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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

4 **Authors**

5 Fu Yang¹, Minghao Zhang¹, Li Zhou^{*,1}, Jikai Liu^{*,1}, Eric Marchioni²

6 ¹The Modernization Engineering Technology Research Center of Ethnic Minority
7 Medicine of Hubei Province, School of Pharmaceutical Sciences, South-Central
8 University for Nationalities, Wuhan 430074, P. R. China

9 ²Equipe de Chimie Analytique des Molécules Bioactives et Pharmacognosie, Institut
10 Pluridisciplinaire Hubert Curien (UMR 7178, CNRS/UDS), 74 route du Rhin, 67400,
11 Illkirch, France

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* To whom correspondence should be addressed. Tel: +86-27-67842267, Email:
zhou2018@scuec.edu.cn

* To whom correspondence should be addressed. Tel: +86-27-67842267, Email:
liujikai@mail.scuec.edu.cn

22 **Abstract**

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

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

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

66 molecular species with PUFA of nutritional interest.

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

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

90 The aim of this study is to identify the molecular species of PC purified from
91 six beans, including soybean, red bean, black bean, red kidney bean, white kidney
92 bean and chickpea. Thin layer chromatography (TLC) was applied for the separation
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
95 with evaporative light scattering detection (HPLC-ELSD) was used to quantify the
96 content of PC. Then, the PC molecular species in six beans were determined by
97 UHPLC-Q-Exactive Orbitrap/MS. Finally, their different compositions in PC
98 molecular species were compared by software-assisted lipidomic analysis. This is a
99 first investigation regarding a deep comprehensive comparison and determination of
100 PC molecular species from these six beans sources. Furthermore, this analytical
101 method can be used to explore new potential sources of bioactive ingredients and
102 evaluate the quality of raw materials.

103 **MATERIALS AND METHODS**

104 **Materials**

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

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

115 **Extraction of total lipids and purification of phospholipids**

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

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

132 **Separation of phospholipid classes by HPLC-ELSD**

133 The determination of phospholipids was completed by HPLC-ELSD (Agilent
134 1260). High purity nitrogen under a pressure of 0.5 MPa was used as nebulizing gas.
135 The drift tube temperature was set at 30°C. The separation of phospholipids was
136 carried out by using a normal phase column (150×3 mm, 3 μm, Inertsil, Japan). The
137 flow rate of mobile phase was 0.6 mL/min, and separations were performed at room
138 temperature using a 25 min linear gradient. The mobile phase A was made up of
139 CHCl₂/CH₃OH (88:12, v/v) and the phase B was made up of CH₃OH/1 M aqueous
140 formic acid (pH was adjusted to 3 by using triethylamine) (84:16, v/v). The gradient
141 started with 100% A, to 80% A by 8 min, then to 60% A by 3 min and holding this
142 proportion for 2 min, then to 40% A by 5 min and return to initial condition in 7 min.
143 The injection volume was 20 μL. The phospholipid classes were identified by
144 comparison of their retention times with those of standard samples which were
145 analyzed under the same condition. The quantification of phospholipid classes from
146 six beans was performed by external standard method, according to the calibration
147 curves of each peak area of corresponding standards.

148 **PC purification by TLC plate**

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

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

157 **Analysis of fatty acids of PC by GC**

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

168 **Separation and identification of PC molecular species by UHPLC-Q-Exactive** 169 **Orbitrap/MS**

170 The analysis of PC from beans was carried on UHPLC system (Dionex, UltiMate,
171 3000 RSLC) equipped with a NucleodurC18HTec column (250 x 4.6 mm, 3 μm). The
172 mobile phase was methanol with 5M ammonium formate. The procedure of
173 separation was implemented in an isocratic elution at a flow rate of 0.6 mL/min for 60
174 min. The column temperature was 60°C. The injection volume was 10 μL. A
175 Q-Exactive Orbitrap mass spectrometry instrument (ThermoFisher Scientific Bremen,

