

Exploration of natural phosphatidylcholine sources from six beans by UHPLC-Q-HRMS

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▶ To cite this version:

Fu Yang, Minghao Zhang, Zhou Li, Jikai Liu , Eric Marchioni. Exploration of natural phosphatidylcholine sources from six beans by UHPLC-Q-HRMS. Journal of Food Science, 2020. hal-02917345

HAL Id: hal-02917345 https://hal.science/hal-02917345v1

Submitted on 19 Aug 2020

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1	Title: Exploration of natural phosphatidylcholine sources from six beans by
2	UHPLC-Q-Exactive Orbitrap/MS
3	Running title: Identification of phosphatidylcholine molecular species in six beans
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22 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 27of PC among the six beans, making up 50.0 mg/g and 34.0 mg/g, respectively. Gas 28 chromatographic analysis showed that soybean fatty acids contained high proportion 29 of polyunsaturated fatty acid (58.78%), and chickpea contained high proportion of 30 DHA (22:6, 2.73%). A total of 49 molecular species were identified by 31 UHPLC-Q-Exactive Orbitrap/MS. (18:2-18:2)PC was predominant in soybean, red 32 33 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. 34 The Principal Component Analysis (PCA) analysis and hierarchical cluster analysis 35 36 indicated that the molecular profiles of chickpea PC were significantly different 37 compared to other beans studied.

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

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

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43

44 **INTRODUCTION**

Beans, originated from America, are the member of the Leguminosae, family 45 Phaseoleae, subfamily Papilionoideae.¹ Numerous cultivars of beans have now been 46 developed, the seeds of which are different in size, shape and color and are called by 47 a variety of name.^{2, 3} There are approximately 20 leguminous species utilized as 48 substantial dietary sources for human nutrition.^{4, 5} Beans contain a variety of nutrients, 49 such as minerals, vitamins, complex carbohydrates, dietary fiber, protein and rather 50 small content lipid.^{6, 7} Although bean lipid content is not in high level, the 51 epidemiological and clinical data have suggested that the low content and high 52 quality of beans lipid is associated with several health benefits which are the 53 treatment of obesity, diabetes mellitus and even cancer, the improvement of serum 54 55 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 56 lecithin, which consists primarily of phospholipids, was widely used in the food 57 industry. The amphiphilic features of phospholipids make lecithin good emulsifying 58 properties and wide cosmetic applications.^{11, 12} The major classes of soy lecithin are 59 phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol 60 (PI) as reported in our previous study.¹³ According to the biodisponibility, 61 phospholipids are better carriers of polyunsaturated fatty acid (PUFA) than 62 triacylglycerols. PC as the predominant class in most of soy lecithin is widely used in 63 food supplementation. Clarification of the molecular structures of PC from various 64 bean sources is an approach to explore and tap the natural PC sources rich in 65

66 molecular species with PUFA of nutritional interest.

Among bean lipids, phospholipids are a minor part in total lipids, which 67 consist a large quantity of neutral lipids. In order to improve the accuracy of the 68 analysis and comprehensively explore the molecular species of bean PC, the neutral 69 lipids were removed to obtain purified PC. Moreover, the high levels of neutral lipids 70 71always affect ionization in ESI sources and desolvation of liquid chromatographic 72 effluent droplets. Hence, additional purification steps are often required to abandon the neutral lipids and further purify PC before molecular species identification. 73 Studies on the separation and purification of phospholipids have been widely 74 investigated by several procedures, such as solventextraction,¹⁴ solid-phase 75 extraction,¹⁵ thin layer chromatography,¹⁶ two-dimensional liquid chromatography¹⁷ 76 and supercritical carbon dioxide extraction.¹⁸ Analysis of phospholipid molecular 77 species is usually performed by liquid chromatography coupled with mass detection 78 (LC-MS). Among MS systems, ESI-MS² is sensitive and results in high specificity 79 80 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 81 Orbitrap mass spectrometry is a recently developed technique with extremely high 82 resolution, sensitivity and mass accuracy. It exhibits strong power for fragment ion 83 scanning enabling recognition of numerous lipid isomers that have proven difficult to 84 measure by normal mass spectrometry.¹⁹ UHPLC-Q-Exactive Orbitrap/MS has been 85 applied for the analysis of lipids in different milks,^{20, 21} marine phospholipids²² or 86 other components in various food sources. However, no studies have been published 87

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

90 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 91 bean and chickpea. Thin laver chromatography (TLC) was applied for the separation 92 93 and collection of PC class based on the adsorption coefficient of different 94 components on the silica gel plate. The high-performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD) was used to quantify the 95 96 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 97 molecular species were compared by software-assisted lipidomic analysis. This is a 98 99 first investigation regarding a deep comprehensive comparison and determination of PC molecular species from these six beans sources. Furthermore, this analytical 100 method can be used to explore new potential sources of bioactive ingredients and 101 evaluate the quality of raw materials. 102

103 MATERIALS AND METHODS

104 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: 150µm) 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).

