PC from hen egg yolk with very low or no toxic solvents. The influences of silica type, sample loading amount, dimension of the glass chromatotube, and mobile phase compositions were studied, and high separation efficiency was attained. The operation conditions was as follow; gradient elution using 200 mL of ethanol followed by 300 mL of 95% ethanol to fractionate PE and PC after removing the neutral lipids with 120 mL of ethyl acetate, 40 mm silica gel bed height of the chromatotube with 22 mm inner diameter, and 0.25 g sample loading. By using this method, 3.69% PE and 2.88% PC were obtained. HPLC-UV was used to identify

the refined PE and PC classes with purity over 96%. The fatty acids composition of egg yolk indicated that PE and PC contained higher percentages of omega-6/omega-3 fatty acids (PE, 7.41; PC, 8.99). The content of linoleic acid in PC (15.21%) was more abundant than PE (10.29%), while the content of arachidonic acid in PC (8.78%) was lower than its content in PE (15.67%).

A new technique for the identification of mitochondrial PLs using an efficient extraction method and a normal-phase HPLC-MS was developed by Kim and Hoppel (2013). The presence of 1,2-dipalmitoyl-*sn*-glycero-3-phospho-N-methylethanolamine as an internal standard and standardization of PLs were used to design the standard curves in order to accurately identify mitochondrial PLs. Chromatographic separation and high sensitivity for each class of PLs allowed the quantitative and qualitative analysis of mitochondrial PLs, including monolysocardiolipin. The application of this method for the identification of rat heart and skeletal muscle mitochondria revealed that the HPLC-MS method considered an influential technique for a reliable and reproducible determination of mitochondrial PLs.

Mitochondrial PLs analysis was performed by using two-dimensional high-performance thin layer chromatography on silica gel 60 plates, impregnated with 2.4% boric acid according to the method modified by Fine and Sprecher (1982). Chloroform/methanol/water/ammonia (120:75:6:2) was used as the first dimension mobile phase, and chloroform/methanol/acetic acid (65:25:10) as the second dimension mobile phase. Spots of PLs classes were visualized by using iodine vapor, and then they were scraped off. Finally, PLs fractions were quantitated according to the procedure reported by Fiske and Subbarow (1925) after the destruction in 70% perchloric

acid at 180°C for three hours. As well, high-performance thin layer chromatography was applied for the identification of the lipids extracted from caprine heart mitochondria. The different fractions of lipids were separated by using a mobile phase system composed of chloroform/methanol/water (65:25:4). Different spots of PLs fractions were visualized and identified with iodine vapor after comparison with separated corresponding standards (Ashavaid and Kumbhat, 2005).

Giuffrida et al. (2013) established and validated an HPLC-ELSD procedure to identify the molecular species of human milk PLs. This method of analysis had the features of robustness to quantify PLs in human milk compared to the other techniques. This method was applied to analyze a large number of human milk samples indicating its applicability for large clinical experiments. In addition, the employment of the internal standard allowed correcting the loss of analyte during sample preparation. Additional investigations are needed to determine the molecular species composition of PLs as previously demonstrated (Montealegre et al., 2012; Russo et al., 2013; Verardo et al., 2013b). LC-MS would be the most suitable technique for the identification of PLs, on the other hand, because of the dependency of the MS response and therefore peak area intensity on the acyl chain length and the degree of unsaturation, the quantification of PLs molecular species required the using of pure standards, which are not always available.

Purification of the PLs classes has been extensively investigated by many researchers in numerous academic fields (Balazs et al., 1996; De Meulenaer et al., 1995; Hanahan, 1997; Hurst et al., 1986; Lendrath et al., 1990; Myher and Kuksis, 1995; Touchstone, 1995; Wang et al.,

2009; Yoon and Kim, 2002). PC and PE were frequently purified by using various chromatography methods, such as TLC, HPLC (Balazs et al., 1996; De Meulenaer et al., 1995; Hurst et al., 1986; Myher and Kuksis, 1995; Yoon and Kim, 2002) and low-pressure column chromatography (LPCC) (Rongsheng et al., 2001). Among these methods, the efficiency of separation by using HPLC considered the best as small diameter packing could be applied. However, the equipment is expensive and the charges of operation and maintenance are high as well. The limitations of TLC method summarized in the difficult online detection and recovery of the products. Consequently, TLC and HPLC were not suitable for the purification PLs classes as industry processes with low cost. Compared with TLC and HPLC methods, LPCC showed a great potentiality for industrial production because of many advantages among them, operation simplicity, convenient gradient elution, and high yield with low cost.

The separation analysis of lipids using the HILIC technique has been widely reported. It is well known that the HILIC-based separation of PLs mainly depends on the polarity of their polar heads; the less polarity the lipids are, the shorter retention times they are detected. Lipids classes were fractionated using a silica-based column in the HILIC analysis method (Lisa et al., 2011). Also, PLs extracted from different foods have been separated by gradient elution in HILIC using acetonitrile and 10 mM of ammonium acetate as a mobile phase at pH 3.0 (Zhao et al., 2011). Furthermore, five classes of PLs were separated by using isocratic elution containing acetonitrile, methanol and 10 mM ammonium acetate (Schwalbe-Herrmann et al., 2010). Different applications of HILIC in PLs analysis were also reported (Bang et al., 2014; Craige Trenerry et al., 2013; Li et al., 2016).

