Title: Exploration of natural phosphatidylcholine sources from six beans by
UHPLC-Q-Exactive Orbitrap/MS

Running title: Identification of phosphatidylcholine molecular species in six beans

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Abstract

BACKGROUND: Bean is a rich source of phosphatidylcholine (PC). This study aims to explore natural PC sources rich in polyunsaturated fatty acid (PUFA) with nutritional interest. PCs from six beans were purified (purity > 98.2%) by thin layer chromatography, and subsequently identified by UHPLC-Q-Exactive Orbitrap/MS.

RESULTS: Results showed that chickpea and soybean contained the highest quantity of PC among the six beans, making up 50.0 mg/g and 34.0 mg/g, respectively. Gas chromatographic analysis showed that soybean fatty acids contained high proportion of polyunsaturated fatty acid (58.78%), and chickpea contained high proportion of DHA (22:6, 2.73%). A total of 49 molecular species were identified by UHPLC-Q-Exactive Orbitrap/MS. (18:2-18:2)PC was predominant in soybean, red bean, red kidney bean and white kidney bean. (16:0-18:1)PC was the major species of chickpea PC and many plasmanyl-PC species and DHA (22:6)-PC were identified. The Principal Component Analysis (PCA) analysis and hierarchical cluster analysis indicated that the molecular profiles of chickpea PC were significantly different compared to other beans studied.

CONCLUSION: The findings suggest that chickpea appears to be an interesting plant source of DHA and ether lipids for dietary supplement.

Keywords: Bean PC, UHPLC-Q-Exactive Orbitrap/MS, Molecular species, Identification, PCA

INTRODUCTION

Beans, originated from America, are the member of the Leguminosae, family Phaseoleae, subfamily Papilionoideae.¹ Numerous cultivars of beans have now been developed, the seeds of which are different in size, shape and color and are called by a variety of name.^{2, 3} There are approximately 20 leguminous species utilized as substantial dietary sources for human nutrition.^{4,5} Beans contain a variety of nutrients, such as minerals, vitamins, complex carbohydrates, dietary fiber, protein and rather small content lipid.^{6, 7} Although bean lipid content is not in high level, the epidemiological and clinical data have suggested that the low content and high quality of beans lipid is associated with several health benefits which are the treatment of obesity, diabetes mellitus and even cancer, the improvement of serum lipid profiles and positive effect on cardiovascular disease risk factors, such as inflammation, platelet activity, and blood pressure.⁸⁻¹⁰ During the last years, soy lecithin, which consists primarily of phospholipids, was widely used in the food industry. The amphiphilic features of phospholipids make lecithin good emulsifying properties and wide cosmetic applications.^{11, 12} The major classes of soy lecithin are phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) as reported in our previous study.¹³ According to the biodisponibility, phospholipids are better carriers of polyunsaturated fatty acid (PUFA) than triacylglycerols. PC as the predominant class in most of soy lecithin is widely used in food supplementation. Clarification of the molecular structures of PC from various bean sources is an approach to explore and tap the natural PC sources rich in

molecular species with PUFA of nutritional interest.

Among bean lipids, phospholipids are a minor part in total lipids, which consist a large quantity of neutral lipids. In order to improve the accuracy of the analysis and comprehensively explore the molecular species of bean PC, the neutral lipids were removed to obtain purified PC. Moreover, the high levels of neutral lipids always affect ionization in ESI sources and desolvation of liquid chromatographic effluent droplets. Hence, additional purification steps are often required to abandon the neutral lipids and further purify PC before molecular species identification. Studies on the separation and purification of phospholipids have been widely investigated by several procedures, such as solvent extraction,¹⁴ solid-phase extraction,¹⁵ thin layer chromatography,¹⁶ two-dimensional liquid chromatography¹⁷ and supercritical carbon dioxide extraction.¹⁸ Analysis of phospholipid molecular species is usually performed by liquid chromatography coupled with mass detection (LC-MS). Among MS systems, ESI-MS² is sensitive and results in high specificity for phospholipid identification. In the last few years, with the development of the lipidomics, great progress has been made in lipid analytical techniques. Q-Exactive Orbitrap mass spectrometry is a recently developed technique with extremely high resolution, sensitivity and mass accuracy. It exhibits strong power for fragment ion scanning enabling recognition of numerous lipid isomers that have proven difficult to measure by normal mass spectrometry.¹⁹ UHPLC-Q-Exactive Orbitrap/MS has been applied for the analysis of lipids in different milks,^{20, 21} marine phospholipids²² or other components in various food sources. However, no studies have been published

yet concerning the comprehensive identification of PC molecular species from different beans by UHPLC-Q-Exactive Orbitrap/MS.

