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

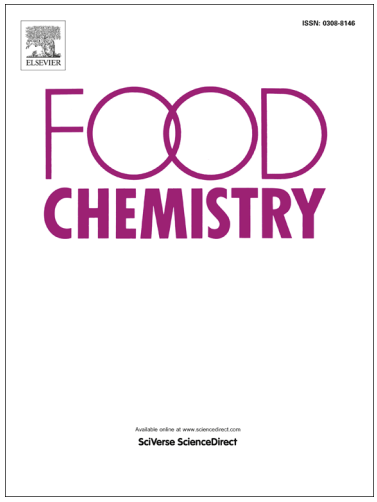
Liang, Peng; Li, Ruifen; Sun, He; Zhang, Min; Cheng, Wenjian; Chen, Lijiao; Cheng, Xinwei; Akoh, Casimir C.

Published in:
Food Chemistry

Link to article, DOI:
10.1016/j.foodchem.2017.11.108

Publication date:
2018

Document Version
Peer reviewed version



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

Peng Liang, Ruifen Li, He Sun, Min Zhang, Wenjian Cheng, Lijiao Chen, Xinwei Cheng, Casimir C. Akoh

PII: S0308-8146(17)31927-1
DOI: https://doi.org/10.1016/j.foodchem.2017.11.108
Reference: FOCH 22085

To appear in: *Food Chemistry*

Received Date: 12 July 2017
Revised Date: 28 November 2017
Accepted Date: 29 November 2017

Please cite this article as: Liang, P., Li, R., Sun, H., Zhang, M., Cheng, W., Chen, L., Cheng, X., Akoh, C.C., Phospholipids composition and molecular species of large yellow croaker (*Pseudosciaena crocea*) roe, *Food Chemistry* (2017), doi: https://doi.org/10.1016/j.foodchem.2017.11.108

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

Peng Liang[a], Ruifen Li[b], He Sun[a], Min Zhang[a], Wenjian Cheng[a], Lijiao Chen[a*], Xinwei Cheng[a], Casimir C. Akoh[c]

[a]College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, 350002, PR, China

[b]Division of Food Technology, National Food Institute, Technical University of Denmark, Kgs. Lyngby, Denmark

[c]Department of Food Science & Technology, University of Georgia, 30602 Athens, GA, USA

All authors contributed equally.

[*]Correspondence should be addressed to Chen Lijiao (chenlijiao606@126.com), College of Food Science, Fujian Agriculture and Forestry University, No. 15, Shangxiadian Road, Cangshan District, Fuzhou City, Fujian Province 350002, PR, China.

Tel/fax: +86 591 83789348

Abstract

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

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

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. Materials and Reagents

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

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

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

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

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

2.3. Fatty acids analysis by GC-MS

2.3.1. Sample preparation

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

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

2.3.2. GC-MS parameter

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

2.4. PLs classes analysis by HPLC-ELSD

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

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

The calibration curve was obtained by injecting 10 μL of serially diluted solutions of 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 different concentrations. All samples were analyzed in triplicate. The calibration curves for each compound were calculated from the area values with known amounts of standards.

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

2.5.1. Sample preparation

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

2.5.2. UPLC parameter

UPLC was equipped with C_{18}CSH column (1 mm × 50 mm, 1.7 μm; Waters Ltd., Elstree, U.K.). The mass spectrometry method of the Xevo G2-S Q-TOF was implemented in order to improve isotopic distribution and mass accuracy and reduce high ion intensities. Two microliters of the samples were injected onto C_{18}CSH column at 55 °C. The mobile phase flow rate was set as 400 μL/min. The mobile phase were A, Acetonitrile (ACN)/Water (60/40%), including 10 mM ammonium formate and 0.1% formic acid; B, IPA/ACN (90/10%), including 10 mM ammonium formate and 0.1% formic acid. Measurements were analyzed in triplicate.

2.5.3. Q-TOF-MS parameter

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

2.5.4. MS Data Preprocessing

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

2.6. Statistics analysis

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

3. Results and discussion

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

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

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

3.2. Analysis of PLs classes using HPLC-ELSD

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

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

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

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

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

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

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

Some characteristic fragmentation ions were confirmed based on comparison with the data of PLs standards. The distinctive phosphocholine headgroup of PC molecules was generated at *m/z* 184 where the product ion $[C_5H_{15}O_4NP]^+$ was yielded in the positive mode (Shen, Wang, Gong, Guo, Dong, & Cheung, 2012; Yan, Li, Xu, & Zhou, 2010), while the unusual tetravalent nitrogen led to the formation of a fragment ion $[M\text{-}CH3]^-$, and then the precursor ion of PC formed the fragment ion $[C_4H_{11}O_4NP]^-$ (m/z 168) in the negative ion mode (Harrison & Murphy, 1995; Yan, Li, Xu, & Zhou, 2010). According to both the fragmentation pattern and molecular weights, a total of 49 PCs were detected. The main PC molecular species were 15:0/19:1&16:0/18:1, O-18:0/22:6&18:0/22:6, 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 of which account for 19.43, 11.52, 13.33 and 15.79% respectively. PC has been regarded

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

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

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

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

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

255 $[C_6H_{12}O_7P]^-$ 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 $[C_6H_{10}O_8P]^-$ 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

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

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

4. Conclusion

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

Conflict of interest

The authors declare that they have no conflicts of interest concerning this article. There was no financial support except those mentioned in the acknowledgments.

Author information

Corresponding Author

*E–mail: chenlijiao606@126.com, Phone: +86 591 83789348. Fax: +86 591 83789348.