5.2. Identification of phospholipids by using nuclear magnetic resonance technique

Recently, ^{31}P nuclear magnetic resonance (NMR) has become an essential technique for the separation and characterization of phosphorus-containing compounds in different matrices. Brinkmann-Trettenes et al. (2012) described a ^{31}P NMR technique for the identification and quantification of PLs content in aqueous samples of different types of PLs in phosphate buffer by using a standard 400 MHz NMR device. In milk and dairy products analysis, this method has been applied to determine PLs, glycerophosphocholine, inorganic phosphate, and casein-bonded phosphoserine (Spyros and Dais, 2009). As well, PLs content and their individual fractions in lipid samples have been characterized by using ^{31}P NMR (Yao and Jung, 2010).

Dairy PLs were analyzed and quantified by using ^{31}P NMR method. The samples were prepared in sodium cholate detergent system, and the ^{31}P NMR procedure was compared with a quantitative two-dimensional thin layer chromatography method for validation. The latter was more sensitive and detected some of the low abundant fractions, which were not detected by ^{31}P NMR. Though, ^{31}P NMR considered more suitable to routine analysis within 36 minutes. The method was also more multipurpose, and sample analysis was possible especially on high PLs-containing matrices without the need to prior extraction of lipids (MacKenzie et al., 2009).

^{31}P NMR spectroscopic analyses have been applied to separate and quantify PLs classes and subclasses because of diverse fatty acids residues; however, unique information about the stereo-specify cannot be obtained without coupling with ^{13}C NMR spectroscopy. In cases in which the disadvantages of ^{13}C NMR are mostly caused by the complication of the spectra and long relaxation time of the insensitive ^{13}C nuclei, leading to crowded, non-quantitative spectra, the

main advantage of ^{31}P NMR spectroscopic investigations of PLs is the simplicity of the spectra, where the unique chemical shift obtained for the single different positioned ^{31}P nucleus in the different PLs classes, and furthermore, the possibility to simply measure the 100% natural abundant ^{31}P in a quantitative way, resulted in a specific identification and quantification of PLs. By using this technique, several foods and food components have been examined to profile the PLs composition, including the profiling of the PLs classes in egg and rat liver (Sotirhos et al., 1986), olive oil (Hatzakis et al., 2008), soybean (Yao and Jung, 2010), and milk (Garcia et al., 2012). Nevertheless, NMR signals of the phosphorus nucleus in the main classes of PLs in ^{31}P NMR spectra were distributed over a relatively narrow range of approximately 2 ppm and subsequently, signals from the different PLs classes frequently overlapped. The two-dimensional NMR technique could be used to avoid the overlapping as kindly established in an NMR spectroscopic profiling of PLs in fish and cheese samples (Kaffarnik et al., 2013).

5.3. Analysis of fatty acid profiles in phospholipids

The distinctive characteristics of PLs mainly depend on the profile of their fatty acids, the differences in which cause significant metabolic and functional variances. Consequently, the molecular structure of PLs has an important influence on the rate and total amount of their consumption (Leray et al., 1993). The biological functions of PLs are strongly correlated to the *sn*-positional distribution of fatty acids. The majority of naturally occurring PLs have a heterogeneous intra-molecular acyl chain structure, typically with saturated acyls at the *sn*-1 position, and unsaturated acyls at *sn*-2 position. Moreover, the variations of the membrane phospholipid fatty acid profile might cause modifications in the physical properties of the

membrane. Therefore, the pattern and abundance of fatty acids linkages at the *sn*-1 or *sn*-2 positions of PLs are critical issues for a better understanding of their biological roles (Donato et al., 2011).

Numerous procedures enable the positional analysis of PLs have been presented. Most of these methods are based on one- or two-enzyme regiospecific enzymatic hydrolysis reactions. Kiełbowicz et al. (2012) developed a simple and fast method for the positional analysis of the fatty acid composition of PC extracted from egg yolk. This method was based on the complete ethanolysis of PC and the separation of reaction products (fatty acids, fatty acids ethyl esters and 2-acyl LPC) by the extraction with water and hexane. The regiospecific analysis showed that the hydroxyl group in the *sn*-1 position of egg yolk PC was mainly esterified by saturated fatty acids, whereas in the *sn*-2 position, predominantly monounsaturated and polyunsaturated fatty acids were detected. A new method for the positional analysis of egg yolk PLs including PC and PE by using liquid chromatography with CAD was described (Kiełbowicz et al., 2015). The results showed that the contents of saturated fatty acids at the *sn*-1 and *sn*-2 positions of PC were 79.4 and 3.8%, respectively. As well, the contents of these fatty acids at the *sn*-1 and *sn*-2 positions of PE were 96.7 and 7.5%, respectively.

The structural information of PLs molecular species from milk was identified by using ESI-MS-MS (Gallier et al., 2010). Milk PLs have longer chain fatty acids than the triglycerides of the lipid core. The acyl chains of milk PLs was reported to be 10- to 24-carbons in length with the major ones being myristic, palmitic, stearic, oleic, and linoleic. Sanchez-Juanes et al. (2009) reported the presence of medium-chain fatty acids (C10-C17), long-chain fatty acids (C18-C20),

and very long-chain fatty acids (C>20) in the whole milk PLs. The long-chain fatty acids were the predominant, whereas SM contained higher amounts of these fatty acids. The presence of the very long chain fatty acids in PLs and the high saturation content of SM fatty acids contributed to the structure of the MFGM by maintaining its rigidity (Fong et al., 2007).

6. Beneficial Health Aspects of Phospholipids

It has been estimated that the average human consumption of dietary PLs approximately in the range of 2 and 8 g/day, introduced with diverse categories of foods, such as milk, eggs, beef, fish oilseeds, and cereal grains (Cohn et al., 2010). Milk and dairy products are expected to be a plentiful source of sphingolipids. According to the US regime, about 116 g of SM, corresponding to 0.01−0.02% w/w of the regime and 0.3−0.4 g/day, is the annual consumption for each person (Vesper et al., 1999).

