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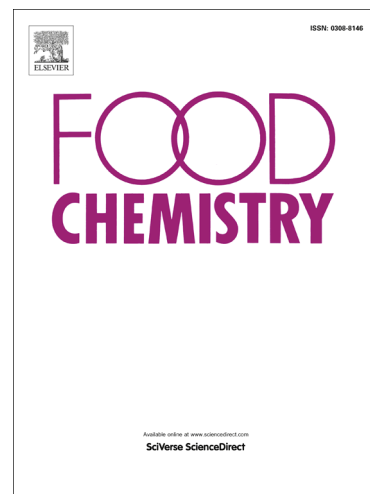
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Phospholipids composition and molecular species of large yellow croaker***(Pseudosciaena crocea)* roe**

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1 Abstract

2 The research aims to study phospholipids (PL) classes and molecular species of large
3 yellow croaker (*Pseudosciaena crocea*) roe. Both gas chromatography-mass spectroscopy
4 (GC-MS) and high-performance liquid chromatography with evaporative light-scattering
5 detection (HPLC-ELSD) were utilized to analyze and identify the PLs fatty acids
6 compositions and classes in the *P. crocea* roe, respectively. Docosahexaenoic acid (DHA,
7 C22:6) and eicosapentaenoic acid (EPA, C20:5) account for 35.0% and 6.9% of the PLs.
8 Phosphatidylcholines (PC), lysophosphatidylcholines (LPC), phosphatidylethanolamines
9 (PE) and phosphatidylinositols (PI) account for $76.36\pm 0.62\%$, $12.30\pm 0.55\%$, $9.12\pm 0.02\%$
10 and $1.09\pm 0.01\%$ of the total PLs, respectively. In addition, the PLs molecular species
11 were characterized by ultra-high performance liquid chromatography-electrospray
12 ionization-quadrupole-time of flight-mass spectrometry (UPLC-Q-TOF-MS). A total of 92
13 PLs molecular species was identified, including 49 PCs, 13 PEs, 10 phosphatidic acids
14 (PAs), 13 phosphatidylserines (PSs), 3 phosphatidylglycerols (PGs), 2 sphingomyelins
15 (SMs), and 2 PIs of the *P. crocea* roe.

16 **Keywords:** *Pseudosciaena crocea* roe; fatty acids composition; phospholipids classes;
17 molecular species.

18 1. Introduction

19 The large yellow croaker (*Pseudosciaena crocea*) has been known for its good taste and
20 high nutritional value among consumers in China (Hui, Liu, Feng, Li, & Gao, 2016; Liu,
21 Chen, Hu, Chen, Zhang, Cao, et al., 2016). In southern part of China, it is regarded as one
22 of the most commercially important marine fish, and possesses the largest yield for a
23 single species in Chinese net-cage farming (J. Zhao, Li, Wang, & Lv, 2012). A total
24 production of approximately 148,600 tons was obtained in 2015 (Yuan & Zhao, 2016).
25 The development and utilization of processed *P. crocea* products has drawn the attention
26 of some researchers in recent years. One by-product in the fish industry which has
27 attracted researcher's interest in PLs (phospholipids) is fish roe. Fish roe has been
28 reported to contain large amounts of n-3 polyunsaturated fatty acids (n-3 PUFAs), mainly
29 eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3)
30 which are recognized to have the functions of preventing the incidence of coronary heart
31 diseases, inflammatory and autoimmune disorders, cancer and so on (Rosa, Scano, Atzeri,
32 Deiana, Mereu, & Dessi, 2012; Q. Wang, Xue, Li, & Xu, 2008). Most of the n-3 PUFAs
33 are present in the PL form, where PC (phosphatidylcholine) is the predominant lipid class
34 (Hayashi, Tanaka, Hibino, Umeda, Kawamitsu, Fujimoto, et al., 1999; Shirai, Higuchi, &
35 Suzuki, 2006). PLs, regarded as major polar lipid components, are mainly known to serve
36 as building blocks for cell membranes, equipped with important physiological and
37 biological functions in almost all known living beings (Burri, Hoem, Banni, & Berge,
38 2012; Suzumura, 2005). n-3 PUFAs-containing PLs would possess more beneficial
39 effects. Some studies have reported that their combination could show more powerful
40 effects on adjusting liver and blood plasma lipid levels (Dasgupta & Bhattacharyya, 2007;
41 Shirouchi, Nagao, Inoue, Ohkubo, Hibino, & Yanagita, 2007). Marine PLs hold more

42 potential applications in pharmaceuticals and cosmetics in addition to the functions of
43 traditional PLs (Burri, Hoem, Banni, & Berge, 2012). Currently, fish roe has been
44 consumed in the products of caviar (the most popular), whole skins, formulations with
45 oils, cheese bases, and salted or smoked products (Bledsoe, Bledsoe, & Rasco, 2003). In
46 the processing of *P. crocea*, its roe becomes a major by-product which is usually thrown
47 away. Furthermore, with the strength of big size for the roe and an annual high yield of *P.*
48 *crocea*, the roe, especially its PLs has more potential to be exploited.