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

189 **Statistical analysis**

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

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

201 **RESULTS AND DISCUSSION**

202 **PC content in different beans**

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

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

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

224 **Fatty acid profiles of purified PC**

225 **Table 2** shows fatty acid profiles of purified PC separated from all kinds of beans
226 investigated. Several fatty acids, including 18:1, 16:0, 18:0 and 18:2 were particularly
227 abundant in the six beans. Especially in soy PC, 18:2 (51.97%) was most predominant
228 among the samples, but in this bean, 22:6 (DHA) were not detected. Moreover,
229 18:3n3 (4.13%) was obviously in higher quantities than that in other five beans PC.
230 The result was in accordance with a previous report.¹² 22:6 was found in five beans,
231 especially in chickpea and red kidney bean, it accounted for 2.73% and 2.53%,
232 respectively. In addition, soy PC contains high levels of PUFA (58.78%) as compared
233 to purified PC from other bean sources. Red beans contain highest proportions of
234 monounsaturated fatty acid (46.77%) and soybean showed lowest level of saturated
235 fatty acid (27.17%). The data on fatty acid profiles were later used in a comparative
236 analysis of molecular species identifications to verify whether the molecular
237 structures of each PC matched its fatty acid profile. Meanwhile, fatty acid profiles
238 provided by GC analysis could help us to determine the molecular structures more
239 accurately and better rule out false positives.

240 **Molecular species of purified PC from various beans**

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

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

256 A representative MS spectra and MS² fragmentation spectra of (18:2-18:2)PC
257 from white kidney bean (*m/z* 782.56671) is shown in **Fig. 2**. One characteristic
258 fragment ion at *m/z* 184.07301 corresponded to polar headgroup [C₅H₁₄NPO₄H]⁺,²³
259 ²⁴ the less intense *m/z* ions at 104.10732 choline [C₅H₁₃NOH]⁺, *m/z* 86.09683
260 dehydrocholine [C₅H₁₂N]⁺, and *m/z* 59.07380 trimethylamine group.²⁵ A fragment ion
261 at *m/z* 599.50067 corresponded to the loss of the polar headgroup. *m/z* 263.51816 was
262 identified as detached linoleic acyl group. *m/z* 502.32800 corresponded to the loss of a
263 linoleic acid. *m/z* 520.33826 corresponded to the loss of a linoleic acyl group.

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 MS² 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.

279 **Profiles of PC molecular species**

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

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

294 **Characterization of PC by PCA**

295 Characterization and discrimination of PC purified from seven beans were
296 investigated by PCA. The first Principal Component (PC1) contributed to 67.4% of
297 the variability in the data set. The second PC (PC2) accounted for 13.1% of the
298 variance in the data. The PC1 and PC2 described 80.5% of the variance and reflected
299 significant variability in the data. The remaining PCs (PC3 to PC5) accounted for 19.5%
300 of the variance and cannot explain significant variability in the data. Thus, only PC1
301 and PC2 were considered for further study according to Kaiser's rule. The loading
302 values in PC1 and PC2 represented significant contributions of individual PC
303 molecular species to total variability (**Supplementary Table 1**). PC1 loading
304 indicated it had high contributions for many molecular species, for example,
305 (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,
306 (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
307 (18:0e-22:6)PC. PC2 loading exhibited high contributions for (16:1-16:1)PC,

308 (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.**
309 **4A, Supplementary Table 1**).