The beneficial health aspects of PLs have been investigated since 1900's in relation to different diseases and symptoms, such as inflammation, cholesterol absorption, coronary heart diseases, and cancer (Cohn et al., 2008; Postle, 2009; Tang et al., 2013). The previous studies indicated that dietary PLs have positive influences on various diseases, seemingly without severe any side effects **(Table 6)**. Additionally, PLs have the ability to decrease the side effects of several medications. These two effects can be somewhat elucidated by the theory that PLs can deliver their fatty acids residues for incorporation into the membranes of cells concerned to diverse diseases, such as immune or cancer cells. The reformed membranes structure is expected to have some influences on the activity of membrane proteins (for example, receptors) through influencing the membranes microstructure; consequently, the cellular membranes characteristics,

for instance of lipid rafts, or through affecting the biosynthesis of fatty acids resulting lipids second messengers. Though, since the fatty acids initially bound to the applied PLs existed in higher amounts in the cellular membrane after their supplementation or consumption, the fatty acids composition of PLs and the class of PLs are fundamental for their effects (Küllenberg et al., 2012).

Several studies have documented the importance of PLs for the growth and development of infants (German, 2011; Tanaka et al., 2013). Therefore, PLs may be of specific interest as functional ingredients; however, the optimal dose and specific PLs class have to be selected. It has been estimated that the average intake of total PLs/day in the newborns of four weeks old was 140 mg/day when the infants were solely fed on mother milk. This average intake was in agreements with the previous literature (Collins et al., 1989; Garcia et al., 2012; Russo et al., 2013), which reported an average consumption of total PLs of 109, 126, and 150 mg/day, respectively. This estimation was based on the hypothesis that the average volume of human milk consumed at this age is 600 mL/day (da Costa et al., 2010).

7. Conclusion and Future Prospects

The studies on PLs from different sources obviously showed that there is no available standardized method for the determination of PLs, and it is definitely one of the main reasons for both the difficulties of comparison and the variability of the results among the different investigations. Currently, PLs have received more attention because of their health-promoting characteristics and advantageous technological functionalities. Distinguished nutraceutical properties of PLs include the association with brain and intestinal immune systems development

in infants, gastrointestinal infection protection, exercise-induced stress reduction, memory improvement in the elderly, and cholesterol absorption lowering. Technological functionalities of PLs-fortified materials are correlated to their emulsifying properties and water-holding capacity resulted in making them potential constituents for the development of emulsion-based foods or emulsions and liposomes for the protection and delivery of drugs and sensitive bioactive nutrients. An effective analytical technique would be important for supporting the evidence of the positive influences of PLs on human health. Future investigations will be helpful to provide a direction for the developing of PLs-fortified products for therapeutic and preventive applications.

Acknowledgments

This work was supported by the National Natural Science Foundation of P.R. China (31601433) and Jiangsu Provincial Natural Science Foundation (BK20140149).

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Phospholipids content in milk and dairy products.

Dairy products	mg PLs/100 mg total lipids	mg PLs/ 1000 mg product	References
Milk (in vat)	0.40	0.09	Avalli and Contarini (2005)
Raw milk	0.71	0.29	Rombaut et al. (2005)
Skim milk	10.7	12.48	Rombaut et al. (2005)
Cram (natural)	0.90	1.98	Avalli and Contarini (2005)
Cream	0.35	1.39	Rombaut et al. (2005)
Cream centrifuged	0.50	0.90	Avalli and Contarini (2005)
Butter	0.20	1.60	Avalli and Contarini (2005)
	0.22	1.81	Rombaut et al. (2005)
Butter milk	4.50	0.90	Avalli and Contarini (2005)
	21.8	0.91	Rombaut et al. (2005)
Cheese (cheddar)	0.47	1.50	Rombaut et al. (2005)
Whey	5.30	0.18	Rombaut et al. (2005)
Quarg	24.70	0.32	Rombaut et al. (2005)

Table 2. Phospholipid classes composition in milk from various animal species and some dairy products.