49 The identified methodologies to characterize and quantify PLs from both biological
50 and food matrixes have developed from the traditional thin layer chromatography (TLC)
51 methods to more advanced mass spectrometry technologies (MS) (Fong, Ma, & Norris,
52 2013). The traditional method has been verified to be time-consuming and large volume
53 of lipid is required. HPLC coupled to an evaporative light-scattering detector (ELSD) is
54 probably the most extensively reported analytical method for PLs class analysis in the
55 food matrixes (Rodriguez-Alcala & Fontecha, 2010). ELSD could create the linearity of
56 complicated calibrations within only a narrow concentration range (Donato, Cacciola,
57 Cichello, Russo, Dugo, & Mondello, 2011). The recently developed technology of
58 reversed phase ultra-high performance liquid chromatography-electrospray
59 ionization-quadrupole-time of flight-mass spectrometry (UPLC-ESI-Q-TOF-MS)
60 possesses the advantages of superior separation, higher resolution, greater sensitivity and
61 faster analysis to comprehensively analyze lipid structure (Y. Wang & Zhang, 2011; Yan,
62 Li, Xu, & Zhou, 2010; Y. Y. Zhao, Wu, Liu, Zhang, & Lin, 2014). Compared to HPLC,
63 UPLC column can be utilized with higher flows and pressures (Sarafian, Gaudin, Lewis,
64 Martin, Holmes, Nicholson, et al., 2014). The soft ionization technique, ESI, coupled
65 with Q-TOF, would be more rapid and sensitive to monitor the molecular species and

66 quantify individual lipid species, most of which possess the specific headgroup
67 fragmentations after collision-induced dissociation (CID), in unfractionated lipid extracts
68 (Pulfer & Murphy, 2003; Y. Wang & Zhang, 2011). GC-MS has been verified as a
69 traditional method to measure the fatty acids in food matrix. But it still has some
70 disadvantages, like tedious operation and poor resolution (Zhou, Gao, Zhang, Xu, Shi, &
71 Yu, 2014).

72 The aim of this study was to fully understand the PLs profile in the roe of *P. crocea*.
73 We used GC-MS and HPLC-ELSD to identify the fatty acids composition and PLs
74 classes of the roe, respectively. In addition, the molecular species of PLs of the roe were
75 also confirmed using UPLC-ESI-Q-TOF-MS which could provide more information.

76 **2. Materials and methods**

77 **2.1. Materials and Reagents**

78 The *P. crocea* roe was kindly provided by Fujian Yuehai Aquatic Food Ltd (Ningde
79 City, Fujian Province). The roe was mixed and kept under refrigeration (0-4°C) for less
80 than 24 h before analysis in the lab of Aquatic Food Products Processing in Fujian
81 Agriculture and Forestry University.

82 Five types of PLs classes were detected in the present study, including
83 phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingophospholipid (SM),
84 phosphatidylinositol (PI) and lysophosphatidylcholine (LPC). Those compounds were all
85 obtained from Sigma-Aldrich for the method of HPLC-ELSD (Dorset, U.K.). 10 lipids
86 standards PC (17:0), LPC (15:0/0:0), PG (15:0/15:0), PC (15:0/15:0), PE (15:0/15:0), SM
87 (d18:1/17:0), PS (17:0/17:0), Cer (d18:1/17:0), DG (17:0/0:0/17:0), and TG
88 (15:0/15:0/15:0) were purchased from Avanti Polar Lipids (Alabaster, Alabama, US) for
89 the method of UPLC-Q-TOF-MS. High-performance liquid chromatography

90 (HPLC)-grade normal hexane, isopropyl alcohol, and methanol were purchased from
91 Merck (Darmstadt, Germany). Other HPLC-grade acetonitrile, formic acid, ammonium
92 formate, Leucine-enkephalin and sodium formate were purchased from Thermo Fisher
93 Scientific (Shanghai City, China).

94 **2.2. Extraction of PLs for the method of GC-MS and HPLC-ELSD**

95 The roe of *P. crocea* was firstly cleaned by removal of the fins, scales and blood
96 vessels and homogenized with a blender. The homogenate was used to extract PLs. The
97 total lipids were extracted from fish roe according to a modified version of the
98 Bligh-Dyer method (Bligh & Dyer, 1959). Briefly, 5.0 g of fish roe homogenate was
99 mixed with 60 mL of chloroform/methanol (2:1, v/v) solution inside a glass tube and
100 extracted for 2 h. Afterwards, the mixture was heated at 65 °C in water bath for 1 h. The
101 filter liquor was collected and solvent evaporated at 38 °C to obtain the final total lipids.
102 PLs were separated from the total lipids by column chromatography on silica gel. Briefly,
103 the activated silica gel was mixed and stirred in the chloroform solution until no bubble
104 appeared, and then was added into chromatographic column slowly. After the silica gel
105 column (26×300 mm) was stable, 3.0 g of the total lipid sample in chloroform solution
106 was loaded. 250 mL of chloroform solution, 100 mL of acetone and 400 mL of methanol
107 were utilized separately to elute neutral lipids, glycolipid and PLs. The phase of methanol
108 was collected and evaporated to obtain PLs at 38 °C. The PLs were stored at -20 °C for
109 further analysis.

110 **2.3. Fatty acids analysis by GC-MS**

111 **2.3.1. Sample preparation**

112 20~50 mg of the extracted PLs was dissolved with 1 mL of 2 mol/L sodium
113 hydroxide in methanol and incubated in a 60 °C water bath for 2 min. Then, 1 mL of 2

114 mol/L methanolic HCl was added and incubated for an additional 5 min. Next, 2 mL of
115 n-hexane was mixed into the solution and kept at room temperature for 1 h. Finally, the
116 upper layer of n-hexane containing the fatty acid methyl esters was collected and dried
117 with anhydrous sodium sulfate before fatty acid compositions analysis.

118 **2.3.2. GC-MS parameter**

119 The fatty acids composition of roe PLs were analyzed by gas chromatograph (GC)
120 (Palo Alto, CA, USA) 6890N equipped with an HP-5 mass spectroscopy (MS) capillary
121 column (30 m×0.25 mm i.d.) connected to an Agilent 5973 mass spectrometer operating
122 in the EI mode (70 eV; m/z) 50-550. The initial column temperature was 140 °C,
123 maintained for 1 min, then increased to 190 °C at the rate of 5 °C/min for 10 min, and
124 then increased to 220 °C at the rate of 5 °C/min and maintained for 10 min. The carrier
125 gas was helium at a flow rate of 1.0 mL/min under 88 kPa, and the injection volume was
126 1 µL with a split ratio of 10:1. Structure assignments were made based on interpretation
127 of mass spectrometer fragmentation and recognized by comparison of retention time.

