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1 **Determination and Comparison of Phospholipid Profiles in Seven Kinds of Egg**  
2 **Types Using UHPLC-ESI-Q-TOF-MS**

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25 **Abstract**

26 Phospholipids from seven egg types were purified (purity > 96%) by solid-phase  
27 extraction cartridges, and subsequently identified and quantified by  
28 ultra-high-performance liquid chromatography-electrospray ionization-quadrupole-time  
29 of flight-mass spectrometry (UHPLC-ESI-Q-TOF-MS). 10 phospholipid classes were  
30 detected and 93 molecular species were characterized. Principal component analysis  
31 (PCA) showed that molecular species and concentration of phospholipids in pigeon and  
32 hen egg yolks had significant difference with other egg types. Hierarchical cluster  
33 analysis further indicated that the phospholipid profiles of pigeon egg yolk were closest  
34 to hen egg yolk, following quail, duck, ostrich, emu and goose egg yolks. The  
35 quantification of several representative molecular species can be considered to be most  
36 responsible for the discrimination among egg yolks phospholipids, for example,  
37 SM(d18:1/16:0) in goose egg yolk and PC(16:0/20:4) in duck egg yolk. This study  
38 provides a basis for better understanding phospholipid profiles of egg yolks and better  
39 evaluating the nutritional value of eggs.

40 **Keywords:** Egg yolk, Phospholipids, UHPLC-ESI-Q-TOF-MS, Molecular species, PCA

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48 **1. Introduction**

49 Eggs are a favorite food all over the world and a good nutrient source for human  
50 health. Egg is inseparable nutrient for most people in daily diet. Egg yolk is a rich source  
51 of phospholipids, which accounts for about 33% in lipid composition, other lipids are 62%  
52 triglycerides and 5% cholesterol (Anton, 2007; Aro, et al., 2009). Phospholipids are  
53 important components of cell membranes which play vital roles in cellular metabolism,  
54 signal transduction and membrane transport (Zhang, Xu, Wang, & Xue, 2019; Harrabi,  
55 Herchi, Kallel, Mayer, & Boukhchina, 2009; Zhou et al., 2010). Phospholipids can be  
56 divided into several classes according to their molecular structures. The common  
57 categories include phosphatidylethanolamine (PE), phosphatidylinositol (PI),  
58 phosphatidylcholine (PC), phosphatidylserine (PS), sphingomyelin (SM),  
59 phosphatidylglycerol (PG) and phosphatidic acid (PA), etc. The difference between  
60 phospholipid classes was the structure of polar headgroup, for example, PC and PE  
61 possess choline group and ethanolamine group, respectively. Each class comprises a  
62 diversity of molecular species due to the structures of the fatty acyl group (length of the  
63 fatty acid chain and number of double bonds). Hence, the different phospholipid classes  
64 possess large structural diversity and complexity.

65 The purification of egg yolk phospholipids from total lipid is needed when  
66 comprehensive analysis of polar lipid classes and their molecular species is aimed.  
67 Because high levels of neutral lipids, such as triglycerides, always affect ionization in  
68 electrospray ionization (ESI) sources and desolvation of liquid chromatographic effluent  
69 droplets, the neutral lipids need to be removed to obtain purified phospholipids. Typically,  
70 many methods including solid-phase extraction, thin layer chromatography,

71 two-dimensional liquid chromatography and supercritical carbon dioxide extraction, were  
72 explored for the separation and purification of phospholipids from foodstuffs (Zhou et al.,  
73 2010; Walczak, Bocian, & Buszewski, 2015; Boselli, & Caboni, 2000; Graves, Beaulieu,  
74 & Drackley, 2007). In general, most of the methods could give good recoveries for most  
75 predominant phospholipid classes.

76 Regarding lipidomics and lipid profiling characterization, mass spectrometry (MS)  
77 has been extensively applied in the analysis of lipids. Particularly, ESI-MS/MS is  
78 sensitive and results in high specificity for phospholipid identification. Triple quadrupole  
79 linear ion trap MS has frequently been used for the identification of molecular species of  
80 phospholipids extracted from various food matrices (Gang et al., 2018; Montealegre,  
81 Sánchez-Hernández, Crego, & Marina, 2013; Herchi et al., 2011). However, in the last  
82 few years, with the development of the lipidomics, great progress has been made in lipid  
83 analytical techniques. One of the most useful techniques for the characterization of  
84 molecular structure of lipids is high resolution mass spectrometry because of its precise  
85 mass accuracy and excellent sensitivity. It exhibits strong power for fragment ion  
86 scanning enabling recognition of numerous lipid isomers that were proved difficult to  
87 measure by low resolution mass spectrometry (Senyuva, Gökmen, & Sarikaya, 2015). For  
88 example, ultra-high performance liquid chromatography-electrospray  
89 ionization-quadrupole-time of flight-mass spectrometry (UHPLC-ESI-Q-TOF-MS) has  
90 been applied for the analysis of lipids in milks (Ali, Zou, Huang, Abed, & Tao, 2017),  
91 marine shellfish (Zhang et al., 2019) or other biological sources (She et al., 2014; Miao et  
92 al., 2015). The information available at present about the detailed molecular species of  
93 egg yolk identified by UHPLC-ESI-Q-TOF-MS is limited. To our knowledge, there is

94 only one literature report that provided the information about molecular species of egg  
95 yolks from duck, hen and quail using UPLC-ESI-Q-TOF-MS (Ali et al., 2017). However,  
96 there are several other eggs, such as goose egg, pigeon egg, ostrich egg and emu egg. In  
97 this study, more kinds of eggs were investigated and the molecular profiles of  
98 phospholipid classes contained in egg yolks were characterized. We aimed to evidence  
99 lipidomic promising analytical ability to provide the information of the molecular  
100 composition of phospholipids in eggs, and to investigate the differences of phospholipid  
101 profiles among seven egg types.

102 Furthermore, although numerous studies have been contributed to characterize  
103 phospholipid profiles in different food matrices, these methods reported only the relative  
104 abundance of the different molecular species of phospholipid classes (Zhou, Zhao,  
105 Ennahar, Bindler, & Marchioni, 2012a; Zhou, Zhao, Ennahar, Bindler, & Marchioni,  
106 2012b; Li et al., 2017; Le Grandois et al., 2009; Wang et al., 2009; Ma et al., 2017). This  
107 study presented the first time a detail elucidation and quantification of each molecular  
108 species of phospholipid classes in seven kinds of eggs. Results from the present study  
109 provided a reference for better evaluation of nutritional value of eggs. In this study,  
110 UHPLC-ESI-Q-TOF-MS was thereby applied to identify and quantify phospholipid  
111 molecular species from seven egg types including hen egg, duck egg, quail egg, pigeon  
112 egg, goose egg, ostrich egg and emu egg. In addition, phospholipid profiles were  
113 investigated with software-assisted lipidomic analysis. This is a first investigation  
114 regarding a deep comprehensive comparison and determination of phospholipid profiles  
115 from various egg types.

## 116 **2. Materials and methods**

117 *2.1. Materials*

118 Fresh hen, duck, goose, quail, pigeon, ostrich and emu eggs were purchased from  
119 local supermarket. Seven standards including  
120 1-pentadecanoyl-2-oleoyl(d7)-sn-glycero-3-phosphocholine (15:0-18:1-d7-PC),  
121 1-pentadecanoyl-2-oleoyl(d7)-sn-glycero-3-phosphoethanolamine (15:0-18:1-d7-PE),  
122 1-pentadecanoyl-2-oleoyl(d7)-sn-glycero-3-[phosphor-rac-(1'-glycrol)]  
123 (15:0-18:1-d7-PG), 1-pentadecanoyl-2-oleoyl(d7)-sn-glycero-3-phosphoinositol  
124 (15:0-18:1-d7-PI), 1-pentadecanoyl-2-oleoyl(d7)-sn-glycero-3-phosphate  
125 (15:0-18:1-d7-PA) and sphingomyelin (d18:1/12:0) were purchased from Avanti Polar  
126 Lipids (Alabaster, AL, USA). All chemicals and solvents used for extraction and analysis  
127 were of high purity and analytical grade. Methanol (LC-MS grade), acetonitrile (LC-MS  
128 grade) and isopropanol (HPLC grade) were provided by Shanhai Forneeds Biological  
129 Technology Co., Ltd. FAME Mix C4-C24 (37 components) was from Supelco.

130 *2.2. Total lipids extraction*

131 Egg yolk powder was obtained by manually removing egg white from fresh eggs,  
132 homogenizing and freeze-drying. The extraction of total lipids from egg yolk powder  
133 (hen, duck, goose, quail, pigeon, ostrich and emu) was carried out following Folch  
134 method (Folch, Lees, & Sloane-Stanley, 1957) with a slight modification. 1.0 g egg yolk  
135 powder was placed in 30 mL of a CHCl<sub>3</sub>/CH<sub>3</sub>OH (2:1, v/v) mixture and shaken at 30°C  
136 for 30 min by water-bathing constant temperature shaker (SHA-C, Nanjing, China). The  
137 mixture was centrifuged at 4000 g for 15 min. The upper layer was transferred into a new  
138 tube and the precipitate was extracted for twice with the same procedure. The combined  
139 organic phases were evaporated by a rotary evaporator at 40°C and the residue was dried

140 under a gentle stream of nitrogen until the obtention of a constant weight. The obtained  
141 total lipids were stored at -20°C until use.

### 142 *2.3. Phospholipid purification*

143 Phospholipids were purified from total lipids according to the procedure of Zhou,  
144 Zhao, Ennahar, Bindler, & Marchioni (2012b). Briefly, 50.0 mg of total lipids were  
145 dissolved in 1.0 mL CHCl<sub>3</sub>, transferred onto a silica gel column (35 cm × 2 cm i. d., 15 g  
146 of Si 60 silica gel, particle size 40-63 μm, Geduran, Merck). Firstly, neutral lipids were  
147 removed by 200 mL of CHCl<sub>3</sub>. Then, the phospholipids were eluted with 150 mL of  
148 CHCl<sub>3</sub>/1 M formic acid (98:2, v/v). After evaporation of the solvent, 16.4 mg of  
149 phospholipids were obtained and stored at -20°C for further use. The purity of  
150 phospholipids isolated from different egg yolks was checked by HPLC hyphenated with  
151 evaporative light scattering detector (ELSD) according to the method of Zhou et al.  
152 (2010).

### 153 *2.4. Gas chromatographic analysis of fatty acids*

154 Fatty acids methyl esters were obtained after transesterification of purified  
155 phospholipids according to our previous study (Zhou et al., 2017). Quantitative analysis  
156 was performed on a 3400 Varian GC equipped with a flame ionization detector (FID).  
157 Separation was made by a SP 2560 column (0.25 mm × 100 m, 0.20 μm). One microlitre  
158 of each sample was injected with a run time of 65 min. Injector was set to 280°C, detector  
159 was set to 285°C and the column was set at 140°C and raised to 220°C at a rate of 5°C  
160 /min, the final temperature was maintained for 49 min. Peaks were identified by  
161 comparison to standards (FAME mix C4-C24). The relative amounts of fatty acids were  
162 calculated by the percent area method with external normalization considering the sum of



163 all areas of the identified fatty acid.