Species	PLs classes (% of total PLs)					Analysis method	References
	PE	PI	PS	PC	SM		
Liquid raw milk	26.9	13.7	4.1	27.5	27.7	^{31}P NMR	Andreotti et al. (2006)
	32.3	9.3	10.5	27.3	20.5	HPLC–ELSD	Avalli and Contarini (2005)
	33.2	5.2	9.3	27.4	25.1	HPLC–ELSD	Rombaut and Dewettinck (2006)
	46.4	5.3	7.4	21.1	19.8	HPLC–ELSD	Rombaut et al. (2007)
	28.5	2.5	11.6	32.7	23.0	HPTLC	Sanchez-Juanes et al. (2009)
	72.3	1.4	11.5	8.0	7.9	HILIC-ELSD-MS	Donato et al. (2011)
	26.4	3.4	2.0	42.8	25.5	ESI-MS/MS	Gallier et al. (2010)
	34.2	7.7	8.6	45.5	4.1	LC-CAD	Kiełbowicz et al. (2013)
Processed milk	20.8	2.3	1.3	40.3	24.1	ESI-MS/MS	Gallier et al. (2010)
Buffalo milk	30.0	4.0	4.0	28.0	32.0	2D-TLC	Morrison (1968)
	29.6	3.4	3.0	27.8	32.1	TLC	Christie (1983)
	21.8	17.5	5.9	21.6	22.1	^{31}P NMR	Murgia et al. (2003)
	31.1	3.95	3.6	29.75	31.6	HPLC–ELSD	Zou et al. (2013)
Cow milk	32.0	5.0	3.0	35.0	25.0	2D-TLC	Morrison (1968)
	35.0	5.0	2.0	30.0	24.0	GC	Cerbulis et al. (1982); Renner et al. (1989)
	31.8	4.7	3.1	34.5	25.2	TLC	Christie (1983)
	25.8	14.0	1.5	26.8	26.8	^{31}P NMR	Murgia et al. (2003)
	23.5	12.0	3.6	24.0	24.2	^{31}P NMR	Andreotti et al. (2006)
	31.5	4.9	8.8	26.0	23.8	HPLC–ELSD	Rombaut et al. (2006)
	42.0	4.8	6.7	19.2	17.9	HPLC–ELSD	Rombaut et al. (2007)
	26.7	7.5	11.7	26.5	20.8	^{31}P NMR	MacKenzie et al. (2009)
	35.9	3.6	11.2	28.7	19.9	HPLC–ELSD	García-Márquez et al. (2013)
	30.23	9.89	7.32	25.20	27.36	HPLC–ELSD	Zou et al. (2013)
	23.42	8.97	9.07	33.12	25.4	HPLC–ELSD	Yao et al. (2016)
Sheep milk	36.0	3.0	3.0	29.0	28.0	2D-TLC	Morrison (1968)
	36.0	3.4	3.1	29.2	28.3	TLC	Christie (1983)
	32.7	4.2	5.0	27.2	26.1	HPLC–ELSD	Rodríguez-Alcalá and Fontecha (2010)
	34.4	4.4	5.2	28.6	27.4	HPLC–ELSD	
	35.85	3.45	3.35	29.6	27.75	HPLC–ELSD	Zou et al. (2013)
Camel milk	30.0	4.3	9.2	19.3	24.6	^{31}P NMR	Garcia et al. (2012)
	36.0	6.0	5.0	24.0	28.0	2D-TLC	Morrison (1968)
	35.9	5.9	4.9	24.0	28.3	TLC	Christie (1983)
	35.5	6.05	4.75	25.55	28.15	HPLC–ELSD	Zou et al. (2013)
Donkey milk	32.0	4.0	4.0	26.0	34.0	2D-TLC	Morrison (1968)
	32.1	3.8	3.7	26.3	34.1	TLC	Christie (1983)

	30.6	4.2	4.0	25.25	35.95	HPLC–ELSD	Zou et al. (2013)

PLs, phospholipids; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; SM, sphingomyelin; NMR, nuclear magnetic resonance; HPLC–ELSD, high-performance liquid chromatography-evaporative light scattering detector; HPTLC, high-performance thin layer chromatography; HILIC-ELSD-MS, hydrophilic interaction liquid chromatography-evaporative light scattering detector-mass spectrometry; ESI-MS/MS, electrospray ionization-mass spectrometry/mass spectrometry; LC-CAD, liquid chromatography-charge aerosol detector 2D-TLC, two-dimension thin layer chromatography; GC, gas chromatography

Species	**PLs classes (% of total PLs)**					**Analysis method**	**References**
	PE	**PI**	**PS**	**PC**	**SM**		
Human milk	26.0	4.0	6.0	28.0	31.0	2D-TLC	Morrison (1968)
	32.0	5.0	4.0	29.0	29.0	GC	Cerbulis et al. (1982); Renner et al. (1989)
	25.9	4.2	5.8	27.9	31.1	TLC	Christie (1983)
	10.1-15.1	9.2-11.7	12.3-18.4	19.3-23.5	36.4-45.5	HPLC–ELSD	Lopez and Ménard (2011)
	19.3	6.1	8.8	28.4	37.5	TLC	Bitman et al. (1984)
	21.3	16.4	-	19.0	43.3	HPLC–ELSD	Rodríguez-Alcalá and Fontecha (2010)
	18.3	3.8	8.1	24.5	29.7	^{31}P NMR	Garcia et al. (2012)
	12.48	7.69	14.36	25.08	40.18	HPLC–ELSD	Zou et al. (2013)
	28.6	4.6	5.9	25.2	35.7	HPLC–ELSD	Giuffrida et al. (2013)
	25.33	7.85	13.12	24.39	29.28	HPLC–ELSD	Yao et al. (2016)
Mare milk	24.3	8.5	10.6	27.8	28.9	^{31}P NMR	Garcia et al. (2012)
Goat milk	33.0	6.0	7.0	26.0	28.0	TLC	Kataoka and Nakae (1973)
	35.4	4.0	3.2	28.2	29.2	GC	Cerbulis et al. (1982); Renner et al. (1989)
	33.2	5.6	6.9	25.7	27.9	TLC	Christie (1983)
	29.2	5.8	7.7	26.3	23.2	HPLC–ELSD	Rodríguez-Alcalá and Fontecha (2010)
	19.92	9.37	14.03	31.64	25.04	HPLC–ELSD	Yao et al. (2016)
Goat buttermilk	35.2	9.8	9.9	24.8	20.3	HPLC–ELSD	Lamothe et al. (2008)
Goat butter serum	27.1	11.7	8.2	26.2	26.8	HPLC–ELSD	
Cream	24.8	8.8	6.7	31.1	17.7	ESI-MS/MS	Gallier et al. (2010)
	26.7	7.5	11.7	26.5	20.8	^{31}P NMR	MacKenzie et al. (2009)
	42.7	6.8	7.2	14.6	28.6	HPLC–ELSD	Avalli and Contarini (2005)