128 **2.4. PLs classes analysis by HPLC-ELSD**

129 HPLC (LC-20A, Shimadzu Corporation, Japan) equipped with an evaporative light
130 scattering detector (ELSD 3300, Alltech, Deerfield, IL) (Sala Vila, Castellote-Bargalló,
131 Rodríguez-Palmero-Seuma, & López-Sabater, 2003) with slight modification was used to
132 measure PLs classes. The operation temperature of ELSD was 50 °C with the nebulizer
133 gas of nitrogen at a flow rate of 2.0 L/min and a pressure of 4.5 MPa. The separation was
134 achieved with a silica column, 250 mm×4.6 mm i.d., 5 µm (Agilent ZORBAX RX-SIL)
135 at 30 °C. The analysis was performed by gradient elution using
136 n-hexane/2-propanol/methanol/1% acetic acid (4:9:5:2, v/v/v/v), with the flow rate of

137 mobile phase at 0.8 mL/min and the evaporation temperature of 60 °C. Measurements
138 were made in triplicate on each sample.

139 The calibration curve was obtained by injecting 10 µL of serially diluted solutions of
140 PE (0.16-2.2 mg/mL) and PC (0.50-8.7 mg/mL) SM, LPC (0.10-3.0 mg/mL) at five
141 different concentrations. All samples were analyzed in triplicate. The calibration curves
142 for each compound were calculated from the area values with known amounts of
143 standards.

144 **2.5. PLs molecular species analysis by UPLC-Q-TOF-MS**

145 **2.5.1. Sample preparation**

146 Approximately 0.1 g of the mixed roe sample was added to 1.4 mL of isopropanol
147 (IPA) in a 2 mL of centrifuge tube, vortex mixed for 1 min, and sonicated for 10 min.
148 Samples were kept in freezer (-20 °C) for 1 h and then frozen centrifuged at 14, 000 g for
149 10 min. The supernatant was collected and 1 mL was filtered into UPLC vials through
150 0.22 µm organic filter. The samples were kept in freezer (-20 °C) for later analysis.

151 **2.5.2. UPLC parameter**

152 UPLC was equipped with C₁₈CSH column (1 mm × 50 mm, 1.7 µm; Waters Ltd.,
153 Elstree, U.K.). The mass spectrometry method of the Xevo G2-S Q-TOF was
154 implemented in order to improve isotopic distribution and mass accuracy and reduce high
155 ion intensities. Two microliters of the samples were injected onto C₁₈CSH column at
156 55 °C. The mobile phase flow rate was set as 400 µL/min. The mobile phase were A,
157 Acetonitrile (ACN)/Water (60/40%), including 10 mM ammonium formate and 0.1%
158 formic acid; B, IPA/ACN (90/10%), including 10 mM ammonium formate and 0.1%
159 formic acid. Measurements were analyzed in triplicate.

160 **2.5.3. Q-TOF-MS parameter**

161 For both positive and negative ion-mode, MS parameters were as follows: capillary
162 voltage was set at 3 kV, cone voltage at 25 V, ESI source temperature at 120 °C,
163 desolvation temperature at 500 °C, desolvation gas flow at 800 L/h, and cone gas flow at
164 50 L/h. Acquisition was performed from m/z 50 to 2000. Leucine enkephalin (m/z
165 556.2771 in ESI⁺, m/z 554.2615 in ESI⁻) was continuously infused at 30 µL/min and
166 used as lock mass correction.

167 **2.5.4. MS Data Preprocessing**

168 MassLynx software version 4.1 was used for MS data acquisition and analysis.

169 **2.6. Statistics analysis**

170 Statistical analysis and calculation of the mean and standard deviation were
171 performed by using Microsoft Excel 2007. The results of triplicate analyses were
172 expressed as means±SE.

173 **3. Results and discussion**

174 **3.1. PLs fatty acids composition of *P. crocea* roe**

175 The PLs fatty acid composition of *P. crocea* roe is presented in Table 1. The main fatty
176 acids were docosahexaenoic acid (C22:6) with a relative percentage of >35%, followed
177 by palmitic acid (C16:0), oleic acid (C18:1), eicosapentaenoic acid acid (C20:5), and
178 stearic acid (C18:0). The percentage of PUFA accounts for 43% of the total PLs, among
179 which considerable amounts of DHA (C22:6) and EPA (C20:5) were found at 35 and
180 6.9%, respectively. Numerous published articles have also indicated a higher
181 concentration of EPA and DHA in the PLs of fish roe. They also detected similar specific
182 fatty acids as shown in Table 1 except for C18:4, C20:4, and C22:5 (Cejas, Almansa,
183 Villamandos, Badı´a, Bolan˜os, & Lorenzob, 2003; Shirai, Higuchi, & Suzuki, 2006). It

184 could be concluded that *P. crocea* roe is a rich source to obtain marine PLs with high
185 contents of EPA and DHA.

186 **3.2. Analysis of PLs classes using HPLC-ELSD**

187 Figure. 1 shows the HPLC-ELSD chromatogram of PLs extracted from the roe of *P.*
188 *crocea*. Corresponding to the PLs standards chromatograms, three PLs classes, PC, PE,
189 and PI, and one LPL class (LPC) were observed in the roe of *P. crocea*. The peak signal
190 of PC was broader. The reason could be that a wide variety of fatty acyl composition is
191 present in this PC molecular species.