The aim of this study is to identify the molecular species of PC purified from six beans, including soybean, red bean, black bean, red kidney bean, white kidney bean and chickpea. Thin layer chromatography (TLC) was applied for the separation and collection of PC class based on the adsorption coefficient of different components on the silica gel plate. The high-performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD) was used to quantify the content of PC. Then, the PC molecular species in six beans were determined by UHPLC-Q-Exactive Orbitrap/MS. Finally, their different compositions in PC molecular species were compared by software-assisted lipidomic analysis. This is a first investigation regarding a deep comprehensive comparison and determination of PC molecular species from these six beans sources. Furthermore, this analytical method can be used to explore new potential sources of bioactive ingredients and evaluate the quality of raw materials.

MATERIALS AND METHODS

Materials

Six beans, including soybeans, red bean, black beans, red kidney beans, white kidney beans and chickpeas were purchased from local market. All the samples were ground into a fine powder (particle size: 150 μ m) at room temperature with wall breaker (Joyoung JYL-Y291, Shandong, China). The obtained powder was stored under -20°C until use.

The phospholipid standards, such as PE, PI, PC, were purchased from Sigma-Aldrich (USA). Solvents for lipid extraction were of chromatographic grade. Other chemicals, including methanol, acetonitrile, chloroform, ammonium formate, formic acid and dichloromethane were purchased from Sigma Aldrich (USA) (HPLC or MS grade). Ultra-pure water was supplied by Milli-Q system (Millipore, USA).

Extraction of total lipids and purification of phospholipids

Beans phospholipids were extracted from beans by the Folch method. In brief, 1.0 g dried bean powder (soybeans, red beans, black beans, red kidney beans, white kidney beans, chickpea) were mixed with 30 mL chloroform/methanol (2:1, v/v) and shaken for 30 min by water-bathing constant temperature shaker (SHA-C, Nanjing, China) at 25°C. The mixture was centrifuged at 4000 g for 15 min. The upper layer was transferred into a new tube and the precipitate was extracted again according to the procedure described above repeated three times. Then, the three combined organic fractions containing the phospholipids were evaporated by a rotary evaporator under reduced pressure and the extracts were collected.

Afterwards, 40mL of acetone was added into the centrifugal tube containing the residue. The tube was placed at -20°C overnight until phospholipids precipitation. The supernatant was removed completely by a rotary evaporator. The precipitate was continually washed 3 times with 30 mL of cold acetone (-20°C) and the organic phase was removed by decantation each time. Finally, the obtained phospholipids were dried under a gentle stream of nitrogen. The phospholipid fractions were stored at -20°C until use.

Separation of phospholipid classes by HPLC-ELSD

The determination of phospholipids was completed by HPLC-ELSD (Agilent 1260). High purity nitrogen under a pressure of 0.5 MPa was used as nebulizing gas. The drift tube temperature was set at 30°C. The separation of phospholipids was carried out by using a normal phase column (150×3 mm, 3 µm, Inertsil, Japan). The flow rate of mobile phase was 0.6 mL/min, and separations were performed at room temperature using a 25 min linear gradient. The mobile phase A was made up of CHCl₂/CH₃OH (88:12, v/v) and the phase B was made up of CH₃OH/1 M aqueous formic acid (pH was adjusted to 3 by using triethylamine) (84:16, v/v). The gradient started with 100% A, to 80% A by 8 min, then to 60% A by 3 min and holding this proportion for 2 min, then to 40% A by 5 min and return to initial condition in 7 min. The injection volume was 20 µL. The phospholipid classes were identified by comparison of their retention times with those of standard samples which were analyzed under the same condition. The quantification of phospholipid classes from six beans was performed by external standard method, according to the calibration curves of each peak area of corresponding standards.