115 Extraction of total lipids and purification of phospholipids

Beans phospholipids were extracted from beans by the Folch method. In brief, 1.0 116 g dried bean powder (soybeans, red beans, black beans, red kidney beans, white 117 118 kidney beans, chickpea) were mixed with 30 mL chloroform/methanol (2:1, v/v) and shaked for 30 min by water-bathing constant temperature shaker (SHA-C, Nanjing, 119 China) at 25°C. The mixture was centrifuged at 4000 g for 15 min. The upper layer 120 121 was transferred into anew tube and the precipitate was extracted again according to the procedure described above repeated three times. Then, the three combined organic 122 fractions containing the phospholipids were evaporated by a rotary evaporator under 123 124 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 133 1260). High purity nitrogen under a pressure of 0.5 MPa was used as nebulizing gas. 134 The drift tube temperature was set at 30°C. The separation of phospholipids was 135 136 carried out by using a normal phase column (150×3 mm, 3 μ m, Inertsil, Japan). The 137 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 138 CHCl₂/CH₃OH (88:12, v/v) and the phase B was made up of CH₃OH/1 M aqueous 139 140 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 141 proportion for 2 min, then to 40% A by 5 min and return to initial condition in 7 min. 142 143 The injection volume was 20 µL. The phospholipid classes were identified by comparison of their retention times with those of standard samples which were 144 analyzed under the same condition. The quantification of phospholipid classes from 145 146 six beans was performed by external standard method, according to the calibration curves of each peak area of corresponding standards. 147

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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, $\nu/\nu/\nu$), 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.

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Analysis of fatty acids of PC by GC

Fatty acids methyl esters were obtained after transesterification of purified 158phospholipids according to our previous study.²³ Gas chromatography (GC) (3400 159Varian) equipped with an autosampler, a flame ionization detector, and an ionic liquid 160 capillary column (SP 2560, 100 m \times 0.25 mm \times 0.2 μ m) was used. The temperature of 161 the injector and the detector was set at 280°C. The analysis was achieved using a 162 temperature gradient program from an initial temperature of 140°C and increased to 163 175°C at a rate of 5°C/min. The total running time was 65 min. Identification of GC 164 165peaks was achieved by comparing their retention times with those of the corresponding standards (FAME mix C4-C24). The percentages of fatty acids were 166 calculated as the ratio of each peak area against the sum of all identified peak areas. 167

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 μ m). 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 μ L. A Q-Exactive Orbitrap mass spectrometry instrument (ThermoFisher Scientific Bremen,

Germany) was applied for the identification and the quantification of PC molecular 176 species in six different beans. The ESI source conditions was set as follows: spray 177 voltage (3.2 kV), sheath gas flow rate (8.4 L/min), the capillary temperature (325°C), 178 auxiliary gas heater temperature (350°C), auxiliary gas flow rate (24.2 L/min). Data 179180 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 181 collision energy of 30 V for each compound in positive mode and negative ion mode. 182 The mass range was set between m/z 50 and 800 for fragmentation products. The 183 184 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 185 areas. To equilibrate the analytical platforms and correct the minor variation from 186 187 different samples, a quality control (QC) sample (cocktail of each sample) was injected regularly after each 5 samples. 188

189 Statistical analysis

190 Lipid were identified and quantified using LipidSearch software version 4.2 (ThermoFisher Scientific Bremen, Germany) based on the accurate mass and 191 192 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 193 the possible elemental composition calculation of empirical chemical formula. 194 Discriminatory analysis of PC composition from various beans was analyzed by 195 Principal Component Analysis (PCA) using MetaboAnalyst software. The first 196 Principal Component (PC1) expresses the most information of original variances and 197

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.