310 **Fig. 4B** shows the score plots of PC in various beans. Red kidney bean and white
311 kidney bean showed negative loadings on PC1 and positive loadings on PC2
312 indicating the PC of these two beans had lower concentration in PC1 and higher
313 concentration in PC2. Meanwhile, soy bean, black bean and red bean had negative
314 loadings on PC1 and PC2. Chickpea showed positive loadings on PC1 and PC2 with
315 highest score on PC1. The result indicated PC molecular species and concentration of
316 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
320 hierarchical cluster analysis of top 25 (concentration) PC molecular species of various
321 beans was given in **Fig. 5**. The cluster analysis indicated that the profiles of chickpea
322 PC were closest to soybean, following white kidney bean, red kidney bean, black soya
323 bean and red bean. The content of molecular species, including (18:0-18:1)PC,
324 (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,
325 (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,
326 (18:0e-16:0)PC, (18:0e-18:1)PC and (16:0-18:1)PC in chickpea, (18:2-18:2)PC,
327 (18:0-20:2)PC and (18:1-20:2)PC in soybean, (15:0-18:3)PC in white kidney bean,
328 (16:0-16:0)PC in red kidney bean, and (18:3-20:3)PC in red bean, appeared
329 significant high quantities compared to those species in other beans. They can be

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

334 **CONCLUSION**

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

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.

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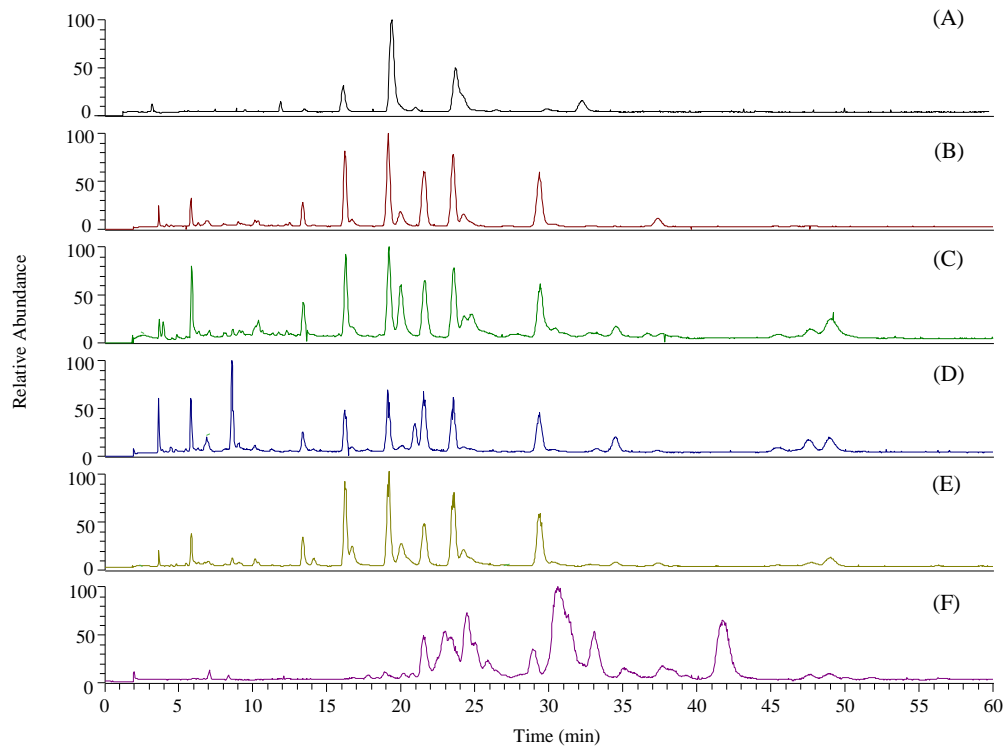
440 **Figure captions**

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).

447 **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.