PC purification by TLC plate

TLC plate was applied for PC isolation. With the developing mixture system of CHCl₃/CH₃OH/NH₃ •H₂O (65:25:4, v/v/v), PC standard samples and the total fatty acid present in different beans were colored by phosphomolybdate on the prepared silica gel plate. The PC of different beans was identified by the *R_f* value comparing with those of standard samples. The silica gel containing PC fractions was collected and

subjected to column chromatography eluted with 100% methanol. The solvent was removed by a rotary evaporator, and then dried under a gentle nitrogen flow. The purity of PC was checked by HPLC-ELSD with the above description.

Analysis of fatty acids of PC by GC

Fatty acids methyl esters were obtained after transesterification of purified phospholipids according to our previous study.²³ Gas chromatography (GC) (3400 Varian) equipped with an autosampler, a flame ionization detector, and an ionic liquid capillary column (SP 2560, 100 m × 0.25 mm × 0.2 μm) was used. The temperature of the injector and the detector was set at 280°C. The analysis was achieved using a temperature gradient program from an initial temperature of 140°C and increased to 175°C at a rate of 5°C/min. The total running time was 65 min. Identification of GC peaks was achieved by comparing their retention times with those of the corresponding standards (FAME mix C4-C24). The percentages of fatty acids were calculated as the ratio of each peak area against the sum of all identified peak areas.

Separation and identification of PC molecular species by UHPLC-Q-Exactive Orbitrap/MS

The analysis of PC from beans was carried on UHPLC system (Dionex, UltiMate, 3000 RSLC) equipped with a NucleodurC18HTec column (250 x 4.6 mm, 3 μm). The mobile phase was methanol with 5M ammonium formate. The procedure of separation was implemented in an isocratic elution at a flow rate of 0.6 mL/min for 60 min. The column temperature was 60°C. The injection volume was 10 μL. A Q-Exactive Orbitrap mass spectrometry instrument (ThermoFisher Scientific Bremen,

Germany) was applied for the identification and the quantification of PC molecular species in six different beans. The ESI source conditions was set as follows: spray voltage (3.2 kV), sheath gas flow rate (8.4 L/min), the capillary temperature (325°C), auxiliary gas heater temperature (350°C), auxiliary gas flow rate (24.2 L/min). Data were collected in positive ionization mode over a mass range between 500 and 900 Da within 0.2 s scan duration. The MS/MS experiments were completed using different collision energy of 30 V for each compound in positive mode and negative ion mode. The mass range was set between m/z 50 and 800 for fragmentation products. The percentage of each PC molecular species from different beans was calculated by the ratio of the peak area of each identified peak against the sum of all identified peak areas. To equilibrate the analytical platforms and correct the minor variation from different samples, a quality control (QC) sample (cocktail of each sample) was injected regularly after each 5 samples.

Statistical analysis

Lipid were identified and quantified using LipidSearch software version 4.2 (ThermoFisher Scientific Bremen, Germany) based on the accurate mass and fragment matching. False positives are checked manually. The exact masses (the mass accuracy was < 5 ppm) of lipids were determined on the MS system, which allowed the possible elemental composition calculation of empirical chemical formula. Discriminatory analysis of PC composition from various beans was analyzed by Principal Component Analysis (PCA) using MetaboAnalyst software. The first Principal Component (PC1) expresses the most information of original variances and

the following is the PC2. The score plots indicate the difference of the various bean PC. The loading plots reflect the contribution of important variables to the PC molecular species from different bean species.