192 As seen from Table 2, PC was the most abundant PLs class in the roe of *P. crocea* with
193 a composition of $76.36 \pm 0.62\%$, accounting for more than half of the total PLs. Followed
194 were LPC and PE with contents of 12.30 ± 0.55 and $9.12 \pm 0.02\%$, respectively. The content
195 of PI was $1.09 \pm 0.01\%$. Wang et al. also detected PE, PC, PI, SM, CL and LPC in squid
196 eggs using HPLC-ELSD and found that the contents of PC and PE were the most (Wang,
197 Xue, & Li, 2008). Similarly, Bledsoe et al. reported that PC and PE were the major PLs
198 components in fish roe (Bledsoe, Bledsoe, & Rasco, 2003). The results indicated that *P.*
199 *crocea* roe would be a valuable source of marine PLs with high PC, LPC and PE levels.

200 **3.3. Characterization of PLs molecular species using UPLC-Q-TOF-MS**

201 In this work, the use of UPLC-Q-TOF-MS provided a full scanning of the roe extracts
202 after IPA precipitation. IPA has been proven to be excellent for sample preparation in one
203 single step that gives a wide range of lipids prior to lipid profiling (Sarafian, et al., 2014).
204 It allows the PLs identification to be faster and more fully elucidated along with a
205 superior separation of UPLC.

206 MS data acquisition and analysis of each peak were processed by MassLynx 4.1
207 which is able to measure all possible molecular formulas corresponding to the observed

208 data. In TOF-MS, the information of element composition can also be provided through
209 mass measurement and isotopic mass distributions.

210 The total ion chromatograms of *P. crocea* are shown in both positive (Figure 2a) and
211 negative (Figure 2c) ion modes, respectively. Large amounts of signal peaks could be
212 seen at 0-18 min, wherein more peaks appeared in the positive ion mode than the
213 negative one. Their corresponding mass spectra were also presented in both positive
214 (Figure 2b) and negative (Figure 2d) ion modes, respectively. The characteristic
215 headgroup fragmentation ions of PLs were identified by analyzing the tandem MS data
216 and searching Lipid Maps Structure Database (<http://www.lipidmaps.org>) through the
217 software Progenesis QI (Lin, Lin, Zhang, Ni, Yin, Qu, et al., 2015; Zhang, Yang, Li, Yao,
218 Qi, Yang, et al., 2016). Accordingly, 92 PLs were identified, including 49 PCs, 13 PEs, 10
219 PAs, 13 PSs, 3 PGs, 2 SMs, and 2 PIs (Table 3).

220 Some characteristic fragmentation ions were confirmed based on comparison with the
221 data of PLs standards. The distinctive phosphocholine headgroup of PC molecules was
222 generated at m/z 184 where the product ion $[C_5H_{15}O_4NP]^+$ was yielded in the positive
223 mode (Shen, Wang, Gong, Guo, Dong, & Cheung, 2012; Yan, Li, Xu, & Zhou, 2010),
224 while the unusual tetravalent nitrogen led to the formation of a fragment ion $[M-CH_3]^-$,
225 and then the precursor ion of PC formed the fragment ion $[C_4H_{11}O_4NP]^-$ (m/z 168) in the
226 negative ion mode (Harrison & Murphy, 1995; Yan, Li, Xu, & Zhou, 2010). According to
227 both the fragmentation pattern and molecular weights, a total of 49 PCs were detected.
228 The main PC molecular species were 15:0/19:1&16:0/18:1, O-18:0/22:6&18:0/22:6,
229 O-16:0/22:6&P-16:0/22:6&16:0/22:6 and P-16:0/20:5&16:0/20:5, the relative abundance
230 of which account for 19.43, 11.52, 13.33 and 15.79% respectively. PC has been regarded

231 as the most important structural PL that constitute cell membranes and pulmonary
232 surfactant.

233 In the positive mode, the fragment ion of $[M+H-141]^+$ was generated through a polar
234 head phosphoryl-ethanolamine in the sn-2 position of PE (Brouwers, Vernooij, Tielens,
235 & Van Golde, 1999). The two unique fragment ions m/z 140 $[C_2H_7O_4NP]^-$ and m/z 196
236 $[C_5H_{11}O_5NP]^-$ were produced in the negative ion mode (Yan, Li, Xu, & Zhou, 2010). 13
237 PEs were identified. The main PE molecular species were 22:6/19:0, 17:2/22:0 and
238 15:0/22:1 with the relative abundance of 60.84, 16.33, and 8.47%, respectively. PEs are
239 non-bilayer preferring lipids and regarded as the key PLs to regulate the fluidity of
240 membranes (Sterin, Cohen, & Ringel, 2004).

241 PA molecular species could be confirmed in the negative ion mode as negatively
242 charged (Knittelfelder, Weberhofer, Eichmann, Kohlwein, & Rechberger, 2014). The
243 most abundant PA molecular species were 19:0/22:1, 14:1/21:0, 16:0/22:1 and 15:1/22:2
244 with the relative abundance of 12.72, 11.85, 18.73 and 24.07%, respectively. PAs can be
245 generated through the hydrolysis of PC, and are major constituents of cell membranes.

246 PS molecular species were confirmed in accordance with the loss of polar headgroup
247 $[M-184]^+$ in the positive ion mode (Theaker, Abdi, Drucker, Boote, & Korachi, 1999),
248 and the neutral loss of serine headgroup (88 units) in the negative ion mode (Murphy &
249 Axelsen, 2011). The predominant PS molecular species were O-20:0/20:5, 18:2/22:0 and
250 O-18:0/20:3 with their relative abundance of 32.72, 29.46, and 12.00%, respectively. PS
251 is a negatively charged PL and usually lies in the membrane leaflets towards the cytosol
252 (Vance & Steenbergen, 2005).