#### 164 2.5. Preparation of stock solutions

165 A stable-isotope labelled standard for each molecular species is the preferred option  
166 for accurate quantification (Pigini, Cialdella, Faranda, & Tranfo, 2006). They have the  
167 same chemical and physical properties as phospholipids to be quantified, but  
168 distinguishable in mass spectra. Hence, 6 d7-phospholipid standards and 1  
169 nonendogenous phospholipid standard were used for the quantification, including  
170 15:0-18:1-d7-PC [standard for PC], 15:0-18:1-d7-PE [standard for PE],  
171 plasmalyethanolamine (PE-O-alkyl-acyl), lysophosphatidylethanolamine (LPE),  
172 phosphatidylethanol (PEtOH) and oxidized phosphatidylethanolamine (OxPE),  
173 15:0-18:1-d7-PG [standard for phosphatidylglycerol (PG)], 15:0-18:1-d7-PI (standard for  
174 PI), 15:0-18:1-d7-PA [standard for phosphatidic acid (PA)] and lysophosphatidic acid  
175 (LPA)] and sphingomyelin (d18:1/12:0) (standard for SM). To equilibrate the analytical  
176 platforms and correct the minor variation from different samples, a quality control (QC)  
177 sample (cocktail of each sample) were injected regularly every 5 samples.

178 Stock solutions were prepared in chloroform/methanol (2:1, v/v), and diluted into a  
179 series of concentrations (10~5000 ng/mL). The 7 phospholipid standards were added in  
180 egg yolk phospholipids and QC samples. The quantification is based on the extracted ion  
181 chromatograms (EICs) of individual phospholipids with  $m/z$  expansion in  $\pm 5$  ppm. Peak  
182 areas of the phospholipid standards and egg yolk phospholipids were integrated from  
183 EICs. The concentration of each phospholipid molecular species was calculated from this  
184 calibration curve constructed from known concentrations of the corresponding standards.

#### 185 2.6. Identification and quantification of phospholipids by UHPLC-ESI-Q-TOF-MS

186 *2.6.1. UHPLC condition*

187 The identification and quantification of phospholipids were performed by a UHPLC  
188 (Shimadzu LC-30A) coupled to a high resolution AB Sciex Q-TOF 6600 mass  
189 spectrometry (HRMS) instrument equipped with an electrospray ionization (ESI) source  
190 (AB Sciex, Toronto, ON, Canada).

191 The purified phospholipids were separated on a Phenomenex Kinetex C18 column  
192 (100 × 2.1 mm, 2.6 μm). Solvent (A) H<sub>2</sub>O: methanol: acetonitrile (1:1:1, v/v/v) with  
193 ammonium acetate (5mM) and solvent (B) isopropanol: acetonitrile (5:1, v/v) with  
194 ammonium acetate (5mM) were employed as mobile phases. The binary gradients used  
195 for the chromatogram were as follows: 80% A for 0.5 min, 80% A to 60% A for 1 min, 60%  
196 A to 40% A for 1.5 min, 40% A to 2% A for 5 min, 2% A to 80% A for 2 min. The total  
197 run time was 10 min at a flow rate 0.4 mL/min. The column temperature was set at 60 °C.  
198 The injection volume was 10 μL.

199 *2.6.2. ESI-Q-TOF-MS conditions*

200 The ESI source conditions were set as follows: spray voltage (5.5 kV), curtain gas  
201 (N<sub>2</sub>, 99.999%, 35 psi, Domnik Hunter), temperature (600 °C), ion source gas1 (nebulizer  
202 gas, 50 psi), ion source gas 2 (turbo gas, 50 psi). Data were collected in both positive and  
203 negative ionization mode over a mass range between 100 ~ 1200 *m/z*. The MS/MS  
204 experiments were performed using collision energy of 35 eV in negative mode. The mass  
205 range was set between 200 ~ 900 *m/z* for fragmentation products.

206 *2.7. Statistical analysis*

207 Phospholipids were identified and quantified using AB SCIEX Analyst 1.6.2  
208 software (Applied Biosystems, Foster City, CA, USA), MS-DIAL and LipidMaps. False

209 positives were checked manually according to the MS/MS fraction analysis.  
210 Discriminatory analysis of molecular composition from various egg yolks were analyzed  
211 by Principal Component Analysis (PCA) and hierarchical cluster analysis using  
212 MetaboAnalyst 4.0 software.

### 213 **3. Results and discussion**

#### 214 *3.1. Analysis of the fatty acids of egg yolk phospholipids*

215 The major fatty acids associated with seven egg yolk phospholipids were C18:1n9,  
216 C16:0, C18:0, C18:2n6 and C20:4n6 (**Supplemental Material Table S1**), which was  
217 almost consistent with the conclusion of Ali et al. (2017). Derived from obtained data, the  
218 fatty acid profiles of seven egg types were significantly different. Oleic acid was the most  
219 abundant fatty acid, occupying 29%, 51%, 30%, 43%, 49%, 51% and 43%, respectively.  
220 C18:3n6 and C18:3n3 were detected in hen egg yolk and ostrich egg yolk, while C17:0,  
221 C20:1 and C20:2 were not found in emu egg yolk. Furthermore, C17:0 was not detected  
222 in duck egg yolk as well. The arachidonic acid (20:4n6) has significant neurocognitive  
223 benefits and plays an essential role in the process of treating cardiovascular disease  
224 (Brenna, 2016). It was found in all egg samples and the content was higher in hen egg  
225 yolk and goose egg yolk than others. Docosahexaenoic acid/eicosapentaenoic  
226 acid-phospholipids (DHA/EPA-phospholipids) are one of the major n-3 long-chain  
227 polyunsaturated fatty acid dietary forms in our diet, have nutritional functions on  
228 antitumor activity, lipid metabolism, and glucose metabolism (Zhang, Xu, Wang, & Xue,  
229 2019). The GC analysis of fatty acid showed that the content of DHA+EPA in pigeon egg  
230 yolk (3.77%) was higher than quail egg yolk (2.30%), duck egg yolk (1.62%), ostrich egg  
231 yolk (1.45%) and emu egg yolk (1.35%), but it was lower than hen egg yolk (4.27%) and

232 goose egg yolk (4.81%). As for polyunsaturated fatty acids (PUFA), hen egg yolk, goose  
233 egg yolk and pigeon egg yolk showed higher content, making up 29%, 28% and 22%,  
234 respectively. The variation in the content of fatty acids in different egg yolks were  
235 probably resulted from different species, growth conditions and even diet the birds were  
236 fed.

### 237 *3.2. Purification of phospholipids*

238 The HPLC Chromatogram of purified phospholipids from seven egg types was given  
239 in **Supplemental Material Figure S1**. The amount of neutral lipids which usually  
240 presented in the first 3 min of the HPLC run, was insignificant. Upscaling to  
241 semi-preparative HPLC did not seem to reduce the quality of the separation of the  
242 phospholipid classes. This procedure enabled collect adequate amounts of phospholipids  
243 fractions. For each egg yolk samples, the purity of phospholipids monitored by  
244 HPLC-ELSD were exhibited to be at least 96%.

### 245 *3.3. Characterization of phospholipids by UHPLC-ESI-Q-TOF-MS*

246 The separation of phospholipid classes of different egg yolks was achieved within a  
247 runtime of 10 min under ESI source in negative ion mode (**Figure 1**). **Supplemental**  
248 **material Figure S2** shows an example of EICs of  $m/z$  455.2201, 480.3114, 885.5588,  
249 773.5440, 703.5684, 816.5811 and 766.5458 for hen egg yolk phospholipids. Although  
250 the chromatogram under positive ionization mode exhibited higher intensities, either  
251 characteristic fragments or fatty acyl identification fragments was not available under  
252 ESI<sup>+</sup> mode for most phospholipids. Only a small part of phospholipids, such as SM was  
253 not detected in negative ion mode while can be detected in positive ion mode. Overall,  
254 the negative ion mode provided more available information for molecular structure

255 identification with lower noise and background than positive ion mode. Furthermore, the  
256 most abundant product ions derived from collision induced dissociation of  $[M-H]^-$  of  
257 phospholipids corresponded to the carboxylate anions and loss of a neutral fatty acid or  
258 ketene from one or both of the fatty acyl chains (Pulfer, & Murphy, 2003). We detected  
259 PE, LPE, OxPE, PE-OH, PG, PI, PA and LPA as  $[M-H]^-$  ions, PC as  $[M+CH_3COO]^-$  ion,  
260 and SM as  $[M+H]^+$  ions. The molecular species within one class were classified on the  
261 basis of the structures of esterified fatty acid chain (double bonds numbers and alkyl  
262 chain length).

263 The molecular structures of each phospholipid were identified by MS/MS  
264 spectrometry. Phospholipids are classified by their different polar headgroups which give  
265 rise to the characteristic fragments in the MS/MS, for example,  $m/z$  140.0132 was  
266 characteristic of the polar head-group of PE, which can be used to distinguish between  
267 different phospholipid classes. Besides, the information of other MS/MS fragments can  
268 help to identify the fatty acid chains of phospholipids.

269 All the characterized phospholipids were quantified based on corresponding standard  
270 curves. The limit of detection (LOD) and limit of quantification (LOQ) corresponded to a  
271 signal-to-noise ratio of 3 and 10, respectively. As shown in **Table 1**, calibration curves  
272 presented linear regression coefficients ( $R^2$ ) higher than 0.99 and LOD was  $\leq 0.8$  ng/mL.  
273 The spiked recovery method was used to evaluate the method. The recoveries of the  
274 seven standards were between 98 and 110%.

#### 275 *3.4. Phospholipid profiles in seven egg types*

276 10 phospholipid classes were detected and 93 phospholipid molecular species were  
277 characterized using UHPLC-ESI-Q-TOF-MS. **Table 2** showed the molecular species

278 identified and quantified of phospholipid classes in seven egg types. 48 species of PE  
279 were identified which was the most abundant among phospholipids. It is worth noting  
280 that ether phospholipids (PE-O-alkyl/acyl) accounted for a high proportion and other  
281 forms of PE such as 1 species of PEtOH, 12 species of LPE and 2 species of OxPE were  
282 also found.