RESULTS AND DISCUSSION

PC content in different beans

Due to the structural features of PC, HPLC-ELSD was used to detect the PC's content in different beans. The content of PC from various beans was quite different (**Table 1**). The total lipids extracted from chickpea and soybean contained the most abundant PC, about 50.0 mg/g and 34.0 mg/g, respectively. The content of red kidney bean and white kidney bean appeared similar, about 11.5 mg/g and 9.0 mg/g, respectively, which are less than the content in red bean (15.0 mg/g), but higher than black bean (4.9 mg/g). According to the different moisture content in various beans, the soybean (7.7 mg/g) exhibited highest level of PC in the dried bean powder. The result was in accordance with the previous report.¹² Chickpea PC also exhibited high level, next to soybean PC. Black bean showed the lowest level of PC in the total lipids, but its content in the dried bean powder, was not the lowest (1.07 mg/g). In general, the soybean and chickpea contained high PC content either in total lipids or dried bean powder. The red bean, white kidney bean and red kidney bean have the similar content of PC.

Furthermore, when considering the high level of structural similarity, the molecular species within a given class probably couldn't be completely resolved and their isomers couldn't be distinguished. That's why some articles reported fewer

phospholipid molecular species. Thus, in the present study, neutral lipids of all the samples were removed by thin layer chromatography in order to obtain pure phospholipids, prior to structural identification. The purities of phospholipids from different beans monitored by HPLC-ELSD were exhibited to be at least 98.2%.

Fatty acid profiles of purified PC

Table 2 shows fatty acid profiles of purified PC separated from all kinds of beans investigated. Several fatty acids, including 18:1, 16:0, 18:0 and 18:2 were particularly abundant in the six beans. Especially in soy PC, 18:2 (51.97%) was most predominant among the samples, but in this bean, 22:6 (DHA) were not detected. Moreover, 18:3n3 (4.13%) was obviously in higher quantities than that in other five beans PC. The result was in accordance with a previous report.¹² 22:6 was found in five beans, especially in chickpea and red kidney bean, it accounted for 2.73% and 2.53%, respectively. In addition, soy PC contains high levels of PUFA (58.78%) as compared to purified PC from other bean sources. Red beans contain highest proportions of monounsaturated fatty acid (46.77%) and soybean showed lowest level of saturated fatty acid (27.17%). The data on fatty acid profiles were later used in a comparative analysis of molecular species identifications to verify whether the molecular structures of each PC matched its fatty acid profile. Meanwhile, fatty acid profiles provided by GC analysis could help us to determine the molecular structures more accurately and better rule out false positives.

Molecular species of purified PC from various beans

The molecular mass peaks present in the chromatograms of purified PC from six

beans were identified by positive UHPLC-Q-Exactive Orbitrap/MS analysis (**Fig. 1**). Soybean PC (**Fig. 1A**) was of simple composition compared with other beans. The profiles of chickpea PC appear different among them (**Fig. 1F**), several ether phospholipids (plasmanyl-PC) were detected, and many isomers were found in chickpea PC samples. The identification of PC molecular structures was realized through the analysis of MS² fractions. The results indicated that the fracture mechanism of glycerol-PC was different from plasmanyl-PC. Under our experimental conditions, the ESI tandem mass spectra of glycerol-PC [M+H]⁺ ions yielded five fragments: (1) A fragments ion [M+H-183]⁺ was a specific fragment corresponding to loss of the polar head-group and enabling distinction with other phospholipid classes; (2) *m/z* values (59, 86 and 104 Da) reflect the fragments derived from polar headgroup; (3) [RCO]⁺ corresponded to the detached fatty acyl residues; (4)[M+H-RCO]⁺ ion corresponded to loss of one of the two acyl groups; (5) [M+H-RCOOH]⁺ ion results from the loss of a fatty acid.

A representative MS spectra and MS² fragmentation spectra of (18:2-18:2)PC from white kidney bean (*m/z* 782.56671) is shown in **Fig. 2**. One characteristic fragment ion at *m/z* 184.07301 corresponded to polar headgroup [C₅H₁₄NPO₄H]⁺,²³ the less intense *m/z* ions at 104.10732 choline [C₅H₁₃NOH]⁺, *m/z* 86.09683 dehydrocholine [C₅H₁₂N]⁺, and *m/z* 59.07380 trimethylamine group.²⁵ A fragment ion at *m/z* 599.50067 corresponded to the loss of the polar headgroup. *m/z* 263.51816 was identified as detached linoleic acyl group. *m/z* 502.32800 corresponded to the loss of a linoleic acid. *m/z* 520.33826 corresponded to the loss of a linoleic acyl group.