253 A characteristic peak $[M-171]^+$ of PGs was formed in the positive ion mode (Pulfer &
254 Murphy, 2003), and two characteristic peaks of m/z 171 $[C_3H_8O_6P]^-$ and m/z 227

255 $[\text{C}_6\text{H}_{12}\text{O}_7\text{P}]^-$ were generated in the negative ion mode (Yan, Li, Xu, & Zhou, 2010). The
256 confirmed PG molecular species and their quantities were P-16:0/20:0 (55.42%),
257 O-16:0/22:2 (38.99%) and 16:0/22:0 (5.59%). PG is also an ubiquitous lipid in the main
258 composition of membranes to perform specific functions. It appears to be essential for
259 photosynthesis and growth in plants (Frentzen, 2004) and may regulate the innate
260 immune in animal (Postle, Heeley, & Wilton, 2001).

261 The fragment ions of SMs were more abundant in the positive ion mode with the
262 presence of the quaternary nitrogen atom therein. SM (d18:1/24:1) was the predominant
263 molecular species with the relative abundance of 78.38% in the *P. crocea roe*, followed
264 by SM d18:2/24:1 21.62%. SMs could be a substitute of PC for being the structural
265 component of biomembranes and also comprise lipid rafts contributing to the regulation
266 of different signaling pathways (Doria, Cotrim, Macedo, Simoes, Domingues, Helguero,
267 et al., 2012).

268 More sufficient information could be obtained for PIs from the negative ion mode, as
269 PIs consist of substantial negative charged fragment ions (Ali, Zou, Lu, Abed, Yao, Tao,
270 et al., 2017). The characteristic fragment ion for PI is $[\text{C}_6\text{H}_{10}\text{O}_8\text{P}]^-$ at m/z 241. The two
271 identified PI molecular species were 12:0/22:1 and 13:0/22:0 with the relative abundance
272 of 29.58 and 70.42%, respectively. PIs have the ability to intervene in communications
273 among cell surface receptors and intracellular organelles (Doria, et al., 2012).

274 Furthermore, from the PLs molecular species detected in the roe of *P. crocea* above,
275 the main fatty acids attached to the sn-1 or sn-2 position of their phosphate group could
276 also be confirmed. The major SFAs were C 14:0, C 16:0 and C 18:0, and the predominant
277 PUFAs, especially the abundance of DHA and EPA in the PCs, PEs, PAs and PSs were
278 consistent with the results obtained from GC-MS analysis. On the other hand, this

279 method of UPLC-Q-TOF-MS gave more detailed results for rapid and sensitive
280 monitoring of PL molecular species than HPLC-ELSD analyzed above from
281 unfractionated lipid extracts.

282 It is possible that the composition of PLs species could be affected by the feeding
283 compositions, rearing conditions, catching season, etc, and the contents may vary a little
284 with different detection methods (Wood, Nute, Richardson, Whittington, Southwood,
285 Plastow, et al., 2004).

286 **4. Conclusion**

287 The *P. crocea* roe was shown to be rich in DHA (C22:6) and EPA (C20:5) as
288 analyzed by GC-MS, and contains large amounts of PC and PE which were determined
289 by HPLC-ELSD analysis. A more detailed information about PLs molecular species were
290 obtained using UPLC-Q-TOF-MS. 92 PLs were identified, including 49 PCs, 13 PEs, 10
291 PAs, 13 PSs, 3 PGs, 2 SMs, and 2 PIs in the *P. crocea* roe. DHA and EPA were verified
292 again as the predominant fatty acids in the *P. crocea* roe. Considering the large
293 production of *P. crocea* and the big size of its roe, the *P. crocea* roe is really worthy of
294 further exploitation for its marine PLs in the future.

295 **Conflict of interest**

296 The authors declare that they have no conflicts of interest concerning this article. There
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Figure captions

Fig. 1 HPLC-ELSD chromatogram of PLs class standards and PLs classes from *P. crocea* roe. (a) standard of PC class; (b) standard of PE class; (c) standard of SM class; (d) standard of LPC class; (e) standard of LPC and PI classes; (f) PLs classes of *P. crocea* roe.

Fig. 2 Total ion chromatogram and mass spectra of *P. crocea* roe. (a) total ion chromatogram in positive mode; (b) mass spectra in positive mode; (c) total ion chromatogram in negative mode; (d) mass spectra in negative mode.

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Tables:

Table 1. Fatty acids composition of total phospholipids from *P. crocea* roe by GC-MS (n=3).

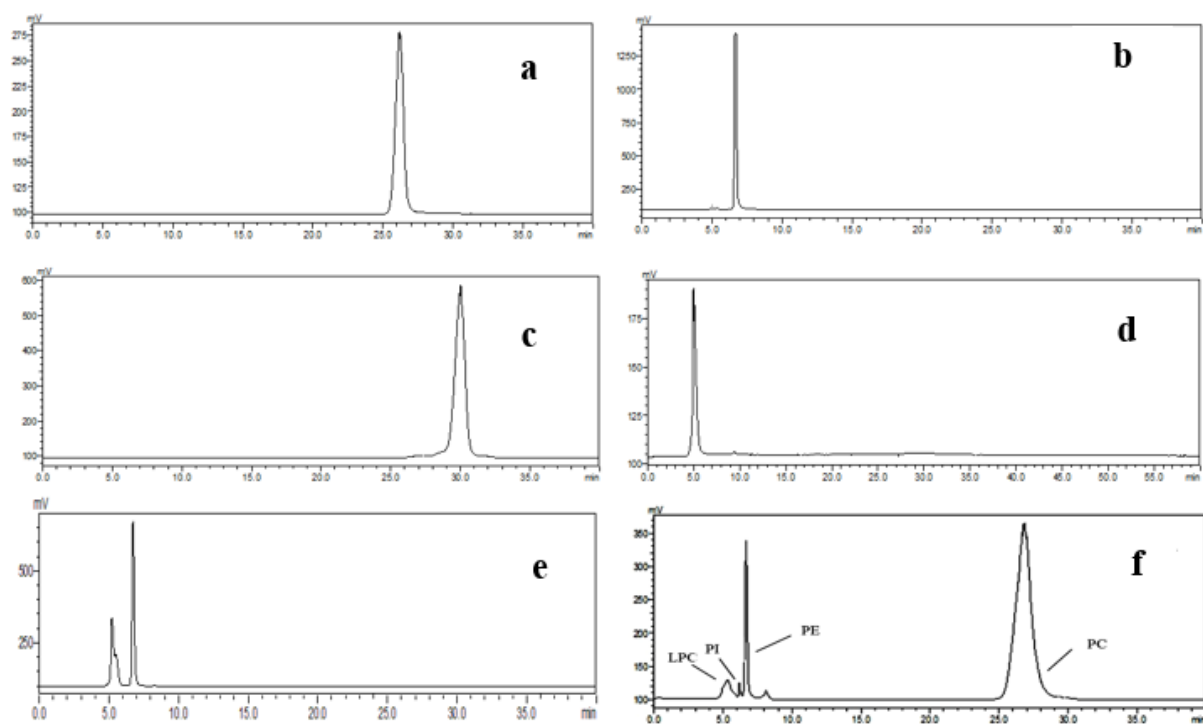
Table 2. Phospholipids composition of the roe of *P. crocea* by HPLC-ELSD (n=3).