283 The ESI tandem mass spectra of PE [M-H]<sup>-</sup> ions yield three fragment categories: (1)  
284 a fragment ion (140 Da) was characteristic of the polar head-group [NH<sub>3</sub>(CH<sub>2</sub>)<sub>2</sub>OPO<sub>3</sub>]<sup>-</sup>;  
285 (2) two fatty acid [RCOO]<sup>-</sup> corresponded to the detached fatty acids; (3) two  
286 [M-H-RCO]<sup>-</sup> resulted from loss of two acyl groups. **Figure 2a** showed the MS/MS  
287 fragmentation spectrum of PE (16:0/18:2) from hen egg yolk at *m/z* 714.5151. *m/z*  
288 140.0132 corresponded to the ethanolamine group. The observed peaks at *m/z* 255.1350  
289 and 279.2347 corresponded to palmitic acid and linoleic acid, respectively. *m/z* 452.2840  
290 and 476.3142 corresponded to the loss of linoleoyl acyl group and palmitoyl acyl group  
291 in the ketene form, respectively. From **Table 2**, we can see that the major PE species from  
292 the seven egg types were PE(18:0/20:4), PE(18:0/18:2), PE(16:0/18:2) and PE(16:0/20:4).  
293 Many PE(O-alkyl/acyl) were detected. The difference between PE and PE(O-alkyl/acyl)  
294 was that one of the two esterified fatty acid was replaced by a saturated ether linkage  
295 (-O-alkyl) (Deeley, Thomas, Truscott, Mitchell, & Blanksby, 2009; Park, Suh, Thomson,  
296 Ramanujam, & Clandinin, 2006). In general, PE(O-alkyl/acyl) were found in marine  
297 organisms or animal tissues (Zhou, Zhao, Ennahar, Bindler, & Marchioni, 2012a;  
298 Chapelle, 1987, Mawatari, Hazeyama, & Fujino, 2016). In our previous study, a  
299 PE(O-alkyl/acyl) was found in krill oil, but not detected in egg yolk by positive mode in  
300 egg yolk by low resolution mass spectrometry (Zhou, Zhao, Ennahar, Bindler, &

301 Marchioni, 2012a). However, many PE(O-alkyl/acyl) were detected in seven egg types by  
302 UHPLC-ESI-Q-TOF-MS in negative ion mode in this study. Apparent disparity to mass  
303 spectrometric studies probably attributed to differences in the instrument response  
304 function. In addition, a total of 14 egg yolk PEs was detected by UHPLC-Q-TOF-MS in  
305 positive ion mode, but no PE(O-alkyl/acyl) was found according to the study of Ali et al.  
306 (2017). One major cause of this phenomenon might be related with the UHPLC condition  
307 and negative ion mode for more accurate identification. **Figure 2b** showed the  
308 fragmentation pathway of PE(O-18:1/18:2) at  $m/z$  726.5488.  $m/z$  279.2321 reflected the  
309 deprotonated linoleic acid.  $m/z$  446.2930 and 464.3142 result from the loss of linoleic  
310 acid and linoleic acyl group, respectively. In addition, most of the PE(O-alkyl/acyl)  
311 contained long chain-polyunsaturated fatty acid. For instance, PE(O-16:1/22:6) ( $m/z$   
312 746.5148) accounted for 83.50 nmol/g in pigeon egg yolk. Furthermore, it is worth noting  
313 that the PE species of emu egg yolk was obviously less abundant than in other egg types,  
314 only 12 molecular species was detected. **Figure 2c** showed the MS/MS fragmentation  
315 spectrum of LPE (18:0) from hen egg ( $m/z$  480.3114).  $m/z$  140.0245 reflected the  
316 ethanolamine group.  $m/z$  283.2643 corresponded to stearic acid.  $m/z$  196.0414  
317 corresponded to the neutral loss of stearic acid. As shown in **Table 2**, LPE(16:0) and  
318 LPE(18:0) were the most abundant species among the seven egg types. 3 molecular  
319 species, such as LPE(O-16:0), PE(16:1) and LPE(20:3) were only identified in hen egg  
320 yolks.

321 The ESI tandem mass spectra of OxPE[M-H]<sup>-</sup> ions showed more diversified  
322 fragmentation patterns, which yields four fragments categories: (1) [RCOO]<sup>-</sup> and  
323 [RCOO+2O]<sup>-</sup> corresponded to a detached fatty acid and a oxidized fatty acid; (2)

324  $[M-H-RCO-2O]^-$  reflected the loss of oxidized fatty acyl group; (3) A fragment ion (196  
325 Da) corresponded to the residue which was formed by the loss of an fatty acyl group and a  
326 fatty acid; (4)  $[M-H-140-RCO-2O]^-$  corresponded to the simultaneous loss of both the  
327 ethanol group (140 Da) and the oxidized fatty acid. A representative MS/MS  
328 fragmentation spectrum of OxPE(18:0/22:5+2O) at  $m/z$  824.5493 was given in **Figure 2d**.  
329  $m/z$  283.2626 and 361.2045 corresponded to the stearic fatty acid (18:0) and oxidized  
330 docosapentaenoic acid (22:5+2O), respectively.  $m/z$  462.0126 referred to the loss of  
331 oxidized docosapentaenoic acid (22:5+2O).  $m/z$  323.0717 reflected the simultaneous loss  
332 of both the ethanol group (140 Da) and oxidized docosapentaenoic acid (22:5+2O).  
333 OxPE(18:0/22:5) was found in almost all the egg yolks except emu egg yolk.  
334 OxPE(18:0/18:2+2O) were detected in hen and pigeon egg yolks, accounted for 3.7  
335 nmol/g vs 14.77 nmol/g, respectively. In addition, a special species, PE-OH(16:0/18:2) in  
336 pigeon and ostrich egg yolks, accounted for 5.4 nmol/g and 1.68 nmol/g, respectively  
337 (**Table 2**). Its MS/MS fragmentation pathway was given in **Figure 2e**.  $m/z$  255.2378 and  
338 279.2290 corresponded to palmitoyl acyl group and linoleoyl acyl group, respectively.

339 PC identification was confirmed in the negative ion mode  $[M+CH_3COO]^-$ . 10  
340 molecular species of PC were detected among the samples (**Table 2**). PC(16:0/18:1) and  
341 PC(16:0/18:2) were species with high concentration. A previous study conducted by  
342 Campos et al. (2016) developed a HILIC-ESI-ion trap-MS method to determine the  
343 molecular species of phospholipids and their changes in eggs from different conditions.  
344 22 molecular species of PC were identified, being PC(16:0/18:1) and PC(16:0/18:2) the  
345 most abundant species detected, which was in agreement with our results. In another  
346 previous study of Pacetti et al. (2005) with the use of LC-ESI-MS for the identification of



347 molecular species of phospholipids in eggs from hens fed diets enriched in seal blubber  
348 oil, PC(16:0/18:1) and PC(16:0/18:2) were major species, which were also in accordance  
349 with our results. **Figure 3a** presented the fragmentation pathway of PC(16:0/18:1) at  $m/z$   
350 818.5958. The fragment ions at  $m/z$  255.2180 and 281.2459 corresponded to deprotonated  
351 palmitic acid and oleic acid, respectively.  $m/z$  480.3052 and 506.1038 reflected the loss of  
352 oleic acid and palmitic acid, as  $[M-CH_3-RCH=C=O]^-$ , respectively.

353 PGs are less abundant than most of the common phospholipid species. Molecular  
354 species of PG form abundant negative ions under ESI-MS conditions, as they would be  
355 expected for these acidic phospholipids with the neutral polar headgroup. 4 molecular  
356 species were detected among the samples. High concentration of PG (16:0/20:1) (52.20  
357 nmol/g) and PG(16:0/18:1) (37.39 nmol/g) were detected in pigeon egg yolk. The  
358 fragmentation pathway of PG(16:0/20:1) at  $m/z$  775.5499 were shown in **Figure 3b** as  
359 example. The observed peaks at  $m/z$  255.2323 and 309.2855 corresponded to  
360 deprotonated palmitic acid and deprotonated eicosanoid acid.  $m/z$  154.0136 corresponded  
361 to the phosphate residue of polar headgroup (170 Da). The peaks observed at  $m/z$   
362 519.3211 and 465.2619 corresponded to the neutral loss of palmitic acid and eicosanoid  
363 acid, respectively.  $m/z$  245.2249 referred to the combination loss of palmitoyl acyl group  
364 and eicosanoid acyl group.  $m/z$  211.2136 derived from the combination loss of palmitic  
365 acid and eicosanoid acid.

366 PI that contains a phosphodiester of the six-carbon sugar inositol as a polar  
367 headgroup was found in all samples. 12 molecular species of PI were identified in the  
368 negative ion mode. Previous literature reports illustrated that the analysis of acidic  
369 phospholipids including PI was typically performed in the negative ion mode (Pacetti,

370 Boselli, Hulan, & Frega, 2016; Koivusalo, Haimi, Heikinheimo, Kostianen, &  
371 Somerharju, 2001). A representative MS/MS fragmentation spectrum of PI(18:0/20:4) at  
372  $m/z$  885.5616 was shown in **Figure 3c**. The observed peaks at  $m/z$  283.0462 and  
373 303.2322 arised from the moieties of stearic acid and arachidonic acid.  $m/z$  418.0431 and  
374 438.6211 corresponded to the simultaneous loss of a fragment of inositol group (163 Da)  
375 and arachidonic acid or stearic acid, respectively. As shown in **Table 2**, PI(18:0/20:4) was  
376 predominant species among them, the concentration was between 30.95~169.24 nmol/g.  
377 PI(18:0/18:2) was second abundant species in most of the samples except for duck, goose  
378 and emu egg yolks. Other major species were PI(16:0/20:4), PI(18:0/18:2), PI(16:0/18:2)  
379 and PI(16:0/18:1). Our results were similar with the literature report of Ali et al. (2017),  
380 but they demonstrated that PI(18:0/18:2) was the most abundant species in duck, hen and  
381 quail egg yolks, while PI(18:0/20:4) was not detected in our study. The reason may be  
382 related with the diet, feed amount or rearing conditions (Zhou, Zhao, Ennahar, Bindler, &  
383 Marchioni, 2012a).

384 PA has the simplest polar headgroup, and serves as a precursor and metabolite in the  
385 biosynthesis and catabolism pathways of phospholipids. Although it is possible to detect  
386 PA in both positive ion and negative ion mode, the informative signals in negative ion  
387 mode are more accurate. 2 molecular species were detected in negative ion mode. The  
388 fragmentation pathway of PA(16:0/18:1) at  $m/z$  673.4848 was shown in **Figure 4a**.  $m/z$   
389 255.2359 and 281.2472 arised from the deprotonation moieties of palmitic and oleic acid,  
390 respectively. The observed peak at  $m/z$  152.9936 referred to the combination loss of  
391 palmitoyl acyl group and oleic acid. A related molecule is lysophosphatidic acid (LPA),  
392 which is produced from lysophospholipids by a plasma enzyme, autotaxin or generated

393 from phospholipids or diacylglycerol and then deacylated by phospholipase A1 or  
394 phospholipase A2 (Aoki, Inoue, & Okudaira, 2008). **Figure 4b** described the fracture  
395 mechanism of LPA(16:0) at  $m/z$  409.2340.  $m/z$  152.9859 reflected the loss of palmitic  
396 acyl group and a hydroxyl group.  $m/z$  313.2505 corresponded to the loss of phosphate  
397 group.  $m/z$  391.2432 corresponds to the loss of H<sub>2</sub>O. **Table 2** described that the  
398 concentrations of PA and LPA species were relatively high compared to other species. In  
399 particular, the content of LPA(16:0) in samples was between 645.17~979.32 nmol/g. The  
400 content of PA(16:0-18:1) in hen egg yolk was highest among other sources.

401 SM is composed of only a single fatty acid esterified to a sphingoid backbone. They  
402 possess a large degree of structural diversity based on the variations in the degree of  
403 unsaturation, hydroxylation, and methylation of long-chain sphingoid base (sphingosine,  
404 sphinganine, etc.) in the nature of the fatty acid, as well as in the nature of the polar  
405 headgroup (Zhou, Zhao, Ennahar, Bindler, & Marchioni, 2012b; Fischbeck, Kruger,  
406 Blaas, & Humpf, 2009). 1 molecular species, SM(d18:1/16:0), was detected in the  
407 different egg yolk samples in positive ion mode. The results were in agreement with  
408 Pacetti et al. (2005). The protonated molecule  $[M+H]^+$  at  $m/z$  703.5759 in details was  
409 shown in **Figure 4c**.  $m/z$  184.0741 corresponded to the phosphocholine group. The signal  
410 observed at  $m/z$  264.2862 reflected sphingosine d18:1 chain base, and  $m/z$  685.5613  
411 referred to the loss of one molecular of H<sub>2</sub>O from fatty acyl chain.