The ESI tandem mass spectra of plasmanyl-PC $[M+H]^+$ ions yielded four fragments: (1) $[M+H-183]^+$ corresponded to the loss of polar headgroup; (2) $[M+H-RCOOH]^+$ corresponded to the loss of a fatty acid; (3) $[M+H-RCOOH-TMA]^+$ corresponded to the loss of a trimethylamine group (TMA, 59 Da) and a fatty acid; (4) $[M+H-RCOOH-104]^+$ resulted from the loss of a fatty acid and a fragment derived from polar headgroup. Through analysis the above fragment information, the molecular structure of plasmanyl-PC could be identified. A representative MS spectra and MS² fragmentation spectra of (16:0e-18:1)PC from chickpea (m/z 746.60339) is given in **Fig. 3**. A fragment ion at m/z 562.62183 reflected the loss of polar headgroup (183 Da); m/z 464.44827 corresponded to the loss of a stearic acid. m/z 360.32010 reflected the loss of a stearic acid and a fragment (104 Da). m/z 405.28946 corresponded to the simultaneous loss of a stearic acid and a trimethylamine group (TMA, 59 Da). The above approaches were applied to identify each molecular species of purified PC from various beans and enabled to identify the PC molecular species from other food sources.

Profiles of PC molecular species

Table 3 presents the relative distributions of various PC molecular species in the six beans studied. 41 species were detected in chickpea PC, number obviously higher than the PC species detected in soybean, red bean, black bean, red kidney bean and white kidney bean, making up 12, 13, 14, 10 and 18. Species determination data for the six beans was in agreement with their fatty acid profiles (**Table 2**), with high quantities of 16:0, 18:0, 18:1, 18:2 and 18:3. From **Table 3**, (16:0-18:1)PC was the

major molecular species in chickpea PC (21.39%), while (18:2-18:2)PC was the predominant molecular species in other bean species, especially in soybean, which accounted for 56.14%. In addition, it was worth noting that many species contain DHA (22:6) in chickpea PC, and many plasmanyl-PC species were found in chickpea PC. Among the plasmanyl-PC species, (16:0e-18:1)PC was relatively abundant, accounted for 2.39%. The variations in the profiles of PC molecular species among the different samples were probably a result of the differences in growing environment, cultivated varieties, plunking time and plant food.

Characterization of PC by PCA

Characterization and discrimination of PC purified from seven beans were investigated by PCA. The first Principal Component (PC1) contributed to 67.4% of the variability in the data set. The second PC (PC2) accounted for 13.1% of the variance in the data. The PC1 and PC2 described 80.5% of the variance and reflected significant variability in the data. The remaining PCs (PC3 to PC5) accounted for 19.5% of the variance and cannot explain significant variability in the data. Thus, only PC1 and PC2 were considered for further study according to Kaiser's rule. The loading values in PC1 and PC2 represented significant contributions of individual PC molecular species to total variability (**Supplementary Table 1**). PC1 loading indicated it had high contributions for many molecular species, for example, (15:0-16:0)PC, (14:0e-22:6)PC, (16:1e-20:3)PC, (18:0e-18:2)PC, (18:0e-18:1)PC, (16:1e-22:6)PC, (16:0e-22:6)PC, (18:2-20:2)PC, (18:0-20:3)PC, (18:1e-22:6)PC and (18:0e-22:6)PC. PC2 loading exhibited high contributions for (16:1-16:1)PC,

(15:0-18:1)PC, (18:2-18:2)PC, (18:1-18:1)PC, (18:1-20:2)PC and (18:0-20:2)PC (**Fig. 4A, Supplementary Table 1**).

Fig. 4B shows the score plots of PC in various beans. Red kidney bean and white kidney bean showed negative loadings on PC1 and positive loadings on PC2 indicating the PC of these two beans had lower concentration in PC1 and higher concentration in PC2. Meanwhile, soy bean, black bean and red bean had negative loadings on PC1 and PC2. Chickpea showed positive loadings on PC1 and PC2 with highest score on PC1. The result indicated PC molecular species and concentration of chickpea had significant difference from other beans.