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451 **Figure 1**

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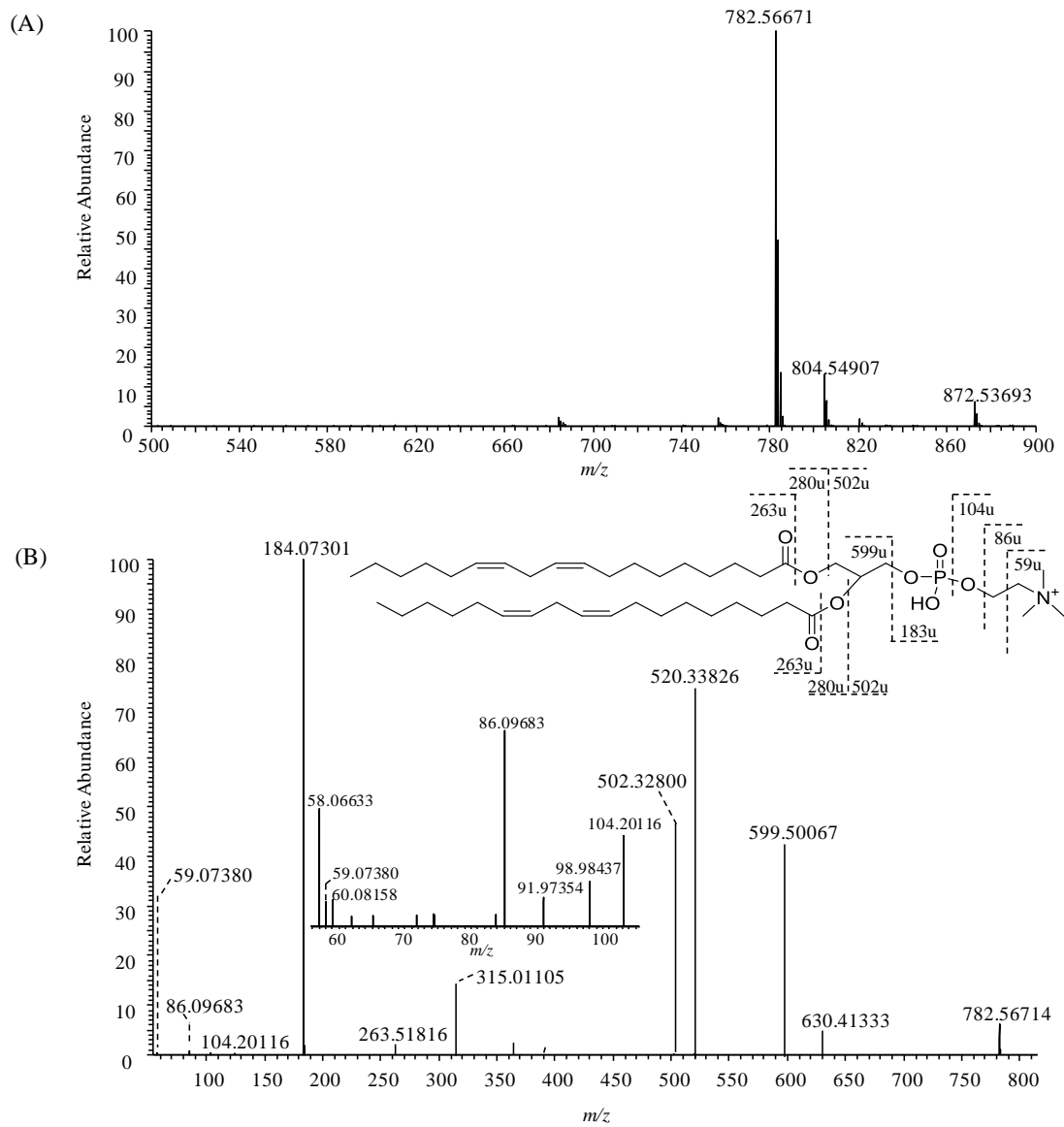
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464 **Figure 2**