### 412 *3.5. Characterization of phospholipid profiles by PCA*

413 PCA involves a mathematical procedure identifying patterns in data set, and aims to  
414 highlight the similarities and differences. PCA is a way of identifying patterns in data and  
415 expressing the data in such a way as to emphasize their similarities and differences (Shin,

416 Craft, Pegg, Dixon Phillip, & Eitenmiller, 2010). The discrimination of phospholipid  
417 profiles from seven egg types were investigated by PCA. The data matrix (a total of 93  
418 molecular species) was subjected to PCA. The first Principal Component (PC1) expresses  
419 the component responsible for the most variance, while PC2 represents the component  
420 responsible for the second-most variance. The score plots indicate the difference of  
421 phospholipid profiles of various egg yolks. The loading plots reflect the contribution of  
422 important variables to the molecular species from different egg yolks.

423 The first Principal Component (PC1) contributed to 46.8% of the variability in the  
424 data set. The second PC (PC2) accounted for 29.7% of the variance in the data was  
425 shown in **Supplemental Material Figure S3**. The PC1 and PC2 described 76.5% of the  
426 variance and reflect significant variability in the data. The remaining PCs (PC3 to PC5)  
427 accounted for 23.5% of the variance and cannot explain significant variability in the data.  
428 Thus, only PC1 and PC2 were considered for further study according to Kaiser criterion.  
429 (Kaiser, & Rice, 1974). The loading values in PC1 and PC2 represented significant  
430 contributions of individual PC molecular species to total variability as presented in  
431 **Figure 5a** and **Supplemental Material Table S2**. PC1 loading was highly contributed by  
432 many molecular species, for example, PE(18:0/20:3), PE(18:0/18:2), PE(18:1/20:1),  
433 PE(18:0/20:1), PE(18:1/20:1), PE(18:0/22:6) and PI(16:0/16:1), and most of them were  
434 from PE. Meanwhile, PC2 loading was positively contributed by PC(18:0/18:2),  
435 PE(O-20:1/18:2), PE(O-18:1/20:3), PE-OH(16:0/18:2), PE(O-16:1/20:4), PI(18:2/20:5)  
436 and PC(16:0/18:2).

437 **Figure 5b** presents the score plots of phospholipid profiles in various egg yolks.  
438 Goose egg yolk showed negative loadings on PC1 and positive loadings on PC2

439 indicating goose egg yolk has lower concentration in PC1 and higher concentration in  
440 PC2. Meanwhile, the egg yolks from emu, quail, ostrich and duck have negative loadings  
441 on PC1 and PC2. Pigeon egg yolk showed positive loadings on PC1 and PC2 with  
442 highest score on PC2. Hen egg yolk exhibited positive loading on PC1 and negative  
443 loading on PC2. The result indicated phospholipid profiles of pigeon and hen egg yolks  
444 had significant difference from other egg yolks.

### 445 *3.6. Diversity and comparison of different groups by hierarchical cluster analysis*

446 Hierarchical cluster analysis provided a clear clustering tendency of egg yolk  
447 samples containing similar profiles of the investigated data. Each colored cell on the map  
448 corresponds to a concentration value in the data table. The hierarchical cluster analysis of  
449 top 25 (concentration) molecular species of various egg yolks was given in **Figure 5c**.  
450 Based on these values, the seven egg samples could be classified correctly into two parts.  
451 The pigeon egg yolk and hen egg yolk were arranged in a single portion and other  
452 samples were classified as the second part based on the composition similarities. It  
453 indicated that the phospholipid profiles of pigeon egg yolk were closest to hen egg yolk,  
454 following quail, duck, ostrich, emu and goose egg yolks. The concentrations of molecular  
455 species, including PE(18:0/22:6), PE(16:0/18:2), PE(16:0/22:6), LPE(18:0), LPE(16:0),  
456 LPE(16:0), PE(16:0/20:4), PE(18:0/20:4) and PE(18:0/22:4), in hen egg yolk, and  
457 PE(O-16:1/22:6), OxPE(18:0/18:2+2O), PE(O-18:1/18:2), PC(16:0/18:2), PC(18:0/18:2),  
458 PE(O-16:1/20:4), PE(O-18:1/20:3) and PE(O-20:1/18:2), in pigeon egg yolk,  
459 SM(d18:1/16:0) and LPA(16:0) in goose egg yolk and PC(16:0/20:4) in duck egg yolk,  
460 appeared in significant high concentrations compared to those species in other egg yolks.

## 461 **4. Conclusion**

462 In summary, we reported a use of UHPLC-ESI-Q-TOF/MS technique to carefully  
463 elucidate and quantify molecular species of different phospholipid classes in seven egg  
464 types. Purified phospholipids (> 96%) were obtained by adsorption column  
465 chromatography. Their molecular structures were further identified by the MS/MS  
466 fragmentation pattern of each informative mass ion. Quantification of phospholipid  
467 molecular species was achieved using d7-phospholipid standards calibration with high  
468 accuracy. The UHPLC-ESI-Q-TOF-MS method was proven to be an effective method for  
469 structural identification and quantification of phospholipid. Discriminatory analysis by  
470 PCA demonstrated that phospholipid profiles of pigeon and hen egg yolks had significant  
471 difference from other egg yolk sources. Hierarchical cluster analysis further indicated that  
472 the phospholipid profiles of pigeon egg yolk were closest to hen egg yolk, following  
473 ostrich, emu, duck, goose and quail egg yolks. The quantitation of some representative  
474 molecular species can be considered to be most responsible for the discrimination among  
475 egg yolks phospholipids. Phospholipids might be as used as molecular fingerprints for  
476 egg analysis, which could be applied to control origination and tagging, and prevent  
477 fraudulent behavior. Furthermore, based on the phospholipid profiles and the content of  
478 DHA+EPA and PUFA, hen egg, goose egg and pigeon egg were probably superior to  
479 other eggs studied. Herein, hen egg maybe the best choice in consideration of taste and  
480 price. Therefore, this study can help scientific research institutions and worker (including  
481 food control laboratories, nutritionists, dieticians and so on) better understand the  
482 molecular composition of phospholipids in eggs and better select or evaluate the  
483 nutritional value of eggs.

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489 analyses.

#### 490 **Supporting information**

491 HPLC Chromatogram of purified phospholipids from seven egg types. Extracted  
492 individual ion chromatograms of phospholipid molecular species. Pairwise score plot for  
493 5 principal components. Fatty acid profiles of purified phospholipids from seven egg  
494 types. Eigen analysis of the correlation matrix loadings of the principal components

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## Figure captions

**Figure 1.** Total ion chromatogram of molecular species from phospholipids under negative ion mode. (a) hen egg yolk; (b) duck egg yolk; (c) goose egg yolk; (d) pigeon egg yolk; (e) quail egg yolk; (f) ostrich egg yolk; (g) emu egg yolk.

**Figure 2.** MS/MS fragmentation pathway of PE(16:0/18:2) ( $m/z$  714.5151) (a), PE(O-18:1/18:2) ( $m/z$  726.5488) (b), LPE (18:0) ( $m/z$  480.3089) (c), OxPE(18:0/22:5) ( $m/z$  824.5493) (d), PE-OH(16:0/18:2) (699.4998) (e) under negative ion mode.

**Figure 3.** (a) MS/MS fragmentation pathway of PC((16:0/18:1) ( $m/z$  818.5958), (b) PG (16:0/20:1) ( $m/z$  775.5499) and (c) PI(18:0/20:4) ( $m/z$  885.5569) under negative ion mode.

**Figure 4.** MS/MS fragmentation pathway of PA(16:0/18:1) ( $m/z$  673.4848) (a) and LPA(16:0) ( $m/z$  409.2340) (b) under negative ion mode, and SM(d18:1/16:0) ( $m/z$  703.5759) (c) under positive ion mode.

**Figure 5.** (a) Loading plot of PC1 and PC2 for phospholipid in seven egg types. (b) Score plots for phospholipids in seven egg types. (c) Hierarchical cluster analysis based on top 25 molecular species in seven egg types. Colors represent different concentrations indicated by the color bar.