Cream (concentrated)	29.9	8.6	15.2	25.9	20.4	HPLC–ELSD	
Butter	31.0	11.9	15.3	24.7	17.1	HPLC–ELSD	
Buttermilk	33.5	2.4	10.3	35.5	18.3	HPLC–ELSD	
	17.0	7.1	8.1	46.1	21.7	HPLC–ELSD	Costa et al. (2010)
Butter milk powder	7.3	7.1	4.0	44.3	23.9	ESI-MS/MS	Gallier et al. (2010)
Buttermilk protein concentrate	25.7	5.8	9.7	27.0	20.4	^{31}P NMR	MacKenzie et al. (2009)
Cheese	11.4	-	-	12.3	13.9	TLC	Weihrauch and Son (1983)
Whey protein concentrate	20.02	14.19	4.32	28.58	28.79	HPLC	Vaghela and Kilara (1996)

PLs, phospholipids; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; SM, sphingomyelin; 2D-TLC, two-dimension thin layer chromatography; GC, gas chromatography; HPLC–ELSD, high-performance liquid chromatography-evaporative light scattering detector; NMR, nuclear magnetic resonance; ESI-MS/MS, electrospray ionization-mass spectrometry/mass spectrometry

Table 3. Phospholipid classes composition in different vegetable oils.

Oils		Phospholipids classes %								Analysis method	Referenc
		PE	PI	PS	PC	LPC	LPE	PG	others		
Soybean	Lecithin	9-12	8-10	1-2	10-15	1-2	1-2			TLC	Wendel (
		13.8	8.0		15.3	0.4			PA: 2.0	^{31}P NMR	MacKenz
	Crude soybean	13.6	12.0		21.9	2.9		2.3	PA: 5.8 others: 3.6	TLC	Gunstone
		22.7	17.2		27.1					TLC-imaging densitometry	Nzai and
	Refined bleached	22.1	16.4		26.8						
	Taekang	23.6	13.5		35.3	0.6				LC-MS/MS	Lee et al.
	GS06	26.2	18.2		35.1	0.6					
	Gaechuck#2	35.8	24.6		49.0	1.3					
Palm		24.0	22.0	traces	36.0	traces	2.0	9.0	PA: 3	TLC	Goh et al.
		21.0	11.0		17.0			37		TLC	Chow and
		22- 26	21- 25		34- 35			5- 7	CL: 7- 8	TLC	Kulkarni
Coconut		24.6	19.0	4.8	34.6	4.6	3.4		6.2		Krishnam (1983)
Linseed		69.3			28.0				SM: 2.7	HPLC	Kivini et
Canola		27.9			69.3				SM: 2.8		
		18.8	19.7	3.1	31.2	0.4	0.4		1.8	TLC-FID	Przybylsk
	Degummed	10.8	28.9	14.6	2.8				PA: 38.4		
Rapseed	Lecithin	29.0	14.0		37.0				GL: 20	TLC	Wendel (
	Monolit	13.0	12.9		74.1					TLC	Ambrose
	Kosto	23.0	11.6		40.2				PA: 7.0 other: 18.0		
	Pomorzanin	27.9	14.4		57.7						
Peanut		18.0	24.0		44.0	traces	traces			TLC	Du Plessi
		13.3	15.7		38.3			2.5	PA: 2.2	TLC HPLC-UV	Singleton Yoshida e

PLs, phospholipids; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PG, Phosphatidylglycerol; PA, phosphatidic acid; CL, cardiolipins; SM, sphingomyelin; GL, glycolipids; TLC, thin layer chromatography; NMR, nuclear magnetic resonance; LC-MS/MS, liquid chromatography-mass spectrometry/mass spectrometry; HPLC, high-performance liquid chromatography; TLC-FID, thin layer

chromatography-flame ionization detector; HPLC-UV, high-performance liquid chromatography-ultraviolet

Oils		Phospholipids classes %							Analysis method	References
		PE	PI	PS	PC	LPC	LPE	PA		
Pine nut			0.07-0.19	0.15-0.33	0.14-0.37				TLC-FID	Miraliakbari an (2008a); Mirali Shahidi (2008b
Pecan nuts			0.2-0.7	0.24-0.47	0.23-0.52					
Walnut			0.22	0.36	0.38					
Hazelnut		30.8	11.7		56.4				Iatroscan TLC-FID	Alasalvar et al.
Corn		4.8	21.1	1.5	43.4	3.5	traces		2D-TLC	Weber (1981)
Sunflower		8.2-16.8	5.1-44.0		8.4-17.1			26.2-78.3	HPLC-UV	Carelli et al. (1
		19.7	26		52		traces		2D-TLC	Chapman (198
		23.0	22		51		traces		TLC	LA et al. (1970
	armavriski	19.6	20.6		58.8			1.1	LC-MS/MS	Gupta (2002)
	peredovik	19.5	15.2		64.2			1.2		
	salyut	17.0	22.4		56.1			4.5		
Cotton seed	13.3	15.7		66.4			2.5	2.2	HPLC-UV	Singleton and S (1995)
Black cumin	25.1	9.56	12.3	46.1	4.23	1.2			HPLC	Ramadan and M
Flax seed	7 days flowering	5.0	35.0		15.0	28.0	7.0	10.0	HPLC-MS	Herchi et al. (2
	56 days flowering	28.0	26.0		18.0	15.0	11.0	2.0		

PLs, phospholipids; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PG, Phosphatidylglycerol; PA, phosphatidic acid; TLC-FID, thin layer chromatography-flame ionization detector; 2D-TLC, two-dimension thin layer chromatography; HPLC-UV, high-performance liquid chromatography-ultraviolet; LC-MS/MS, liquid chromatography-mass spectrometry-mass spectrometry; HPLC-MS, high performance liquid chromatography-mass spectrometry

Table 4. Phospholipids content of egg products.