201 RESULTS AND DISCUSSION

202 PC content in different beans

Due to the structural features of PC, HPLC-ELSD was used to detect the PC's 203 content in different beans. The content of PC from various beans was quite different 204 (Table 1). The total lipids extracted from chickpea and soybean contained the most 205 206 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, 207 respectively, which are less than the content in red bean (15.0 mg/g), but higher than 208 209 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 210 result was in accordance with the previous report.¹² Chickpea PC also exhibited high 211 level, next to soybean PC. Black bean showed the lowest level of PC in the total lipids, 212 but its content in the dried bean powder, was not the lowest (1.07 mg/g). In general, 213 the soybean and chickpea contained high PC content either in total lipids or dried 214 bean powder. The red bean, white kidney bean and red kidney bean have the similar 215 content of PC. 216

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 225investigated. Several fatty acids, including 18:1, 16:0, 18:0 and 18:2 were particularly 226 abundant in the six beans. Especially in soy PC, 18:2 (51.97%) was most predominant 227 228 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. 229 The result was in accordance with a previous report.¹² 22:6 was found in five beans, 230 231 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 232to purified PC from other bean sources. Red beans contain highest proportions of 233 234 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 235 analysis of molecular species identifications to verify whether the molecular 236 structures of each PC matched its fatty acid profile. Meanwhile, fatty acid profiles 237 provided by GC analysis could help us to determine the molecular structures more 238 accurately and better rule out false positives. 239

240 Molecular species of purified PC from various beans

241 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). 242 Soybean PC (Fig. 1A) was of simple composition compared with other beans. The 243 profiles of chickpea PC appear different among them (Fig. 1F), several ether 244 phospholipids (plasmanyl-PC) were detected, and many isomers were found in 245 chickpea PC samples. The identification of PC molecular structures was realized 246 through the analysis of MS² fractions. The results indicated that the fracture 247 mechanism of glycerol-PC was different from plasmanyl-PC. Under our experimental 248 conditions, the ESI tandem mass spectra of glycerol-PC [M+H]+ ions yielded five 249 fragments: (1) A fragments ion [M+H-183]⁺ was a specific fragment corresponding to 250 loss of the polar head-group and enabling distinction with other phospholipid classes; 251 (2) m/z values (59, 86 and 104 Da) reflect the fragments derived from polar headgroup; 252 (3) $[RCO]^+$ corresponded to the detached fatty acyl residues; (4) $[M+H-RCO]^+$ ion 253 corresponded to loss of one of the two acyl groups; (5) [M+H-RCOOH]⁺ ion results 254from the loss of a fatty acid. 255

A representative MS spectra and MS^2 fragmentation spectra of (18:2-18:2)PC 256 from white kidney bean (m/z, 782.56671) is shown in **Fig. 2**. One characteristic 257 fragment ion at m/z 184.07301 corresponded to polar headgroup $[C_5H_{14}NPO_4H]^+$,²³, 258 ²⁴ the less intense m/z ions at 104.10732 choline $[C_5H_{13}NOH]^+$, m/z 86.09683 259 dehydrocholine $[C_5H_{12}N]^+$, and m/z 59.07380 trimethylamine group.²⁵ A fragment ion 260 at m/z 599.50067 corresponded to the loss of the polar headgroup. m/z 263.51816 was 261 identified as detached linoleic acyl group. m/z 502.32800 corresponded to the loss of a 262 linoleic acid. m/z 520.33826 corresponded to the loss of a linoleic acyl group. 263

264	The ESI tandem mass spectra of plasmanyl-PC [M+H]+ ions yielded four
265	fragments: (1) $[M+H-183]^+$ corresponded to the loss of polar headgroup; (2)
266	[M+H-RCOOH] ⁺ corresponded to the loss of a fatty acid; (3) [M+H-RCOOH-TMA] ⁺
267	corresponded to the loss of a trimethylamine group (TMA, 59 Da) and a fatty acid; (4)
268	[M+H-RCOOH-104] ⁺ resulted from the loss of a fatty acid and a fragment derived
269	from polar headgroup. Through analysis the above fragment information, the
270	molecular structure of plasmanyl-PC could be identified. A representative MS spectra
271	and MS^2 fragmentation spectra of (16:0e-18:1)PC from chickpea (m/z 746.60339) is
272	given in Fig. 3. A fragment ion at m/z 562.62183 reflected the loss of polar headgroup
273	(183 Da); m/z 464.44827 corresponded to the loss of a stearic acid. m/z 360.32010
274	reflected the loss of a stearic acid and a fragment (104 Da). m/z 405.28946
275	corresponded to the simultaneous loss of a stearic acid and a trimethylamine group
276	(TMA, 59 Da). The above approaches were applied to identify each molecular species
277	of purified PC from various beans and enabled to identify the PC molecular species
278	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 286 predominant molecular species in other bean species, especially in soybean, which 287 accounted for 56.14%. In addition, it was worth noting that many species contain 288 DHA (22:6) in chickpea PC, and many plasmanyl-PC species were found in chickpea 289 PC. Among the plasmanyl-PC species, (16:0e-18:1)PC was relatively abundant, 290 accounted for 2.39%. The variations in the profiles of PC molecular species among 291 the different samples were probably a result of the differences in growing 292 environment, cultivated varieties, plunking time and plant food. 293