**Figure 1**

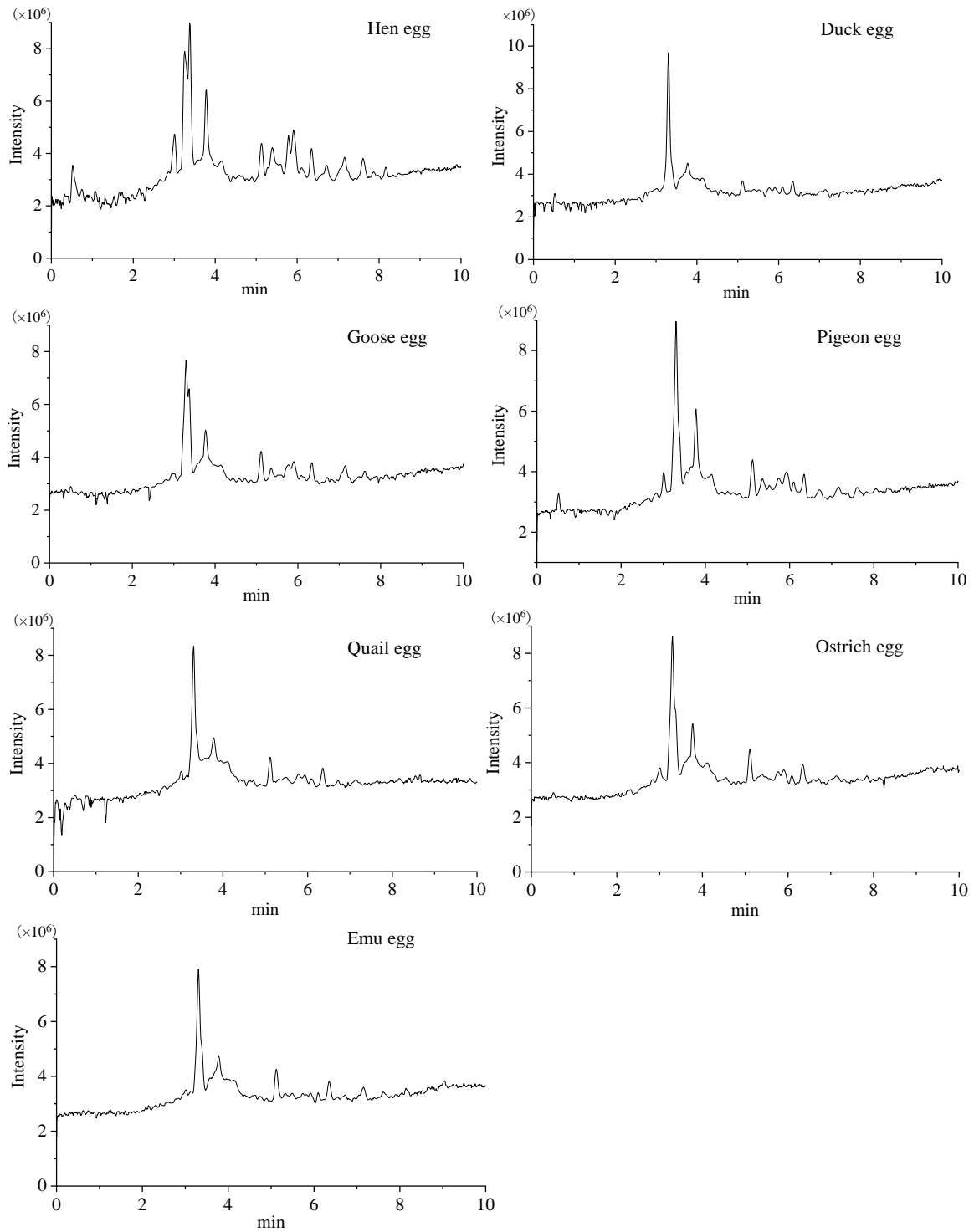
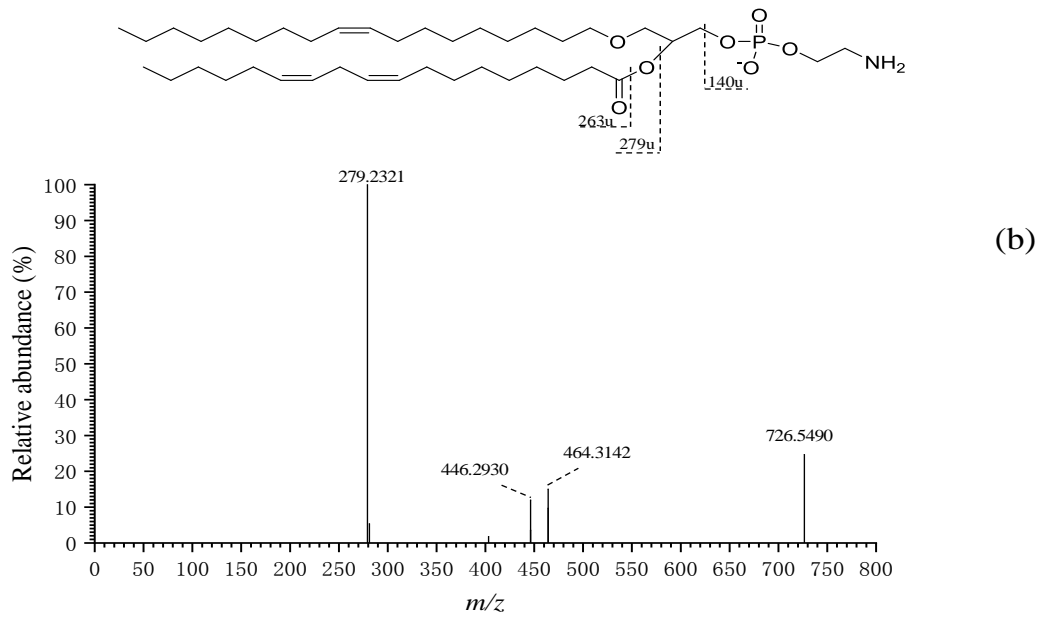
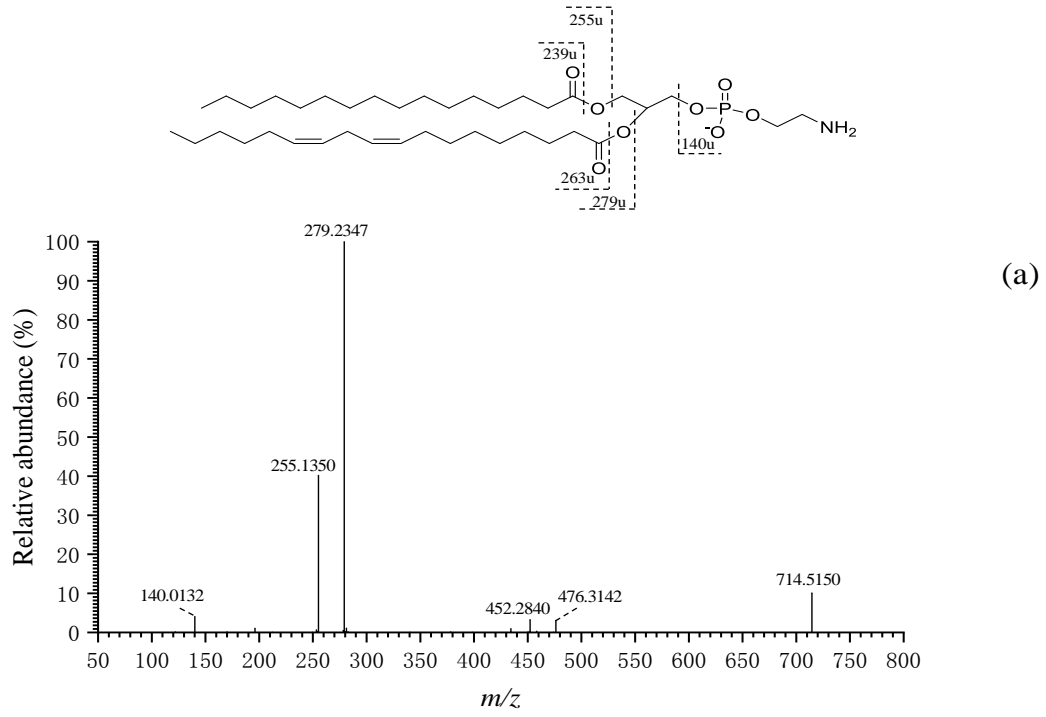


Figure 2







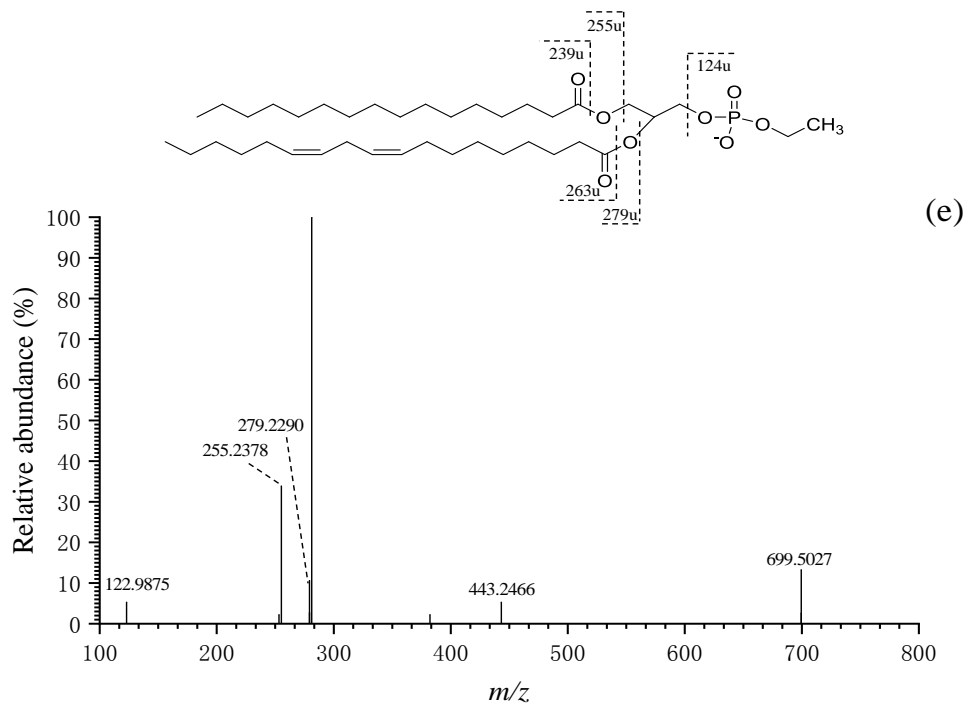
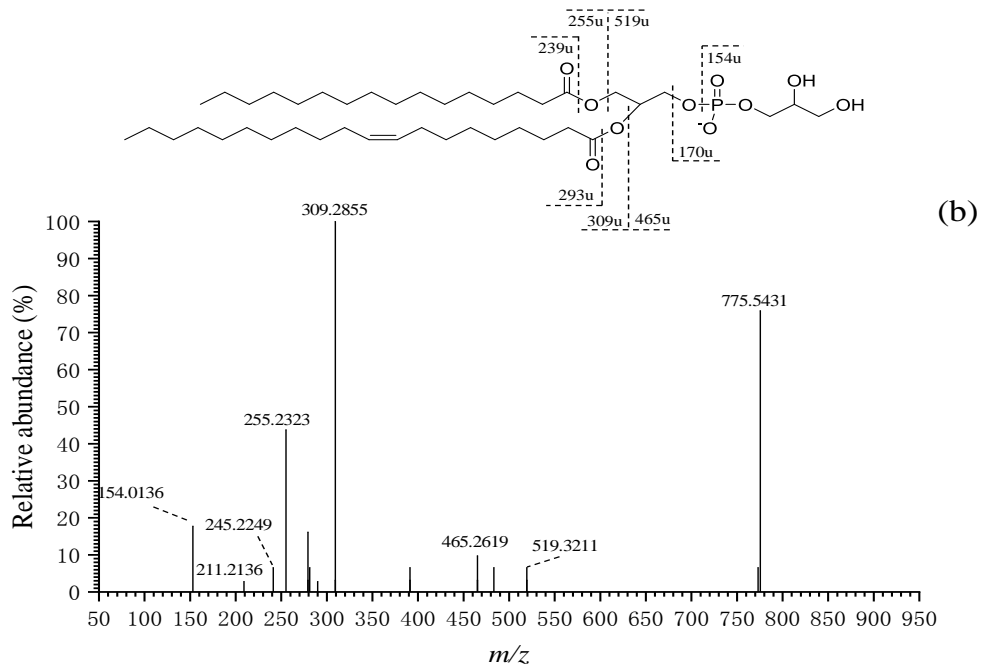
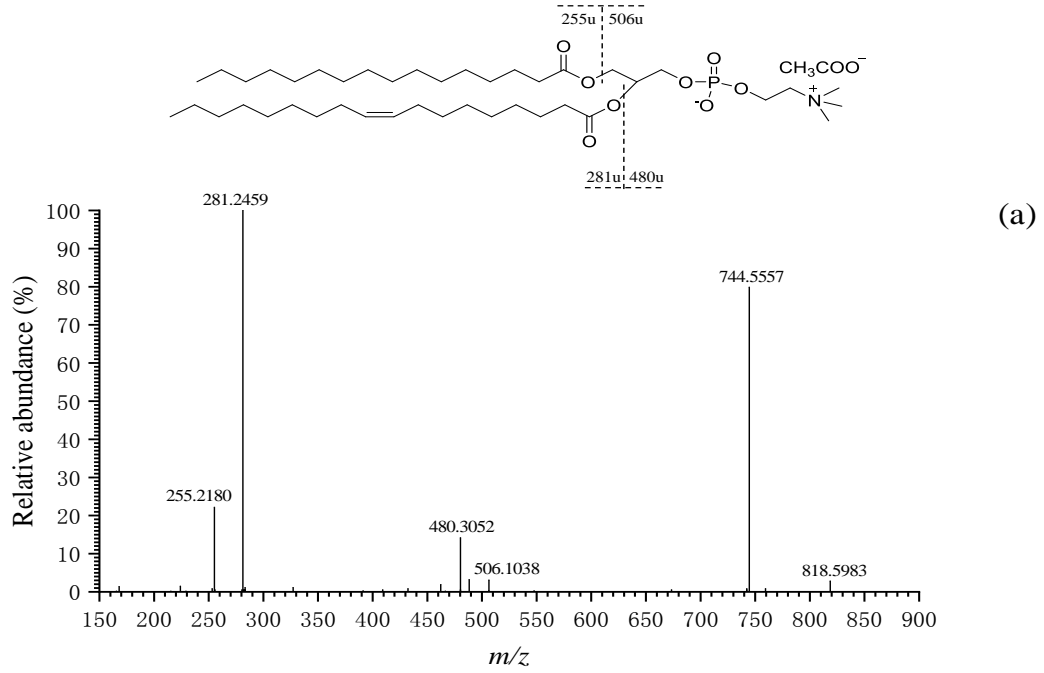