Egg Products		% total lipids	% of phospholipids						Analysis method	References
			PE	PI	PS	PC	SM	LPC		
Eggs, whole	Chicken	31.4	16.0			77.0	2.4	2.4	TLC, GC	Christie and Moore (1972)
	Duck	26.5	16.0			75.6	2.4	2.7		
	Goose	25.0	18.8			74.0	3.0	2.3		
	Turkey	29.8	12.9			81.5	2.1	2.2		
	Quail	29.7	12.0			80.7	1.9	2.3		
Egg, chicken	White	2.8	traces			1.2	0.9		TLC, GC	Sato et al. (1973)
	Powder		22.09			10.76			TLC, GC	Privett et al. (1962)
	Yolk		15.0			72.8	2.5	5.8	TLC	Rhodes and Lea (1957)
			20.9		0.7	73.1	4.6		TLC	Monma et al. (1970); Weihrauch and Son (1983)
			15.0		3.3	58.4	19.0		HILIC-MS	Zhao et al. (2011)
			31.3	5.90	6.96	46.0		9.72	GC–PFPD	Adeyeye (2012)
	Albumen		17.0	2.95	4.69	63.1		12.2		
	Dried egg yolk		14.1	0.6		84.2	1.1		HPLC–ELSD	Boselli and Caboni (2000)
			10.1	1.1		87.8	1.0			
Plasma yolk	Quail		11.3		4.3	74.8	6.5	3.1	TLC	Cioceanu et al. (2007)

PLs, phospholipids; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; SM, sphingomyelin; LPC, lysophosphatidylcholine; TLC, thin layer chromatography; GC, gas chromatography; HILIC-MS, hydrophilic interaction liquid chromatography-mass spectrometry; GC-PFPD, gas chromatography-pulse flame photometric detector; HPLC–ELSD, high-performance liquid chromatography- evaporative light scattering detector

Table 5. Composition of mitochondrial phospholipids.

Mitochondria	% of total phospholipids								References
	PE	PG	PI	PC	SM	CL	PS	Others	
Caprine heart	32.58		0.74	47.11	1.61	13.07	1.5	LPC: 0.77	Ashavaid and Kumbhat (2005)
Sheep heart	16-35	4.0	1-5	43-46	2.0	10-15	1.0	PA: 2	Getz et al. (1968); Strickland and Benson (1960)
Sheep adrenal cortex	11.0		5.0	35.0	1.0	6.0	5.0	PA: 1	Getz et al. (1968)
Sheep kidney	16.0		1.0	33.0	7.0	7.0	1.0	PA: 2	
Bovine heart	30-37	11.0	6-10	38-45	3.0	16-20	1.0	Lyso: 6	Strickland and Benson (1960); Wheeldon et al. (1965)
Maize	34.0	3.0	6	49				DPG: 8	Guillot-Salomon et al. (1997)
Rice (mg/mL)	0.5560		0.0203	0.0070			0.1441	PA: 0.5042	He et al. (2007)
Potato (inner membrane)	29-33	5.0	7-24	27-33		19.0			McCarty et al. (1973)
Potato (outer membrane)	25-64		0-10	36-53		12.0			
Trout liver	31.2	9.5	6.4	49.0	2.1		1.6		Zabelinskii et al. (1999)
Rat lunge	27.0		22.0	45.0	8.0	7.0	9.0		Hallman and Gluck (1975)
Rat brain	32.0	2.0	5.0	55.0		2.0	4.0		Strickland and Benson (1960)
Rat heart (nmol/mg mitochondria protein)	165.5	4.1	14.3	152.3		39.5		MLCL: 0.7	Kim and Hoppel (2013)
Rat muscle (nmol/mg mitochondria protein)	183.7	2.7	20.4	195.5		47.7		MLCL: 1.1	
Rat liver	26.2	5.6	8.5	55.9	2.3		1.3		Zabelinskii et al. (1999)
	34.4	0.1	5.4	44.3	0.9	13.5	0.5		de Kroon et al. (1997)
Outer membrane vesicles (*Neurospora crassa*)	27.5	0.2	13.4	54.7	0.7	0.3	2.1		
Neurospora crassa	39.9	1.0	4.5	36.7		11.8	0.7	Lyso: 2.3	
Outer membrane vesicles (*Neurospora crassa*)	32.7	0.4	9.2	50.2		3.1	0.6	Lyso: 1.2	
Saccharomyces cerevisiae (inner membrane)	29.0	1.0	8.0	36.0		19.0	2.0	Lyso: 3	Bottema and Parks (1980)
Saccharomyces cerevisiae (outer membrane)	22.0	1.0	20.0	43.0		6.0	2.0	Lyso: 2	

PE, phosphatidylethanolamine; PG: Phosphatidylglycerol; PI, phosphatidylinositol; PC, phosphatidylcholine; SM, sphingomyelin; CL, cardiolipin; PS, phosphatidylserine; LPC: lysophosphatidylcholine; PA, phosphatidic acid; DPG, diphosphatidylglycerol; MLCL: monolysocardiolipin; Lyso, lysophospholipids

Table 6. Beneficial health aspects of phospholipids on different diseases.