Diversity and comparison of different groups by hierarchical cluster analysis

Hierarchical cluster analysis provides intuitive visualization of a data table. Each colored cell on the map corresponded to a concentration value in the data table. The hierarchical cluster analysis of top 25 (concentration) PC molecular species of various beans was given in **Fig. 5**. The cluster analysis indicated that the profiles of chickpea PC were closest to soybean, following white kidney bean, red kidney bean, black soya bean and red bean. The content of molecular species, including (18:0-18:1)PC, (16:1e-22:6)PC, (16:1-20:2)PC, (16:1e-18:2)PC, (16:0e-22:6)PC, (15:0-16:0)PC, (14:0e-22:6)PC, (18:1e-22:6)PC, (18:0e-18:2)PC, (18:0e-22:6)PC, (18:0-20:3)PC, (18:0e-16:0)PC, (18:0e-18:1)PC and (16:0-18:1)PC in chickpea, (18:2-18:2)PC, (18:0-20:2)PC and (18:1-20:2)PC in soybean, (15:0-18:3)PC in white kidney bean, (16:0-16:0)PC in red kidney bean, and (18:3-20:3)PC in red bean, appeared significant high quantities compared to those species in other beans. They can be

considered to be most responsible for the discrimination among the six bean types, which adds important capability for more comprehensive evaluation of the nutritional value of beans. Moreover, the results demonstrated that the profiles of chickpea PC had key difference among the six beans studied.

CONCLUSION

In this paper, a separation and identification approach of beans PC species was developed by UHPLC-Q-Exactive Orbitrap/MS analysis. The fatty acids profiles of PC molecules were confirmed by GC analysis. Chickpea PC presented obviously more species than other bean sources, in particular, many ether phospholipids (plasmanyl-PC) were found in chickpea which were not detected in other beans. The PCA analysis and hierarchical cluster analysis indicated that the molecular profiles of chickpea PC were significantly different from other beans studied. The experimental findings suggest that chickpeas appear to be an interesting plant source of DHA and ether lipids for dietary supplement. In addition, soybean is a rich source of PUFA, in particular, 18:2 which accounted for high proportion in purified PC. Nevertheless, their stability during processing or cooking should be considered, because the beans used for human consumption are usually processed by different methods, such as frying, steam or boiling. Whether these processing methods affect the profiles of molecular structures of PC would be investigated in our further study.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this

paper.

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Figure captions

Figure 1. Total ion chromatogram of molecular species from purified PC under positive ion mode. (A) soybean; (B) red bean; (C) black bean; (D) red kidney bean; (E) white kidney bean; (F) Chickpea

Figure 2. MS/MS fragmentation under positive ion mode of white kidney bean (18:2-18:2)PC at m/z 782.56671 (A) and white kidney bean (16:0e-18:1)PC at m/z 746.60339 (B).

Figure 3. Loading plot of PC1 and PC2 (A) and score plots (B) for six beans PC.

Figure 4. Hierarchical cluster analysis based on top 25 molecular species in six beans.

Colors represent different concentrations indicated by the color bar.

Figure 1

Figure 2

Figure 3

483 (B)

485 **Figure 4**

Figure 5

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Table1. PC content in total lipids and in dry powder of various beans

Beans	Content in total lipids (mg/g)	Content in dried powder (mg/g)
black bean	4.9±0.6	1.07±0.08
soybean	34±7	7.70±0.28
red bean	15.0±1.4	0.48±0.03
red kidney bean	11.5±0.7	0.42±0.02
white kidney bean	9.0±4.2	0.32±0.02
chickpea	50.0±8.5	3.43±0.12

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Table 2. Percentages of Fatty acids of purified PC from various beans