Figure 3



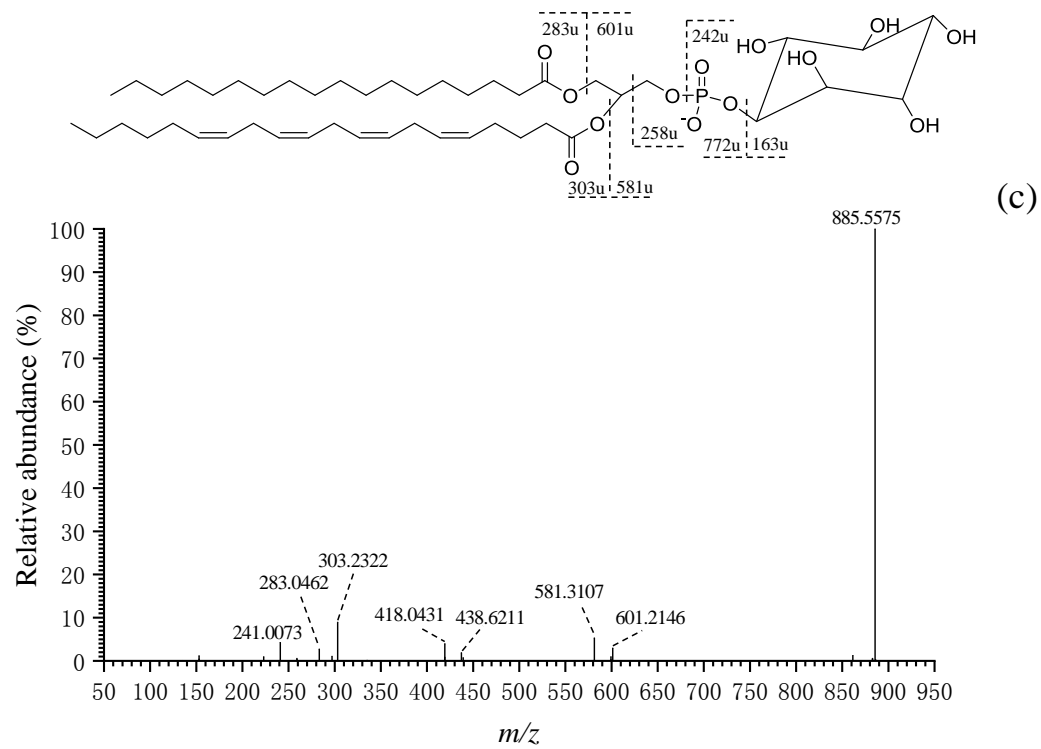
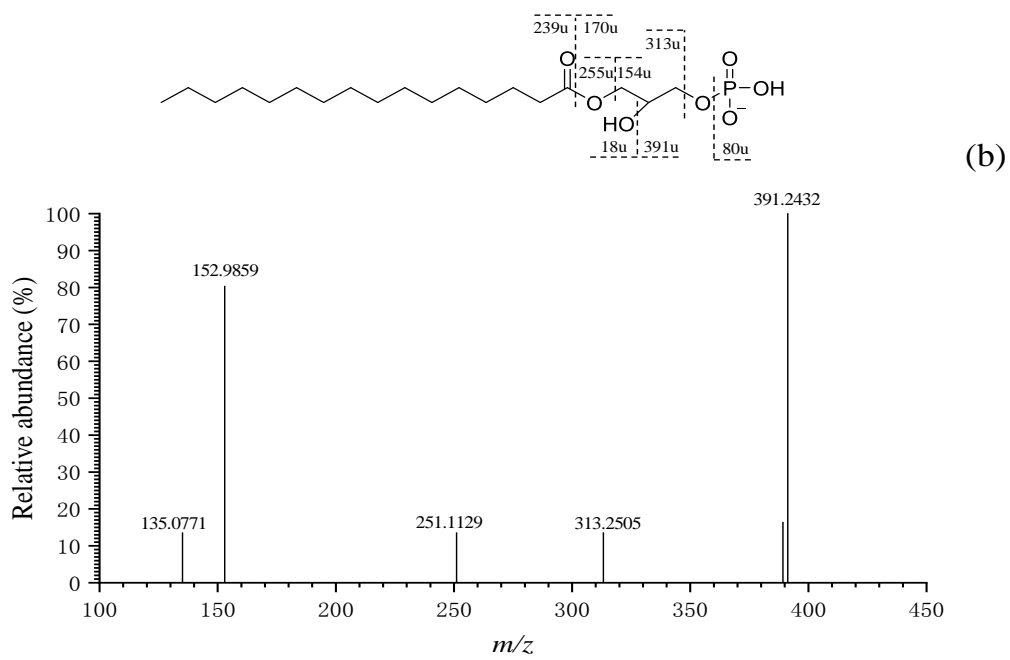
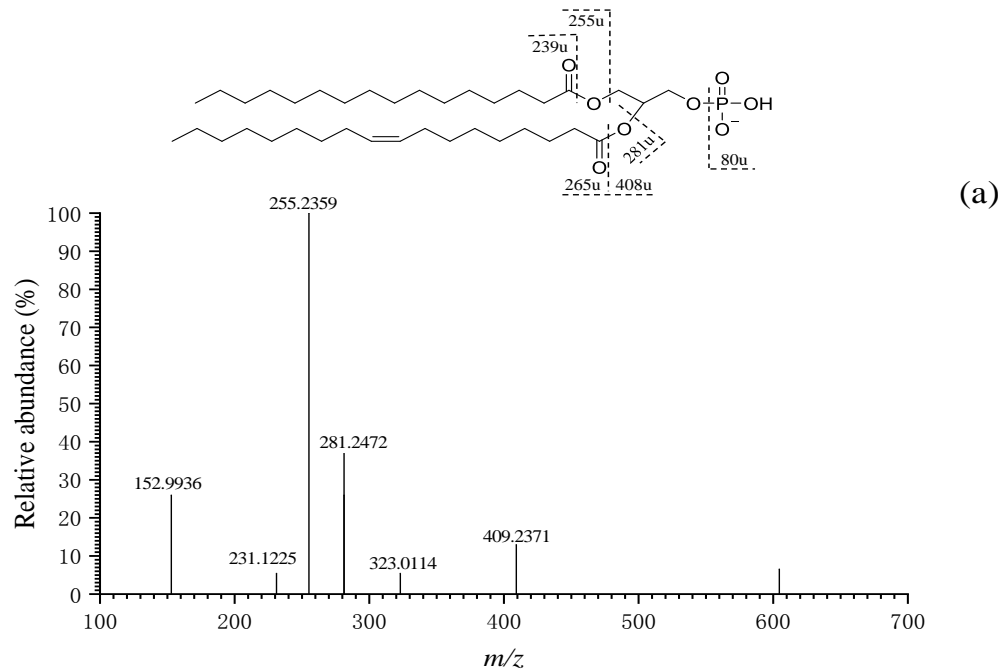
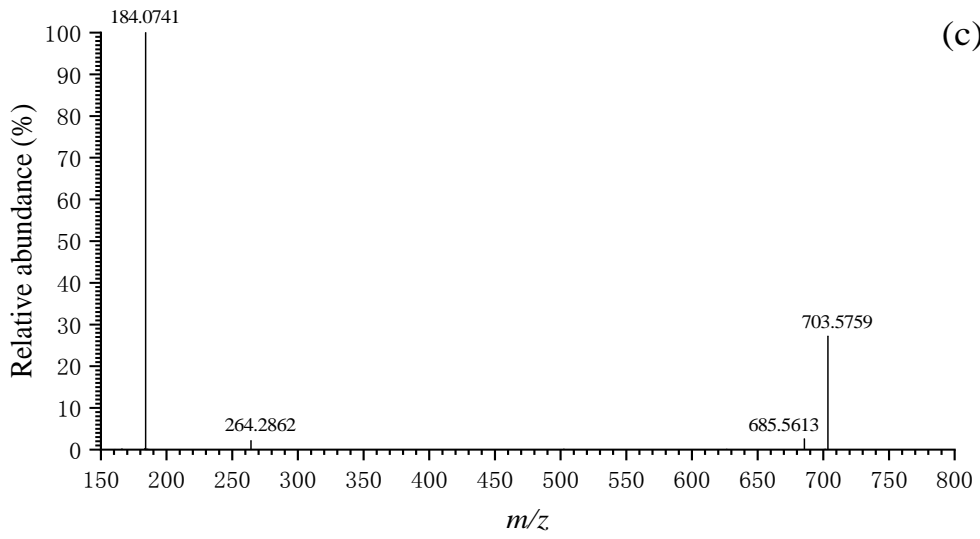
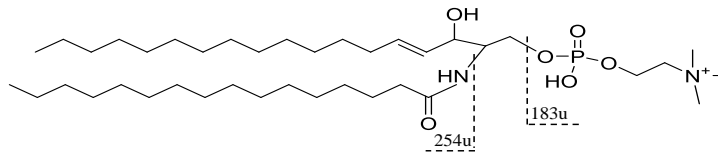


Figure 4

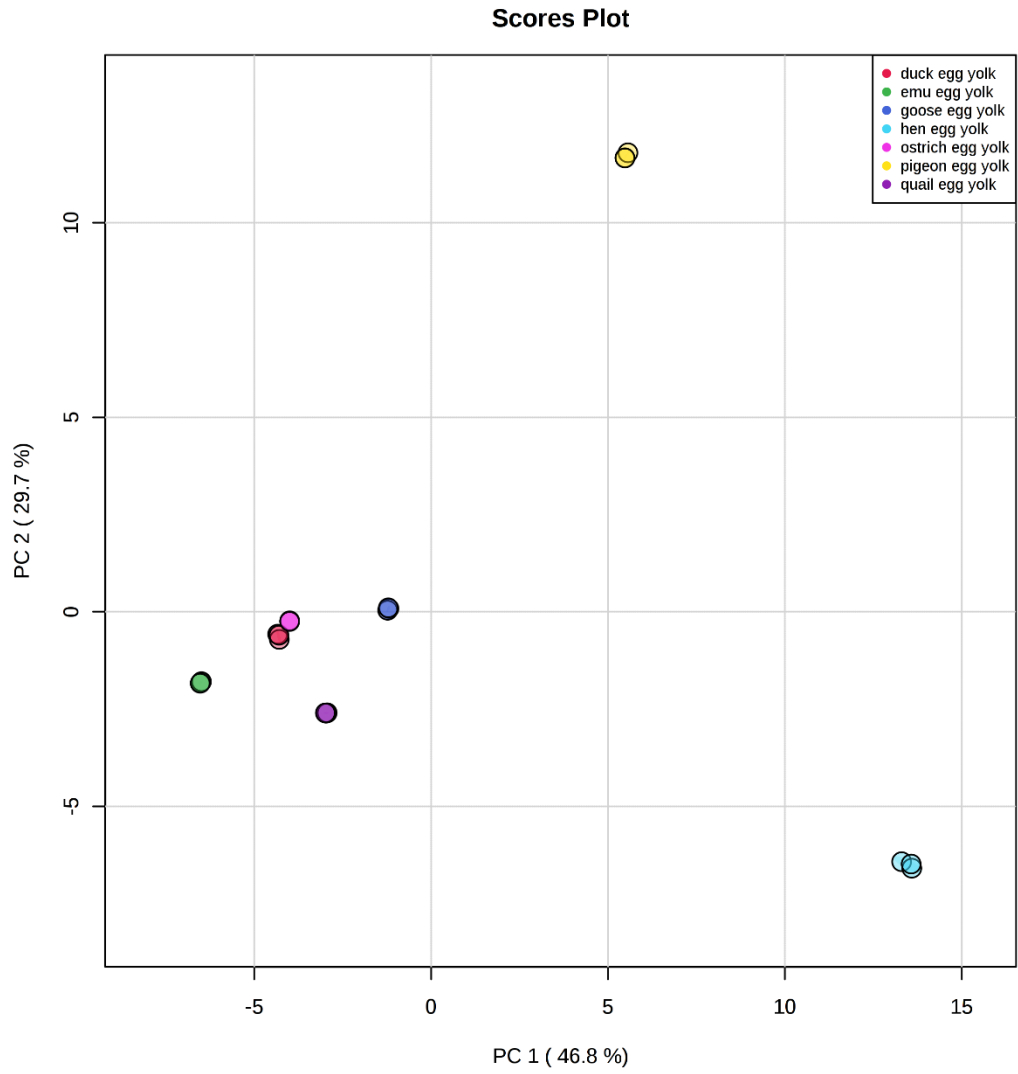




(c)