294 Characterization of PC by PCA

Characterization and discrimination of PC purified from seven beans were 295 investigated by PCA. The first Principal Component (PC1) contributed to 67.4% of 296 297 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 298 significant variability in the data. The remaining PCs (PC3 to PC5) accounted for 19.5% 299 300 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 301 values in PC1 and PC2 represented significant contributions of individual PC 302 molecular species to total variability (Supplementary Table 1). PC1 loading 303 indicated it had high contributions for many molecular species, for example, 304 (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, 305 (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 306 (18:0e-22:6)PC. PC2 loading exhibited high contributions for (16:1-16:1)PC, 307

309 **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.

317 Diversity and comparison of different groups by hierarchical cluster analysis

318 Hierarchical cluster analysis provides intuitive visualization of a data table. Each 319 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 320 beans was given in Fig. 5. The cluster analysis indicated that the profiles of chickpea 321 322 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, 323 (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, 324 (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, 325 (18:0e-16:0)PC, (18:0e-18:1)PC and (16:0-18:1)PC in chickpea, (18:2-18:2)PC, 326 (18:0-20:2)PC and (18:1-20:2)PC in soybean, (15:0-18:3)PC in white kidney bean, 327 (16:0-16:0)PC in red kidney bean, and (18:3-20:3)PC in red bean, appeared 328 significant high quantities compared to those species in other beans. They can be 329

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.

334 CONCLUSION

In this paper, a separation and identification approach of beans PC species was 335 developed by UHPLC-Q-Exactive Orbitrap/MS analysis. The fatty acids profiles of 336 PC molecules were confirmed by GC analysis. Chickpea PC presented obviously 337 338 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 339 PCA analysis and hierarchical cluster analysis indicated that the molecular profiles of 340 341 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 342 ether lipids for dietary supplement. In addition, soybean is a rich source of PUFA, in 343 344 particular, 18:2 which accounted for high proportion in purified PC. Nevertheless, their stability during processing or cooking should be considered, because the beans 345 used for human consumption are usually processed by different methods, such as 346 frying, steam or boiling. Whether these processing methods affect the profiles of 347 molecular structures of PC would be investigated in our further study. 348

349 **Conflicts of interest**

350 The authors declare that they have no known competing financial interests or 351 personal relationships that could have appeared to influence the work reported in this

352 paper.

353 Acknowledgements

This work was supported by grants from National Natural Science Foundation of China (31501521) and the Fundamental Research Funds for the Central Universities (CZY19032). The authors thank the Analytical & Measuring Center, School of Pharmaceutical Sciences, South-Central University for Nationalities for the spectral analyses.

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- 440 **Figure captions**
- 441 Figure 1. Total ion chromatogram of molecular species from purified PC under
- 442 positive ion mode. (A) soybean; (B) red bean; (C) black bean; (D) red kidney bean; (E)
- 443 white kidney bean; (F) Chickpea
- 444 Figure 2. MS/MS fragmentation under positive ion mode of white kidney bean
- 445 (18:2-18:2)PC at m/z 782.56671 (A) and white kidney bean (16:0e-18:1)PC at m/z
- 446 746.60339 (B).
- Figure 3. Loading plot of PC1 and PC2 (A) and score plots (B) for six beans PC.
- 448 **Figure 4.** Hierarchical cluster analysis based on top 25 molecular species in six beans.
- 449 Colors represent different concentrations indicated by the color bar.





Figure 1





481 (A)



(B)







Figure 5

493	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	$15.0{\pm}1.4$	0.48 ± 0.03					
	red kidney bean	11.5 ± 0.7	0.42 ± 0.02					
	white kidney bean	$9.0{\pm}4.2$	0.32 ± 0.02					
	chickpea	50.0±8.5	3.43±0.12					
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Table1, PC content in total lipids and in dry powder of various beans

530	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	$35.24{\pm}2.01$	43.93±2.53	$40.74{\pm}1.92$		
C18:2n6c	$51.97 {\pm} 2.88$	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		
ΣΡυγΑ	58.78±3.82	17.17±0.54	18.33±0.48	21.51±1.31	14.53 ± 1.26	17.45 ± 1.43		

Table 2 D f E c .: f: ı

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

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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	$0.14{\pm}0.01$
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
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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	^a Percentages	of all	identified mol	lecular species	and Results	are expressed a	as mean ± SD.	
536	^b molecular s	tructures	s provide carbo	n number, num	ber of double	bonds, alkylacyl	(ether) species,	
537	such as 14:00	e.						
538	^c nd means no	ot detect	ed.					
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