Table 3. Phospholipids molecular species of the roe of *P. crocea* by UPLC-Q-TOF-MS (n=3).

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Figure graphics

Fig. 1



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Fig. 2

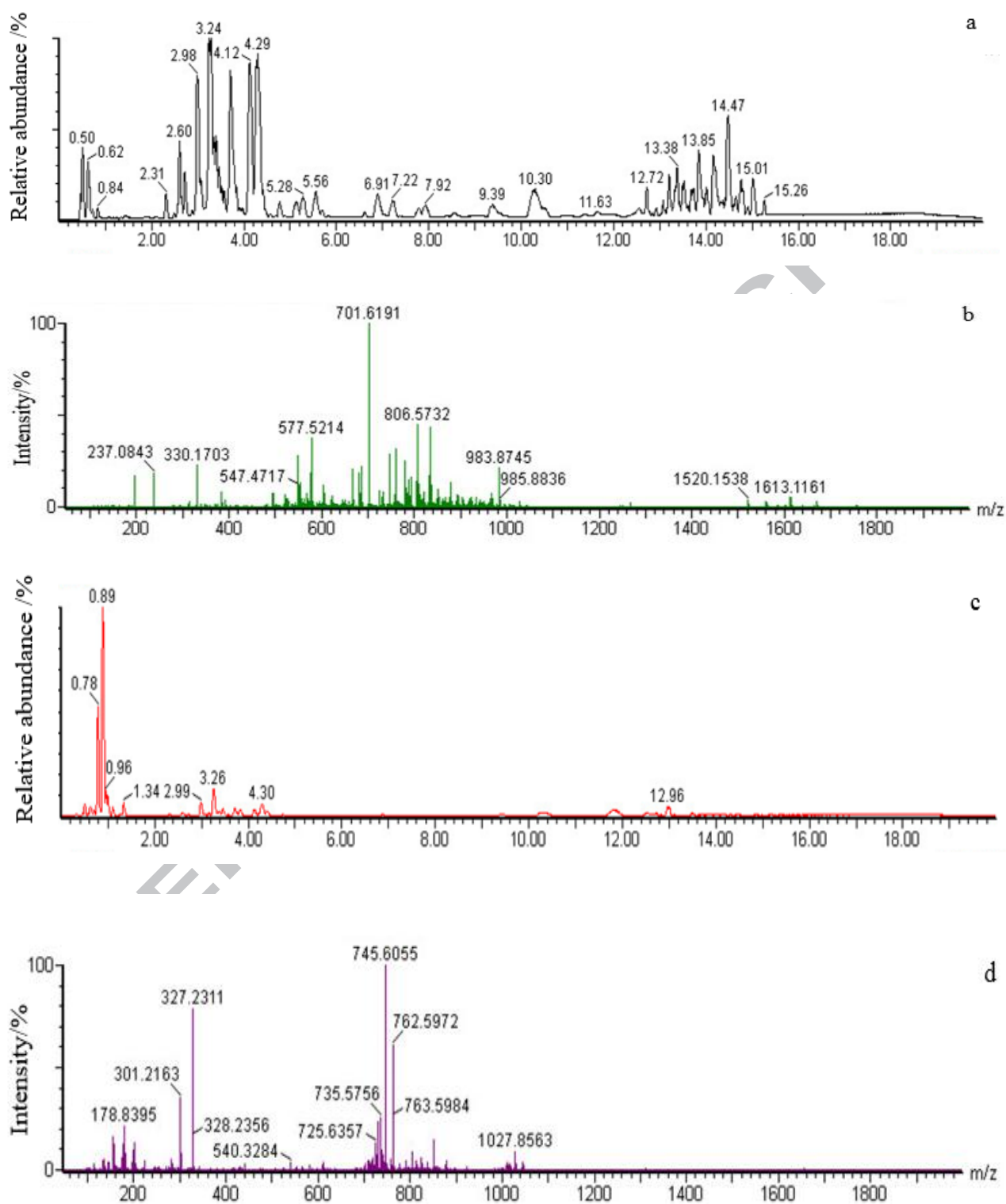


Table 1

Fatty acids	Content (%)
C 14:0	0.79±0.02
C 16:0	23.22±1.31
C 16:1	2.61±0.23
C 17:0	1.58±0.11
C 17:1	0.21±0.01
C 18:0	7.19±0.20
C 18:1	15.07±0.87
C 18:2	1.04±0.02
C 18:3	0.21±0.01
C 20:0	0.29±0.01
C 20:1	0.39±0.02
C 20:5	6.89±0.54
C 22:6	35.01±1.43
C 22:1	0.21±0.01
Σ EPA+DHA	41.90±1.97
Σ SFP	33.07±1.65
Σ MUFA	18.28±1.14
Σ PUFA	43.15±2.00

Table 2

Phospholipid	Content (%)
PC	76.36±0.62
LPC	12.30±0.55
PE	9.12±0.02
PI	1.09±0.01

Data are expressed as w/w of total phospholipids and represent means±standard deviation of three replicate determinations.