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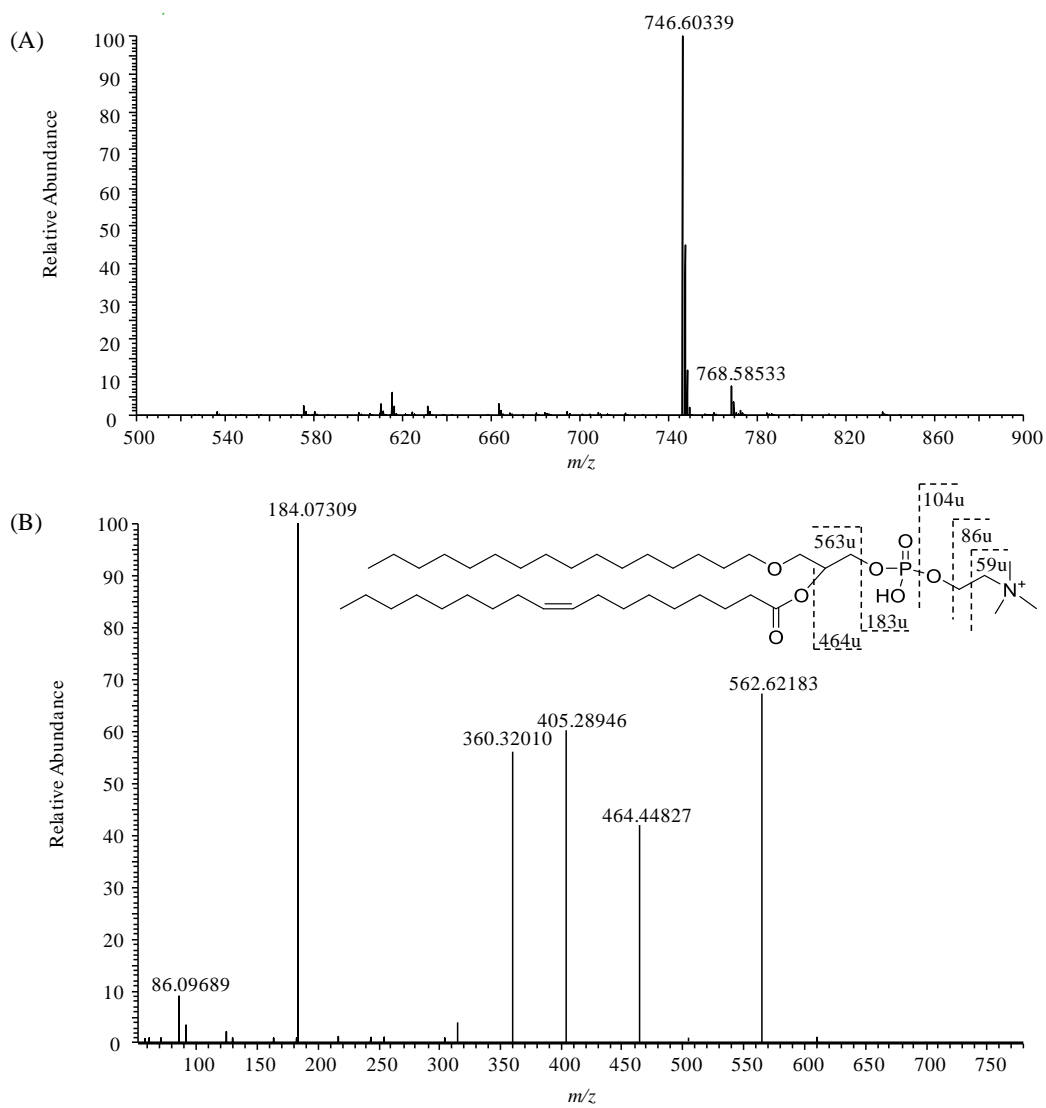
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472 **Figure 3**