Disease	Phospholipid action	References
Cardiovascular	Milk and dairy products consumption associated with reducing the occurrence of obesity	Choi et al. (2005); Pereira et al. (2002)
	Oxidized PLs could be an indicative marker of coronary artery disease or might represent a potential target for therapeutic intervention	Ashraf et al. (2009)
	High-fat diet with PLs-rich dairy extract caused a major decrease in the liver weight, total liver lipid, liver triglycerides and total cholesterol and serum lipids	Wat et al. (2009)
	PLs fortifications changed the plasma cholesterol quantity but did not influence the LDL/HDL ratio	Keller et al. (2013)
Inflammation and gastrointestinal infections	Milk PLs exert a strong gastro-protective function in humans, mainly in the duodenal mucosa	Kivinen et al. (1992); Kivinen et al. (1995)
	Egg PLs safeguard the mucosa of intestinal tract against the adhesion of pathogenic micro-organisms, decreasing the risk of acute diarrhea	Carlsson (1998)
	PS offered function as an endogenous regulator of immune and anti-inflammatory reactions	Carr et al. (1992), Gaitonde et al. (2011); Ponzin et al. (1989); Yamazaki et al. (1997)
	Sphingolipids have positive effects against gastrointestinal infections	Sprong et al. (2002)
	Consuming 3 eggs/day for 12 weeks caused a reduction in plasma C-reactive protein and an increase in adiponectin in overweight males	Ratliff et al. (2008)
	PLs have an active role in decreasing the development of arthritis	Hartmann et al. (2009)
	Dietary egg PC reduced inflammation in the gastrointestinal tract	
	Increased dietary PC absorption before collagen-induced arthritis associated with enhancing the anti-inflammatory protection	Erős et al. (2009)
	PC-enriched food as a pretreatment, but not as a therapy, could employ positive effects on the functional, morphological and microcirculatory features of chronic arthritis	
	Consumption of MFGM-enriched milk had a protective influence against gastrointestinal infections, resulted in a significant reduction in the number of short febrile episodes	Veereman-Wauters et al. (2012)
	Egg consumption improved the circulating plasma inflammatory markers in adults	Blesso et al. (2013)
Cancer	Sphingolipids inhibit the early and the late stages of colon carcinogenesis, in tests on mice in which tumor genesis was caused by an inherited genetic imperfection or chemically resulted by a chemical agent	Dillehay (1994)
	Sphingolipids fortification reduced the amount of aberrant crypt foci and, with longer feeding, also the amount of adenocarcinomas	
	Sphingolipids have a protective action against damage from γ-irradiation and chemical agents	Vesper et al. (1999)
	Inhibition of colon carcinogenesis/colorectal cancer prevention	Berra et al. (2002); Dillehay (1994); Schmelz et al. (1996); Snow et al. (2010)
	Milk PLs, especially SM, act upon skin cells protecting them against the influence of UV radiation	Russell et al. (2010)
	Dietary sphingolipids affected breast cancer	Schmelz et al. (2007)
Cholesterol absorption	SM affected different aspects of cholesterol transport and metabolism	Vesper et al. (1999)
	SM decreased the intestinal absorption of cholesterol and fats	Eckhardt et al. (2002); Noh and Koo (2004); Nyberg et al. (2000)
	Phytosphingosine reduced dietary cholesterol and free fatty acids absorption in animals by 50% and 40%, respectively	Duivenvoorden et al. (2006)
	Egg PC and SM reduced the lymphatic absorption of cholesterol	Jiang et al. (2001); Noh and Koo (2003)
	Milk SM is more powerful in inhibiting the intestinal absorption of cholesterol than egg SM	Noh and Koo (2004)
	PLs reduced the hepatic accumulation of intestinal cholesterol and increased the fecal cholesterol secretion	Kamili et al. (2010)
	Dietary PE decreased serum cholesterol in rats	Imaizumi et al. (1983); Imaizumi et al. (1991)
	PLs limited cholesterol absorption from gastrointestinal tract, and effective in liver therapy (steatosis, alcohol intoxication)	Ambroziak and Cichosz (2013)

Disease	Phospholipids action	References
Nervous system	Yolk PLs consumption relieved Alzheimer disease symptoms	Juneja et al. (1997)
	Dietary SM contributed to the myelination of the central nervous system	Oshida et al. (2003)
	Protective function of milk PLs on endoplasmic reticulum stress induced neuronal cell death, consumption of milk PLs or milk products reduced the risk of some neurodegenerative diseases	Nagai (2012)
	SM-fortified milk had a positive association with the neurobehavioral development in infants	Tanaka et al. (2013)
Alzheimer	PLs act as neuroprotectively, regulate brain activity, improve memory and resistance to stress, reduce depression risk, Alzheimer and Parkinson diseases	Ambroziak and Cichosz (2013)
Liver diseases	Studies showed an improvement of hepatic symptoms and an enhancement of liver functions with soybean PC supplementation in patients presenting nonalcoholic liver damage	Lieber et al. (1990)
	Soybean PC supplementation prevented lipid accumulation in liver, through reactivating the ethanol induced inhibition of mitochondrial fatty acids oxidation, thus decreasing liver damage	Navder et al. (1997)
	Soybean PC enhancement hemodynamics, intrahepatic cholestasis, liver function and in the general lipid metabolism	Golochevskaia et al. (1996)
	PC supported the recovery of liver after toxic or chronic viral damage	Niederau et al. (1997)