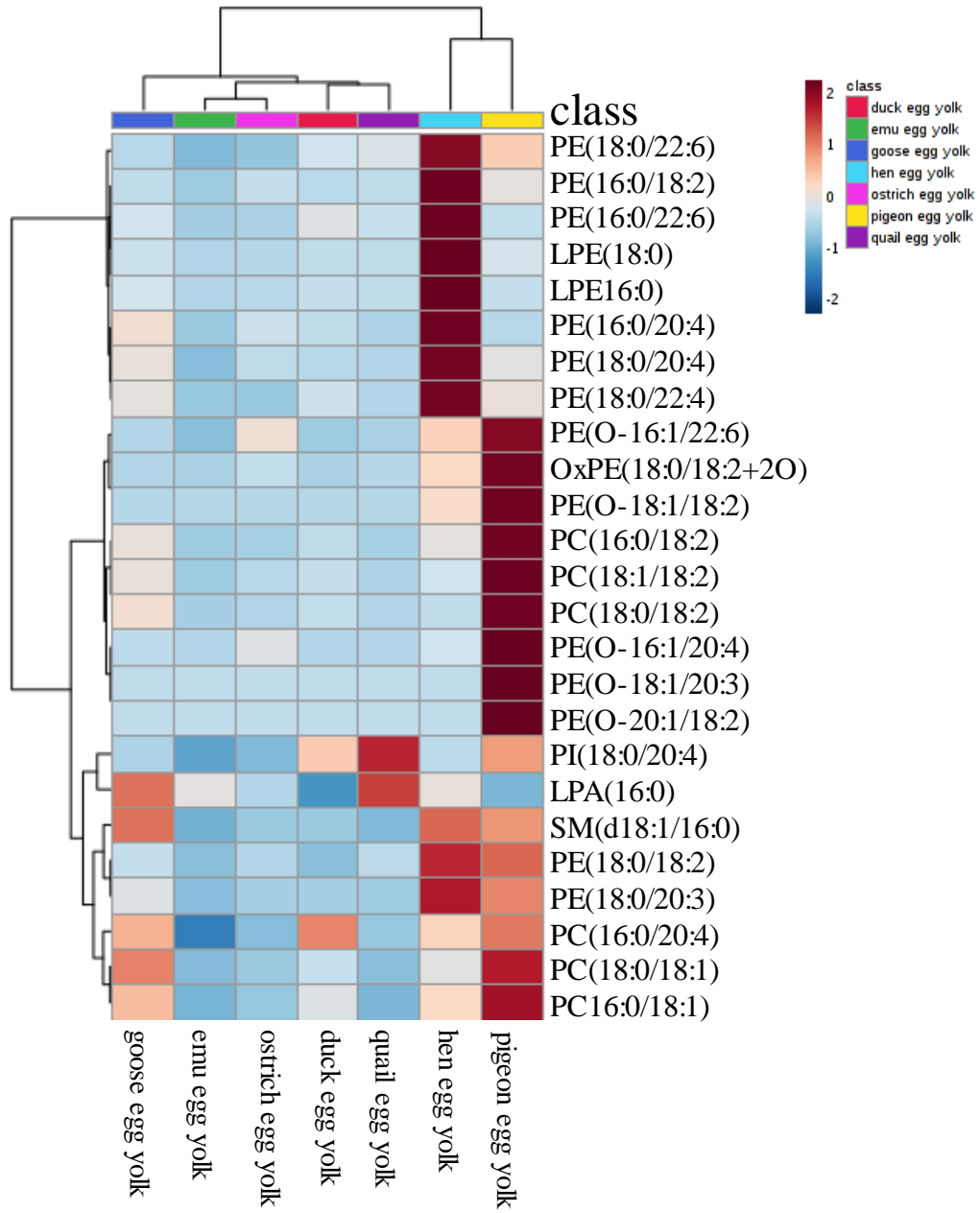


(b)





(c)



**Table 1.** Calibration curves, retention time (RT), linear regression coefficients ( $R^2$ ), limit of detection (LOD) and limit of quantification (LOQ) of seven phospholipid standards

Standards	Formula	RT (min)	$m/z$	Equations	$R^2$	LOD (ng/mL)	LOQ (ng/mL)
15:0-18:1-d7-PE	$C_{38}H_{67}D_7NO_8P$	4.06	709.5519	$y = 2.0415x + 0.1129$	0.9982	0.5	1.7
15:0-18:1-d7-PI	$C_{42}H_{72}D_7O_{13}P$	2.93	828.5625	$y = 3.3967x - 0.1603$	0.9993	0.3	1.0
15:0-18:1-d7-PC	$C_{41}H_{73}D_7NO_8P$	4.74	811.6189	$y = 1.5318x - 0.1809$	0.9988	0.6	2.0
15:0-18:1-d7-PA	$C_{36}H_{62}D_7O_8P$	3.07	666.5097	$y = 2.9346x - 1.2760$	0.9961	0.4	1.3
15:0-18:1-d7-PS	$C_{39}H_{67}D_7NO_{10}P$	3.08	753.5417	$y = 1.4189x - 0.5214$	0.9942	0.8	2.6
15:0-18:1-d7-PG	$C_{39}H_{68}D_7O_{10}P$	3.01	740.5464	$y = 4.2594x - 0.1083$	0.9981	0.2	0.7
d18:1/12:0-SM	$C_{35}H_{71}N_2O_6P$	3.34	647.5123	$y = 1.9735x - 0.0236$	0.9921	0.5	1.7

plasmalyethanolamine (PE-O-alkyl-acyl), lysophosphatidylethanolamine (LPE)], phosphatidylethanol (PEtOH) and oxidized phosphatidylethanolamine (OxPE),

**Table 2.** Molecular species profiles of purified phospholipids from seven egg types

Precursors	<i>m/z</i>	Error (ppm)	RT (min)	Species	Concentrations (nmol/g)							
					Hen	Duck	Goose	Pigeon	Quail	Ostrich	Emu	
<b>PE</b>												
[M-H] <sup>-</sup>	688.4958	+5.08	5.27	16:0/16:1	30.76	5.53	7.78	3.97	10.66	24.99	nd	
[M-H] <sup>-</sup>	698.5128	-0.29	5.69	O-16:1/18:2	14.20	nd	2.48	52.06	3.98	18.95	nd	
[M-H] <sup>-</sup>	700.5293	+0.86	5.76	O-16:0/18:2	16.91	1.19	nd	14.92	3.80	1.41	nd	
[M-H] <sup>-</sup>	700.5326	+5.57	6.10	O-16:1/18:1	41.79	9.20	12.55	105.39	16.04	59.09	1.21	
[M-H] <sup>-</sup>	702.5483	+5.70	6.18	O-16:0/18:1	30.98	5.53	9.33	24.77	14.95	15.88	4.58	
[M-H] <sup>-</sup>	712.4940	+2.38	5.01	16:1/18:2	8.36	nd	nd	nd	nd	1.33	nd	
[M-H] <sup>-</sup>	712.4955	+4.49	5.15	16:0/18:3	1.14	nd	nd	nd	nd	4.75	nd	
[M-H] <sup>-</sup>	714.5151	+4.06	5.41	<b>16:0/18:2</b>	499.73	48.02	55.06	121.55	52.98	61.26	10.65	
[M-H] <sup>-</sup>	716.5284	+6.70	5.79	16:0/18:1	2.27	nd	nd	nd	nd	nd	nd	
[M-H] <sup>-</sup>	718.5428	+5.01	6.24	16:0/18:0	4.65	nd	nd	nd	nd	nd	nd	
[M-H] <sup>-</sup>	722.5172	+5.81	5.65	O-16:1/20:4	6.57	nd	2.05	79.77	nd	11.25	nd	
[M-H] <sup>-</sup>	724.5321	+4.69	5.79	O-18:2/18:2	11.43	nd	3.99	56.36	nd	4.10	nd	
[M-H] <sup>-</sup>	726.5463	+2.75	6.19	O-18:2/18:1	9.74	6.23	14.37	25.45	nd	9.41	nd	
[M-H] <sup>-</sup>	726.5488	+6.19	6.25	O-18:1/18:2	40.56	nd	3.17	135.40	3.97	9.39	nd	
[M-H] <sup>-</sup>	728.5261	+3.43	5.68	17:0/18:2	7.54	nd	nd	nd	nd	2.04	nd	
[M-H] <sup>-</sup>	728.5618	+2.61	6.68	O-18:1/18:1	28.17	3.70	8.69	54.80	3.82	5.63	nd	
[M-H] <sup>-</sup>	730.5413	+2.87	6.07	O-17:0/18:1	8.70	nd	3.08	2.95	2.43	2.79	nd	
[M-H] <sup>-</sup>	730.5777	+2.87	6.76	O-18:0/18:1	32.86	nd	6.46	21.03	15.51	1.96	3.23	
[M-H] <sup>-</sup>	736.4948	+3.39	4.96	16:1/20:4	7.58	nd	nd	nd	nd	2.65	nd	
[M-H] <sup>-</sup>	738.5129	+6.63	5.37	<b>16:0/20:4</b>	402.30	43.25	113.19	35.23	26.00	56.59	2.85	
[M-H] <sup>-</sup>	740.5275	+5.27	5.50	18:1/18:2	6.79	nd	4.66	nd	nd	1.11	nd	
[M-H] <sup>-</sup>	740.5568	-4.19	4.40	O-22:3/15:0	10.08	9.90	7.57	13.34	9.52	7.30	7.59	
[M-H] <sup>-</sup>	742.5430	+5.11	5.95	<b>18:0/18:2</b>	582.06	22.78	126.25	474.04	110.66	92.91	23.22	
[M-H] <sup>-</sup>	742.5443	+6.89	4.38	18:1/18:1	97.05	85.55	41.25	46.73	22.61	23.18	5.11	
[M-H] <sup>-</sup>	744.5580	+4.80	6.35	18:0/18:1	1.95	nd	nd	nd	nd	nd	nd	
[M-H] <sup>-</sup>	746.5148	+2.41	5.55	O-16:1/22:6	32.44	4.78	9.19	83.50	7.17	26.37	nd	
[M-H] <sup>-</sup>	748.5314	+4.68	5.88	O-16:1/22:5	27.31	4.22	13.39	18.14	2.94	18.24	nd	
[M-H] <sup>-</sup>	748.5345	+7.75	5.63	O-16:0/22:6	25.74	nd	3.78	nd	16.41	nd	nd	
[M-H] <sup>-</sup>	750.5505	+8.26	5.79	O-16:0/22:5	25.86	nd	2.96	nd	4.68	nd	nd	
[M-H] <sup>-</sup>	750.5478	+4.46	6.04	O-16:1/22:4	5.44	nd	7.68	15.31	nd	6.64	nd	
[M-H] <sup>-</sup>	752.5655	+7.44	6.48	O-18:1/20:3	nd	nd	nd	15.77	nd	nd	nd	
[M-H] <sup>-</sup>	754.5787	+4.11	6.82	O-20:1/18:2	nd	nd	nd	6.53	nd	nd	nd	