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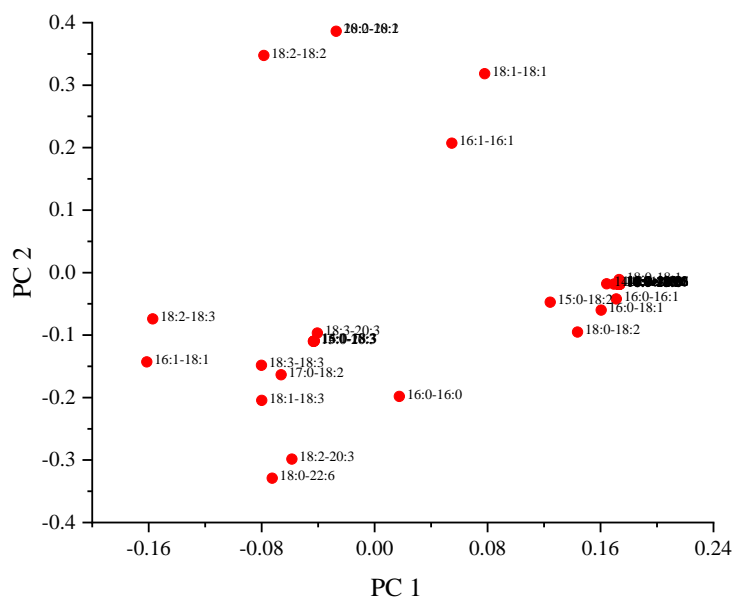
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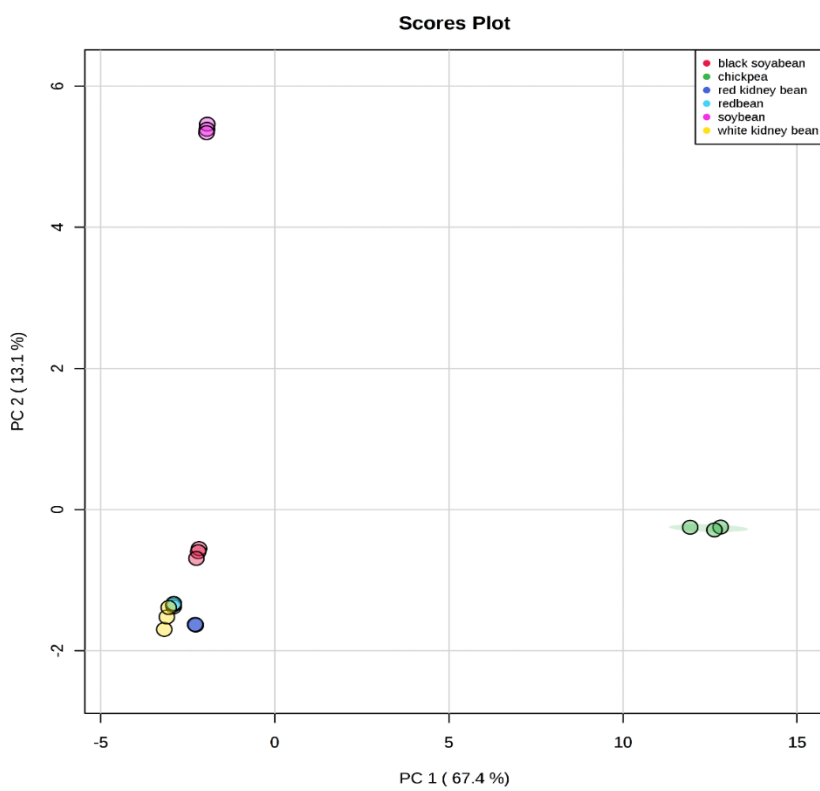
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481 (A)