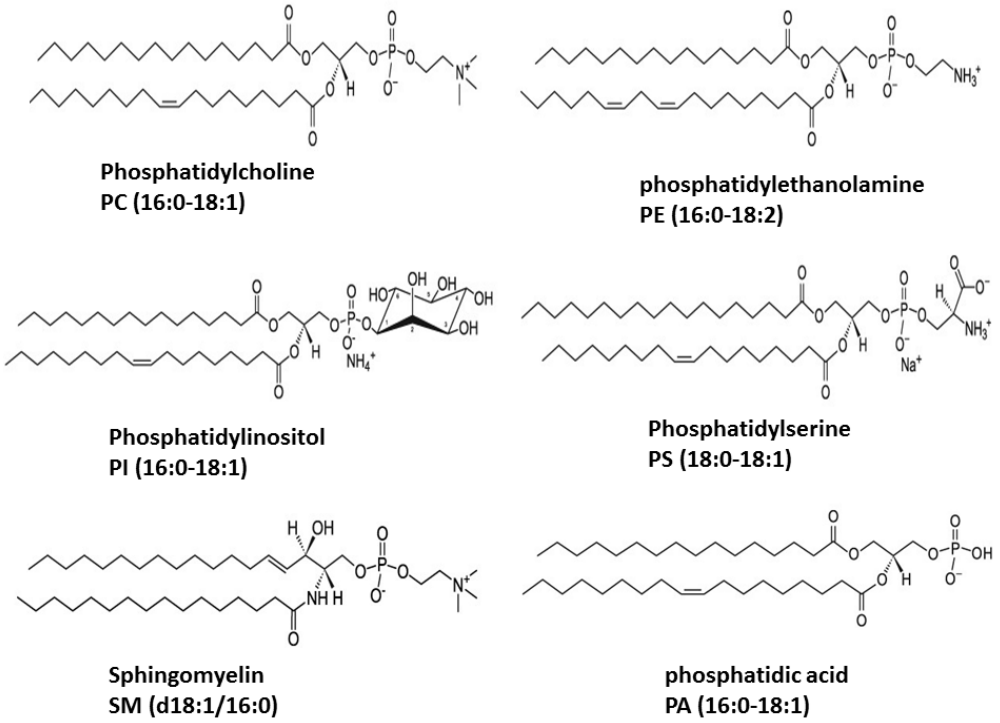

Fig. 1. Chemical structure of the major classes of phospholipids

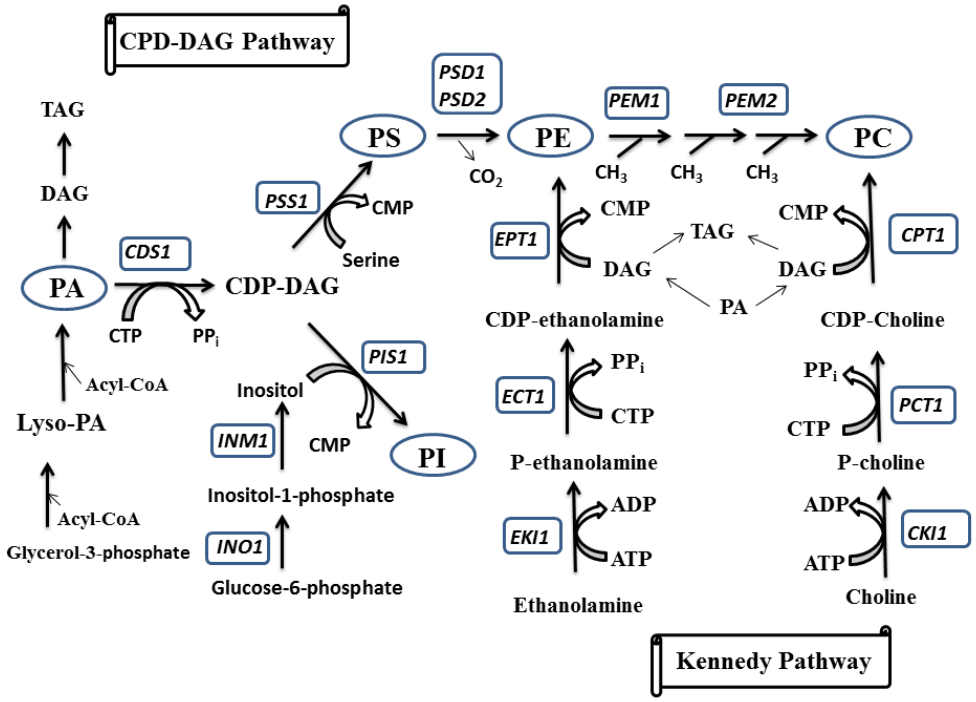

Fig. 2 Phospholipid biosynthetic pathways. The enzymes CDS1- encoded CDP-DAG synthase, PSS1-encoded PS synthase, PSD1/PSD2-encoded PS decarboxylase, PEM1-encoded PE methyltransferase and the PEM2-encoded PL methyltransferases catalyze reactions that lead to the formation of PC by the CDP-DAG pathway. CKI1-encoded choline kinase, PCT1-encoded choline-P cytidylyltransferase, and CPT1-encoded choline phosphotransferase catalyze reactions that lead to the formation of PC by the CDP-choline branch of the Kennedy pathway. EKI1-encoded ethanolamine kinase, ECT1-encoded ethanolamine-P cytidylyltransferase, and EPT1-encoded ethanolamine phosphotransferase catalyze reactions that lead to the formation of PE by the CDP-ethanolamine branch of the Kennedy pathway. INO1-encoded inositol 3-phosphate synthase and INM1-encoded inositol 3-phosphate phosphatase catalyze reactions that lead to the formation of inositol, which is utilized for the formation of PI. Abbreviations: PA, phosphatidate;

CDP-DAG, cytidine 5'diphosphate-diacylglycerol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; DAG, diacylglycerol; TAG, triacylglycerol. (Choi, 2007)