Fatty acids	Soy	Red	Black soya	Red kidney	White kidney	Chickpea
C14:0	nd	nd	nd	nd	0.28±0.04	0.31±0.05
C15:0	nd	nd	0.31±0.03	nd	0.2±0.0	0.3±0.0
C16:0	19.31±0.45	28.51±0.89	27.21±1.12	27.88±1.04	27.30±1.28	27.11±1.08
C16:1	0.7±0.1	3.79±0.28	2.87±0.19	2.14±0.11	2.94±0.23	1.72±0.14
C17:0	nd	0.13±0.06	nd	nd	0.13±0.06	nd
C18:0	7.86±0.35	7.42±0.27	9.65±0.44	13.08±0.82	10.69±0.74	12.37±0.92
C18:1n9t	nd	0.16±0.04	0.26±0.02	0.15±0.02	nd	nd
C18:1n9c	13.35±0.62	42.82±2.38	41.37±2.12	35.24±2.01	43.93±2.53	40.74±1.92
C18:2n6c	51.97±2.88	11.24±1.3	12.17±1.04	13.38±0.41	9.55±0.96	9.67±0.84
C18:3n6	nd	0.36±0.02	0.24±0.01	0.21±0.03	1.69±0.01	0.12±0.04
C18:3n3	4.13±0.42	0.28±0.04	0.13±0.03	0.23±0.01	0.11±0.01	0.15±0.06
C20:2	0.86±0.08	nd	nd	nd	nd	0.09±0.02
C20:3n3	1.82±0.06	4.52±0.96	4.46±0.82	5.16±1.02	3.18±0.28	4.69±0.32
C22:6	nd	0.77±0.04	1.33±0.06	2.53±0.13	1.61±1.02	2.73±0.41
ΣSFA	27.17±1.78	36.06±1.85	37.17±2.07	40.96±2.36	38.60±1.68	40.09±2.42
ΣMUFA	14.05±1.36	46.77±3.21	44.5±3.1	37.53±2.66	46.87±2.78	42.46±2.71
ΣPUFA	58.78±3.82	17.17±0.54	18.33±0.48	21.51±1.31	14.53±1.26	17.45±1.43