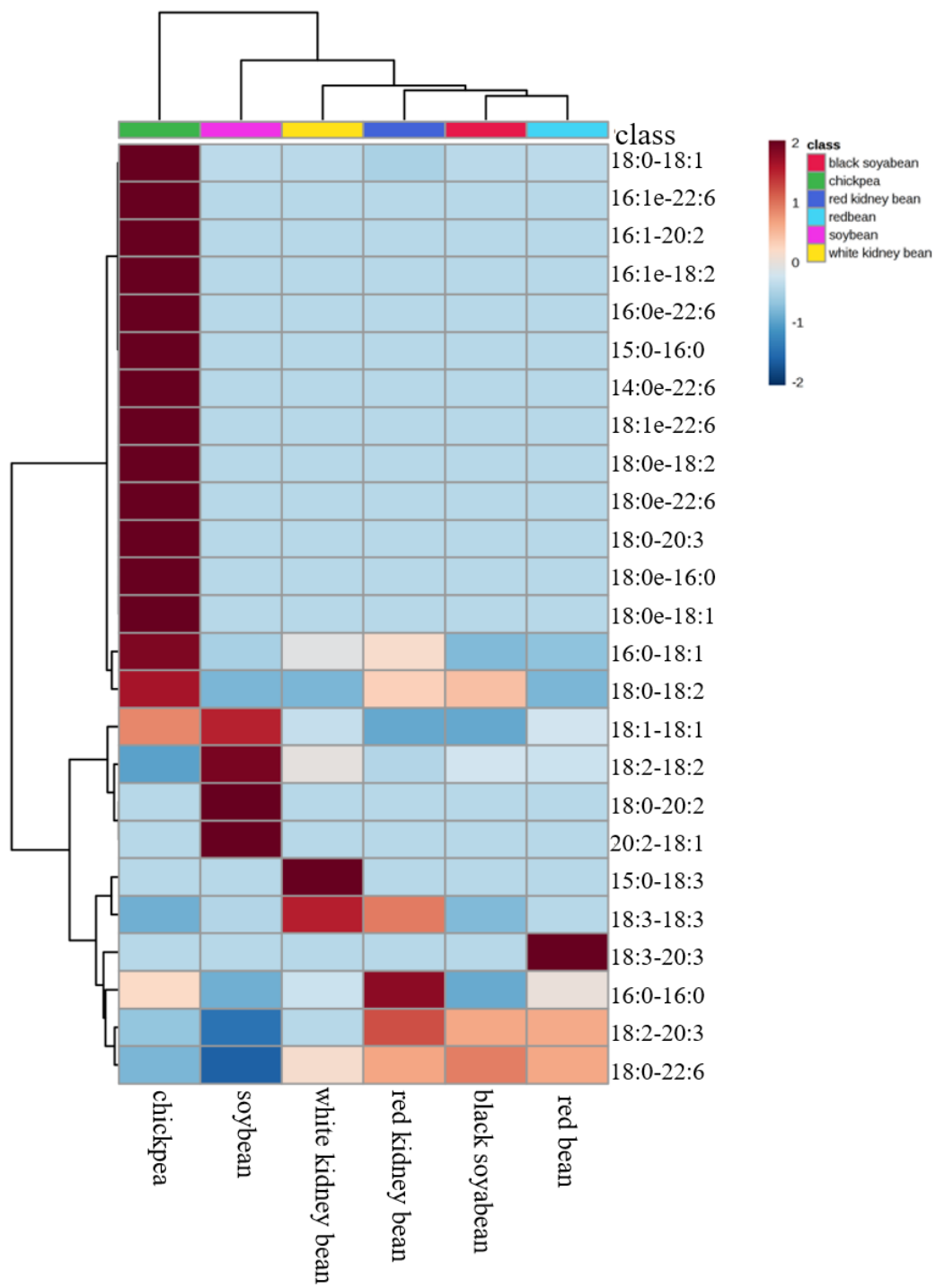
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485 **Figure 4**



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487 **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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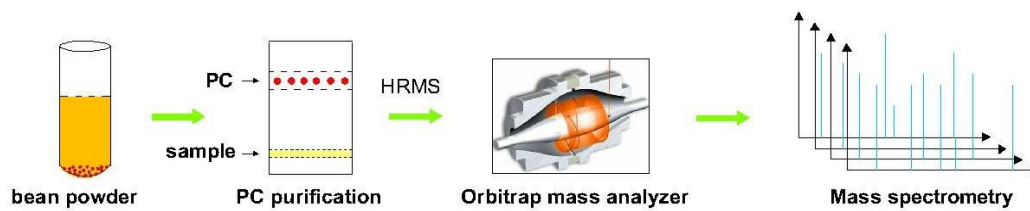
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571 **Graphic Abstract**



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