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Table 3

Class	m/z observed	Molecular formula	Ion (m/z)	Acyl chains (sn1/sn2)	Relative abundance (%)
PC	756.5546	C ₄₀ H ₈₀ NO ₈ P	32:0	10:0/22:0	0.48±0.01
	730.5388	C ₄₀ H ₇₆ NO ₈ P	32:2	14:1/18:1	0.44±0.31
	748.5860	C ₄₁ H ₈₂ NO ₈ P	33:0	10:0/23:0	0.20±0.00
	784.5865	C ₄₂ H ₈₄ NO ₈ P	34:0	10:0/24:0	0.32±0.02
	804.5750	C ₄₂ H ₈₂ NO ₈ P	34:1	15:0/19:1 & 16:0/18:1	19.43±0.47
	758.5706	C ₄₂ H ₈₀ NO ₈ P	34:2	12:0/22:2	2.52±0.05
	752.5230	C ₄₂ H ₇₄ NO ₈ P	34:5	14:0/20:5	0.18±0.02
	1554.1508	C ₄₃ H ₈₄ NO ₇ P	35:2	O-18:0/17:2	0.14±0.01
	814.6093	C ₄₄ H ₉₀ NO ₇ P	36:0	O-14:0/22:0	0.09±0.01
	796.6202	C ₄₄ H ₈₈ NO ₇ P	36:1	O-16:0/20:1 & 18:0/18:1	3.36±0.07
	786.6013	C ₄₄ H ₈₄ NO ₈ P	36:2	17:2/19:0	0.75±0.05
	768.5906	C ₄₄ H ₈₂ NO ₇ P	36:3	P-16:0/20:3	0.92±0.01
	790.5735	C ₄₄ H ₈₂ NO ₇ P	36:4	O-16:0/20:4	0.64±0.01
	764.5594	C ₄₄ H ₇₈ NO ₇ P	36:5	P-16:0/20:5 & 16:0/20:5	11.52±0.01
	778.5390	C ₄₄ H ₇₆ NO ₈ P	36:6	18:2/18:4	0.48±0.06
	794.5725	C ₄₅ H ₈₀ NO ₈ P	37:5	18:4/19:1	0.31±0.01
	818.6074	C ₄₆ H ₈₆ NO ₇ P	38:3	P-18:0/20:3	1.14±0.01
	794.6066	C ₄₆ H ₈₄ NO ₇ P	38:5	O-16:0/22:5 & 18:0/20:5	0.83±0.01
	792.5919	C ₄₆ H ₈₂ NO ₇ P	38:6	O-16:0/22:6 & P-16:0/22:6 & 16:0/22:6	13.33±0.05
	804.5548	C ₄₆ H ₇₈ NO ₈ P	38:7	22:6/16:1	2.41±0.16
	822.6038	C ₄₇ H ₈₄ NO ₈ P	39:5	20:4/19:1	0.14±0.01
	820.5883	C ₄₇ H ₈₂ NO ₈ P	39:6	17:0/22:6	1.59±0.04
	818.5704	C ₄₇ H ₈₀ NO ₈ P	39:7	22:6/17:1	1.49±0.07
	816.5551	C ₄₇ H ₇₈ NO ₈ P	39:8	22:6/17:2	0.05±0.01
	826.5391	C ₄₈ H ₇₆ NO ₈ P	40:10	18:4/22:6	0.15±0.02
	838.6332	C ₄₈ H ₈₈ NO ₈ P	40:4	18:0/22:4	0.10±0.01
	836.6177	C ₄₈ H ₈₆ NO ₈ P	40:5	18:0/22:5 & 18:3/22:2	1.73±0.07
	776.6157	C ₄₃ H ₇₅ O ₇ P	40:6	O-18:0/22:6 & 18:0/22:6	15.79±0.24
	832.5862	C ₄₈ H ₈₂ NO ₈ P	40:7	18:1/22:6	5.71±0.12
	848.6184	C ₄₉ H ₈₆ NO ₈ P	41:6	19:0/22:6	0.65±0.06
	854.5705	C ₅₀ H ₈₀ NO ₈ P	42:10	20:4/22:6	0.62±0.04
	852.5551	C ₅₀ H ₇₈ NO ₈ P	42:11	20:5/22:6	1.62±0.22
	864.6482	C ₅₀ H ₉₀ NO ₈ P	42:5	20:0/22:5	0.03±0.00
	862.6336	C ₅₀ H ₈₈ NO ₈ P	42:6	22:0/22:6	0.64±0.06
	860.6181	C ₅₀ H ₈₆ NO ₈ P	42:7	20:1/22:6	0.95±0.03
	880.5861	C ₅₀ H ₈₄ NO ₈ P	42:8	20:2/22:6	0.13±0.01
	878.5711	C ₅₂ H ₈₀ NO ₈ P	44:12	22:6/22:6	3.60±0.30
	916.6805	C ₅₂ H ₉₆ NO ₈ P	44:4	22:0/22:4	0.17±0.02
	890.6644	C ₅₂ H ₉₂ NO ₈ P	44:6	22:0/22:6 & 22:4/22:2	0.08±0.01
	888.6494	C ₅₂ H ₉₀ NO ₈ P	44:7	22:6/22:1	0.17±0.02
	PE	1546.1638	C ₄₂ H ₈₄ NO ₈ P	37:0	15:0/22:0
836.5237		C ₄₉ H ₇₄ NO ₈ P	44:12	22:6/22:6	0.71±0.05
822.6024		C ₄₇ H ₈₄ NO ₈ P	42:5	20:5/22:0	0.48±0.04