531 SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty
532 acid. nd means not detected.

Table 3. Molecular species profiles of each purified PC^a

[M+H] ⁺	Structure ^b	Error	Soy	Red	Black soy	Red kidney	White kidney	Chickpea
692.55735	14:0e-16:0	-2.19	nd	nd	nd	nd	nd	0.06±0.02
706.53669	14:0-16:0	-2.04	nd	nd	nd	nd	nd	0.55±0.13
716.55712	14:0e-18:2	-2.44	nd	nd	nd	nd	nd	0.04±0.03
718.57283	14:0e-18:1	-2.35	nd	nd	nd	nd	nd	0.21±0.01
720.55195	15:0-16:0	-2.54	nd	nd	nd	nd	nd	0.11±0.00
720.58832	16:0e-16:0	-2.57	nd	nd	nd	nd	nd	0.23±0.04
728.52516	14:0-18:3	3.68	nd	nd	nd	nd	0.07±0.01	nd
730.53607	16:1-16:1	-2.82	0.18±0.02	nd	0.22±0.07	nd	0.09±0.01	0.16±0.03
730.53617	14:0-18:2	-2.68	nd	nd	nd	nd	nd	0.14±0.01
732.55164	16:0-16:1	-2.92	nd	0.30±0.02	0.21±0.06	nd	0.25±0.02	3.02±0.04
734.56740	16:0-16:0	-2.76	0.25±0.01	0.93±0.01	0.22±0.04	2.29±0.03	0.71±0.04	1.08±0.03
742.53996	15:0-18:3	2.46	nd	nd	nd	nd	0.25±0.03	nd
742.57332	16:1e-18:2	-1.62	nd	nd	nd	nd	nd	0.06±0.00
744.55285	15:0-18:2	-1.25	nd	nd	0.31±0.03	nd	nd	0.36±0.01
744.58799	16:0e-18:2	-2.93	nd	nd	nd	nd	nd	0.45±0.03
746.60339	16:0e-18:1	-3.25	nd	nd	nd	nd	nd	2.39±0.08
748.62012	18:0e-16:0	-1.80	nd	nd	nd	nd	nd	0.02±0.01
754.54074	16:1-18:3	3.46	nd	nd	nd	nd	0.13±0.01	nd
758.56683	16:1-18:1	-3.43	13.76±0.34	18.28±0.04	17.21±1.03	17.57±0.15	17.48±1.12	8.3±0.4
760.58411	16:0-18:1	-1.28	2.78±0.08	1.40±0.05	0.81±0.06	8.14±0.18	6.20±0.81	21.39±0.33
762.59924	16:0-18:0	-1.95	nd	nd	nd	nd	nd	0.21±0.02
764.55714	14:0e-22:6	-2.26	nd	nd	nd	nd	nd	0.26±0.00
768.58811	16:1e-20:3	-2.68	nd	nd	nd	nd	nd	0.44±0.01
770.56803	15:0-20:3	-1.82	nd	nd	nd	nd	0.20±0.04	nd
772.58798	17:0-18:2	3.75	nd	0.31±0.02	nd	nd	0.32±0.03	nd
772.61883	18:0e-18:2	-3.42	nd	nd	nd	nd	nd	0.86±0.04
774.63494	18:0e-18:1	-2.81	nd	nd	nd	nd	nd	0.52±0.02
778.53619	18:3-18:3	-2.49	1.27±0.05	4.40±0.22	2.32±0.08	6.32±0.31	7.04±0.21	0.03±0.00
780.55157	18:2-18:3	-2.83	12.75±0.24	18.84±0.54	19.01±0.35	11.59±0.88	20.03±1.22	0.66±0.02
782.56671	18:2-18:2	-3.48	56.14±0.31	22.14±1.81	23.10±1.41	19.58±0.28	25.87±1.53	10.94±0.03
782.57068	18:1-18:3	+1.60	nd	4.07±0.06/	5.07±0.06	1.45±0.05	2.06±0.72	0.34±0.01
784.58618	16:1-20:2	+1.40	nd	nd	nd	nd	nd	0.62±0.03
786.60162	18:0-18:2	+1.13	nd	nd	4.19±0.12	3.70±0.17	nd	8.13±0.34
786.60120	18:1-18:1	+0.60	11.4±0.3	3.32±0.01	nd	nd	2.90±0.06	8.38±0.41
788.61377	18:0-18:1	-3.31	0.80±0.09	0.77±0.10	0.68±0.01	nd	0.65±0.04	16.70±1.63
790.57205	16:1e-22:6	-3.12	nd	nd	nd	nd	nd	0.04±0.01
792.58797	16:0e-22:6	-2.78	nd	nd	nd	nd	nd	0.18±0.03
804.55135	16:1-22:6	-3.02	nd	nd	nd	nd	nd	0.16±0.02
806.56966	18:3-20:3	+0.29	0.15±0.02	14.26±0.03	14.31±0.03	18.34±0.02	7.19±0.02	5.41±0.19
808.58221	18:2-20:3	-3.55	nd	nd	nd	nd	nd	1.57±0.08
810.59886	18:2-20:2	-2.31	nd	nd	nd	nd	nd	0.56±0.06

810.60227	18:1-20:3	1.90	nd	nd	nd	nd	nd	nd
812.61404	18:0-20:3	-2.88	nd	nd	nd	nd	nd	0.30±0.02
812.61612	20:2-18:1	-0.32	0.24±0.02	nd	nd	nd	nd	nd
814.63489	18:0-20:2	+3.39	0.28±0.04	nd	nd	nd	nd	nd
818.60384	18:1e-22:6	-2.42	nd	nd	nd	nd	nd	0.11±0.01
820.61960	18:0e-22:6	-2.28	nd	nd	nd	nd	nd	0.13±0.04
830.56748	18:2-22:6	-2.35	nd	nd	nd	nd	nd	0.94±0.02
834.59784	18:0-22:6	-3.46	nd	10.98±0.22	12.34±0.03	11.02±0.01	8.56±0.12	3.94±0.42

535 ^aPercentages of all identified molecular species and Results are expressed as mean ± SD.

536 ^bmolecular structures provide carbon number, number of double bonds, alkylacyl (ether) species,
537 such as 14:0e.

538 ^cnd means not detected.

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571 **Graphic Abstract**

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