[M-H] <sup>-</sup>	762.5111	+4.20	5.27	16:0/22:6	208.01	43.29	33.29	24.02	26.26	10.23	5.40
[M-H] <sup>-</sup>	764.5240	+0.52	5.45	18:1/20:4	117.98	20.61	43.10	11.96	17.86	26.99	2.66
[M-H] <sup>-</sup>	766.5423	+4.04	5.91	<b>18:0/20:4</b>	956.93	127.67	282.99	254.84	118.45	146.41	14.66
[M-H] <sup>-</sup>	768.5568	+2.47	6.14	18:0/20:3	36.01	3.27	10.15	24.88	2.25	3.25	nd
[M-H] <sup>-</sup>	770.5718	+1.56	6.46	18:1/20:1	12.20	0.00	3.80	10.20	nd	nd	nd
[M-H] <sup>-</sup>	772.5327	+5.18	5.63	O-18:2/22:6	7.22	2.08	4.15	31.74	nd	nd	nd
[M-H] <sup>-</sup>	772.5873	+1.42	6.91	18:0/20:1	6.38	nd	2.25	5.56	nd	nd	nd
[M-H] <sup>-</sup>	774.5493	+6.46	6.09	O-18:1/22:6	6.13	nd	2.76	nd	3.67	nd	nd
[M-H] <sup>-</sup>	776.5636	+4.76	6.18	O-18:0/22:6	19.90	2.93	3.08	6.26	22.58	nd	nd
[M-H] <sup>-</sup>	778.5762	+0.77	6.60	O-18:1/22:4	6.70	nd	7.05	16.78	nd	nd	nd
[M-H] <sup>-</sup>	778.5785	+3.72	6.54	O-18:0/22:5	35.28	nd	1.14	nd	5.56	nd	nd
[M-H] <sup>-</sup>	780.5947	+4.48	6.72	O-18:0/22:4	21.03	nd	2.34	3.99	3.00	nd	nd
[M-H] <sup>-</sup>	788.5248	+1.52	5.36	18:1/22:6	9.75	4.18	2.91	3.14	4.59	nd	nd
[M-H] <sup>-</sup>	790.5425	+4.17	5.80	18:0/22:6	95.77	23.70	17.25	43.30	26.54	8.38	4.21
[M-H] <sup>-</sup>	792.5581	+4.03	6.15	18:0/22:5	141.64	17.45	43.96	12.72	8.99	11.59	nd
[M-H] <sup>-</sup>	794.5729	+2.89	6.32	18:0/22:4	26.37	3.68	6.47	6.75	1.98	nd	nd
<b>PE-OH</b>											
[M-H] <sup>-</sup>	699.4998	+4.00	5.72	16:0/18:2	nd	nd	nd	5.4	nd	1.68	nd
<b>LPE</b>											
[M-H] <sup>-</sup>	438.2630	+0.91	2.50	15:0	4.79	5.49	4.17	6.18	5.27	3.97	4.12
[M-H] <sup>-</sup>	438.2986	-0.91	2.99	O-16:0	7.04	nd	nd	nd	nd	nd	nd
[M-H] <sup>-</sup>	450.2639	+2.89	2.38	16:1	3.61	nd	nd	nd	nd	nd	nd
[M-H] <sup>-</sup>	452.2783	0	2.76	16:0	239.84	14.03	24.63	13.09	10.25	4.79	nd
[M-H] <sup>-</sup>	464.3143	-0.65	3.09	O-18:1	7.42	nd	2.35	nd	2.69	nd	nd
[M-H] <sup>-</sup>	466.3311	+1.72	3.47	O-18:0	11.15	nd	nd	nd	2.75	nd	nd
[M-H] <sup>-</sup>	476.2781	-0.42	2.55	18:2	34.61	3.81	7.25	61.77	9.59	4.72	nd
[M-H] <sup>-</sup>	480.3089	+4.16	3.23	18:0	419.46	22.31	33.33	49.84	18.06	7.36	2.52
[M-H] <sup>-</sup>	500.2780	-0.60	2.54	20:4	39.82	nd	10.26	42.02	7.56	3.90	nd
[M-H] <sup>-</sup>	502.2967	+5.57	2.76	20:3	3.10	nd	nd	nd	nd	nd	nd
[M-H] <sup>-</sup>	524.2783	0	2.50	22:6	21.54	3.64	7.46	24.25	11.83	3.16	nd
[M-H] <sup>-</sup>	526.2944	+0.95	2.77	22:5	41.48	2.93	10.52	5.30	3.72	2.17	nd
<b>OxPE</b>											
[M-H] <sup>-</sup>	774.5359	+8.78	4.47	18:0/18:2+2O	3.70	nd	nd	14.77	nd	nd	nd
[M-H] <sup>-</sup>	824.5493	+5.58	5.95	18:0/22:5+2O	10.14	2.64	3.09	9.79	3.12	2.31	nd
<b>PC</b>											
[M+CH <sub>3</sub> COO] <sup>-</sup>	790.5649	+5.69	5.22	16:0/16:1	87.14	111.74	211.36	120.25	42.61	178.46	41.26
[M+CH <sub>3</sub> COO] <sup>-</sup>	814.5631	+3.31	4.92	16:1/18:2	129.27	34.94	63.52	131.89	30.17	35.93	17.24

[M+CH <sub>3</sub> COO] <sup>-</sup>	816.5810	+6.12	5.34	16:0/18:2	1156.53	642.03	1241.58	4255.32	395.23	387.96	278.62
[M+CH <sub>3</sub> COO] <sup>-</sup>	818.5958	+5.01	5.73	16:0/18:1	1783.42	1367.30	2098.15	3614.85	480.53	686.58	475.86
[M+CH <sub>3</sub> COO] <sup>-</sup>	840.5803	+5.08	5.30	16:0/20:4	245.91	312.73	275.86	320.26	143.21	128.87	63.00
[M+CH <sub>3</sub> COO] <sup>-</sup>	842.5933	+1.90	5.44	18:1/18:2	137.63	118.35	187.84	620.90	83.28	96.86	61.12
[M+CH <sub>3</sub> COO] <sup>-</sup>	844.6126	+6.28	5.88	18:0/18:2	302.96	311.90	625.30	1892.91	224.69	230.50	175.12
[M+CH <sub>3</sub> COO] <sup>-</sup>	846.6256	+3.07	6.31	18:0/18:1	274.89	223.34	480.44	628.00	127.77	151.38	121.00
[M+CH <sub>3</sub> COO] <sup>-</sup>	864.5805	+5.20	5.22	16:0/22:6	88.49	43.08	68.67	80.78	65.93	nd	28.69
[M+CH <sub>3</sub> COO] <sup>-</sup>	866.5956	+4.50	5.53	16:0/22:5	103.78	66.94	67.55	nd	59.20	nd	nd
<b>PG</b>											
[M-H] <sup>-</sup>	747.5183	+0.13	4.58	16:0/18:1	12.61	3.12	5.46	37.39	3.79	14.64	nd
[M-H] <sup>-</sup>	771.5226	+5.70	4.42	16:0/20:3	1.99	nd	nd	nd	nd	nd	nd
[M-H] <sup>-</sup>	773.5392	+6.98	4.65	16:0/20:2	3.98	1.62	2.51	16.02	1.34	1.34	nd
[M-H] <sup>-</sup>	775.5499	+0.52	4.94	16:0/20:1	9.00	2.39	5.97	52.20	4.22	1.51	nd
<b>PI</b>											
[M-H] <sup>-</sup>	807.5096	+8.30	4.15	16:0/16:1	0.25	0.03	0.07	0.09	0.05	0.04	0.03
[M-H] <sup>-</sup>	833.5223	+4.44	4.25	16:0/18:2	12.82	4.40	3.00	11.11	17.74	5.40	2.58
[M-H] <sup>-</sup>	835.5376	+4.07	4.47	16:0/18:1	8.96	4.26	6.28	4.92	32.20	11.99	2.53
[M-H] <sup>-</sup>	855.5109	+9.35	4.05	16:0/20:5	0.07	0.30	0.02	0.21	0.20	0.16	0.05
[M-H] <sup>-</sup>	857.5223	+4.31	4.24	16:0/20:4	14.67	20.73	8.27	2.79	22.88	8.88	6.07
[M-H] <sup>-</sup>	861.5520	+2.44	4.59	18:0/18:2	18.50	8.21	8.05	33.33	49.90	15.29	4.98
[M-H] <sup>-</sup>	863.5724	+8.00	4.87	18:0/18:1	4.35	2.83	3.40	6.81	20.33	7.95	2.05
[M-H] <sup>-</sup>	879.5092	+7.16	3.90	18:2/20:5	0.00	0.04	nd	0.07	0.00	0.03	0.00
[M-H] <sup>-</sup>	881.5224	+4.31	4.08	18:1/20:5	0.18	0.22	0.03	0.11	0.27	0.05	0.09
[M-H] <sup>-</sup>	883.5401	+6.68	4.35	18:0/20:5	1.44	1.43	0.76	0.33	6.21	1.10	1.96
[M-H] <sup>-</sup>	885.5569	+7.90	4.58	18:0/20:4	65.05	105.89	60.00	125.99	169.24	43.19	30.95
[M-H] <sup>-</sup>	887.5735	+9.01	4.73	18:0/20:3	4.62	3.36	8.13	4.63	7.17	3.57	0.57
<b>PA</b>											
[M-H] <sup>-</sup>	671.4678	+2.98	5.33	16:0/18:2	220.96	132.61	277.57	330.15	141.54	203.85	202.19
[M-H] <sup>-</sup>	673.4848	+5.05	5.70	16:0/18:1	351.72	208.09	304.66	288.87	241.89	231.15	257.72
<b>LPA</b>											
[M-H] <sup>-</sup>	409.2340	-5.13	2.72	16:0	775.87	597.56	930.25	645.17	979.32	701.30	765.50
<b>SM</b>											
[M+H] <sup>+</sup>	703.5759	-9.10	5.03	d18:1/16:0	31672	5912	30542	27027	3502	6097	2323

nd means not detected; RT means retention time.

# Graphic Abstract