	814.6339	C ₄₄ H ₈₅ O ₈ P	41:1	19:0/22:1	0.12±0.02
	806.5709	C ₄₆ H ₈₀ NO ₈ P	41:6	22:6/19:0	60.84±0.89
	776.5655	C ₄₅ H ₇₈ NO ₇ P	40:6	P-18:0/22:6	0.94±0.07
	808.5870	C ₄₄ H ₈₄ NO ₈ P	39:2	17:2/22:0	16.33±0.53
	824.6182	C ₄₅ H ₈₈ NO ₈ P	40:1	18:0/22:1	0.11±0.02
	768.5553	C ₄₃ H ₇₈ NO ₈ P	38:4	18:3/20:1	0.29±0.02
	764.5237	C ₄₃ H ₇₄ NO ₈ P	38:6	P-16:0/22:6	5.53±0.28
	766.5438	C ₄₃ H ₇₆ NO ₈ P	38:5	P-18:1/20:4	1.00±0.05
	810.6025	C ₄₄ H ₈₆ NO ₈ P	39:1	17:0/22:1	4.96±0.28
	782.5705	C ₄₂ H ₈₂ NO ₈ P	37:1	15:0/22:1	8.47±0.11
PA	842.6641	C ₄₆ H ₈₉ O ₈ P	43:1	21:0/22:1	2.01±0.35
	816.6504	C ₄₄ H ₈₇ O ₈ P	41:0	19:0/22:0	10.97±0.77
	814.6339	C ₄₄ H ₈₅ O ₈ P	41:1	19:0/22:1	12.72±0.56
	776.6157	C ₄₃ H ₇₅ O ₇ P	40:6	O-18:0/22:6	4.76±0.12
	1556.0977	C ₄₄ H ₇₉ O ₈ P	41:4	20:4/21:0	8.18±0.29
	800.6169	C ₄₃ H ₈₃ O ₈ P	40:1	18:0/22:1	3.14±0.25
	802.6331	C ₄₃ H ₈₅ O ₈ P	40:0	18:0/22:0	3.58±0.33
	706.5387	C ₃₈ H ₇₃ O ₈ P	35:1	14:1/21:0	11.85±1.81
	772.5865	C ₄₁ H ₇₉ O ₈ P	38:1	16:0/22:1	18.73±0.50
	754.5389	C ₄₀ H ₇₃ O ₈ P	37:3	15:1/22:2	24.07±0.49
	PS	846.6031	C ₄₉ H ₈₈ NO ₁₀ P	43:4	22:4/21:0
818.6004		C ₄₈ H ₈₈ NO ₉ P	42:4	O-20:0/22:4	6.08±0.09
1694.1742		C ₄₆ H ₈₆ NO ₉ P	40:3	O-20:0/20:3	1.51±0.18
806.5701		C ₄₆ H ₈₂ NO ₉ P	40:5	O-20:0/20:5	32.72±0.47
808.5861		C ₄₆ H ₈₆ NO ₁₀ P	40:2	18:2/22:0	29.46±1.05
1600.1438		C ₄₄ H ₈₂ NO ₉ P	38:3	O-18:0/20:3	12.00±0.28
744.5544		C ₄₁ H ₈₀ NO ₉ P	35:1	O-16:0/19:1	2.68±0.10
858.5996		C ₄₅ H ₉₀ NO ₉ P	39:0	O-18:0/21:0	1.89±0.03
1544.0897		C ₄₂ H ₇₈ NO ₉ P	36:3	O-16:0/20:3	1.51±0.07
828.5546		C ₄₃ H ₈₄ NO ₉ P	37:1	O-18:0/19:1	2.48±0.31
1570.1002		C ₄₂ H ₈₀ NO ₉ P	36:2	O-16:0/20:2	1.05±0.07
PG	830.5698	C ₄₃ H ₈₆ NO ₉ P	37:0	O-16:0/21:0	6.10±0.44
	1598.1164	C ₄₃ H ₈₂ NO ₉ P	37:2	O-20:0/17:2	1.04±0.07
	1564.1147	C ₄₂ H ₈₃ O ₉ P	36:0	P-16:0/20:0	55.42±1.13
SM	1616.1414	C ₄₄ H ₈₅ O ₉ P	38:2	O-16:0/22:2	38.99±0.94
	1652.1690	C ₄₄ H ₈₇ O ₁₀ P	38:0	16:0/22:0	5.59±0.36
SM	811.6698	C ₄₇ H ₉₁ N ₂ O ₆ P	42:3	d18:2/24:1	21.62±0.26
	813.6856	C ₄₇ H ₉₃ N ₂ O ₆ P	42:2	d18:1/24:1	78.38±0.26
PI	854.5708	C ₄₃ H ₈₁ O ₁₃ P	34:1	12:0/22:1	29.58±1.40
	894.6021	C ₄₄ H ₈₅ O ₁₃ P	35:0	13:0/22:0	70.42±1.40

Note: The 'O-' prefix is used to indicate the presence of an alkyl ether substituent, whereas the 'P-' prefix is used for the 1Z-alkenyl ether (Plasmalogen) substituent, and 'd-' prefix is used to indicate that sphingene possesses two hydroxyl groups.

Highlights

- Docosahexaenoic acid (DHA, C22:6) and eicosapentaenoic acid (EPA, C20:5) were the major polyunsaturated fatty acids from phospholipids (PLs) of *Pseudosciaena crocea* roe.
- Both HPLC-ELSD and UPLC-Q-TOF-MS were used to identify PLs classes and molecular species of phospholipids from *Pseudosciaena crocea* roe, respectively.
- PC and PE were detected as the predominant PLs classes in *P. crocea* roe.

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