ORCID

Lijiao Chen: 0000-0002-1892-4119

Peng Liang: 0000-0002-8974-2398

Acknowledgments

The authors thank the Natural Science Foundation of Fujian Province (2016J0116), and the support from Chinese Scholarship Council for Studying Abroad. The project was also supported by Fujian Agriculture and Forestry University (612014042).

Reference

Ali, A. H., Zou, X., Lu, J., Abed, S. M., Yao, Y., Tao, G., Jin, Q., & Wang, X. (2017). Identification of phospholipids classes and molecular species in different types of egg yolk by using UPLC-Q-TOF-MS. *Food Chemistry, 221*, 58-66.

Bledsoe, G. E., Bledsoe, C. D., & Rasco, B. (2003). Caviars and fish roe products. *Critical Reviews in Food Science and Nutrition, 43*(3), 317-356.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*(8), 911–917.

Brouwers, J. F. H. M., Vernooij, E. A. A. M., Tielens, A. G. M., & Van Golde, L. M. G. (1999). Rapid separation and identification of phosphatidylethanolamine molecular species. *The Journal of Lipid Research, 40*(1), 164-169.

Burri, L., Hoem, N., Banni, S., & Berge, K. (2012). Marine omega-3 phospholipids: metabolism and biological activities. *International Journal of Molecular Sciences, 13*(11), 15401-15419.

Cejas, R. J., Almansa, E., Villamandos, E. J., Badı´a, P., Bolan˜os, A., & Lorenzob, A. (2003). Lipid and fatty acid composition of ovaries from wild fish and ovaries and eggs from captive fish of white sea bream (*Diplodus sargus*). *Aquaculture, 216*, 299-313.

Dasgupta, S., & Bhattacharyya, D. k. (2007). Dietary Effect of Eicosapentaenoic Acid (EPA) Containing Soyphospholipid. *Japan Oil Chemists' Society, 56*(11), 563-568.

Donato, P., Cacciola, F., Cichello, F., Russo, M., Dugo, P., & Mondello, L. (2011). Determination of phospholipids in milk samples by means of hydrophilic interaction liquid chromatography coupled to evaporative light scattering and mass spectrometry detection. *Journal of Chromatography A, 1218*(37), 6476-6482.

Doria, M. L., Cotrim, Z., Macedo, B., Simoes, C., Domingues, P., Helguero, L., & Domingues, M. R. (2012). Lipidomic approach to identify patterns in phospholipid profiles and define class differences in mammary epithelial and breast cancer cells. *Breast Cancer Research*

and Treatment, 133(2), 635-648.

Fong, B., Ma, L., & Norris, C. (2013). Analysis of phospholipids in infant formulas using high performance liquid chromatography-tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 61*(4), 858-865.

Frentzen, M. (2004). Phosphatidylglycerol and sulfoquinovosyldiacylglycerol: anionic membrane lipids and phosphate regulation. *Current Opinion in Plant Biology, 7*(3), 270-276.

Harrison, K. A., & Murphy, R. C. (1995). Negative electrospray ionization of glycerophosphocholine lipids: formation of $[M–15]^-$ ions occurs via collisional decomposition of adduct anions. *Journal of Mass Spectrometry, 30*(12), 1772-1773.

Hayashi, H., Tanaka, Y., Hibino, H., Umeda, Y., Kawamitsu, H., Fujimoto, H., & Amakawa, T. (1999). Beneficial effect of salmon roe phosphatidylcholine in chronic liver disease. *Current Medical Research and Opinion, 15*(3), 177-184.

Hui, G., Liu, W., Feng, H., Li, J., & Gao, Y. (2016). Effects of chitosan combined with nisin treatment on storage quality of large yellow croaker (*Pseudosciaena crocea*). *Food Chemistry, 203*, 276-282.

Knittelfelder, O. L., Weberhofer, B. P., Eichmann, T. O., Kohlwein, S. D., & Rechberger, G. N. (2014). A versatile ultra-high performance LC-MS method for lipid profiling. *Journal of Chromatography B Analytical Technologies in the Biomedical and Life Sciences, 951-952*, 119-128.

Lin, L., Lin, H., Zhang, M., Ni, B., Yin, X., Qu, C., & Ni, J. (2015). A novel method to analyze hepatotoxic components in Polygonum multiflorum using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry. *Journal of Hazardous Materials, 299*, 249-259.

Liu, Y. Y., Chen, X. F., Hu, J. W., Chen, Z. W., Zhang, L. J., Cao, M. J., & Liu, G. M. (2016). Purification and Characterization of Protamine, the Allergen from the Milt of Large

Yellow Croaker (*Pseudosciaena crocea)*, and Its Components. *Journal of Agricultural and Food Chemistry, 64*(9), 1999-2011.

Murphy, R. C., & Axelsen, P. H. (2011). Mass spectrometric analysis of long-chain lipids. *Mass Spectrometry Reviews, 30*(4), 579-599.

Postle, A. D., Heeley, E. L., & Wilton, D. C. (2001). A comparison of the molecular species compositions of mammalian lung surfactant phospholipids. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 129*(1), 65-73.

Pulfer, M., & Murphy, R. C. (2003). Electrospray mass spectrometry of phospholipids. *Mass Spectrometry Reviews, 22*(5), 332-364.

Rodriguez-Alcala, L. M., & Fontecha, J. (2010). Major lipid classes separation of buttermilk, and cows, goats and ewes milk by high performance liquid chromatography with an evaporative light scattering detector focused on the phospholipid fraction. *Journal of Chromatography A, 1217*(18), 3063-3066.

Rosa, A., Scano, P., Atzeri, A., Deiana, M., Mereu, S., & Dessi, M. A. (2012). Effect of storage conditions on lipid components and color of Mugil cephalus processed roes. *Journal of Food Science, 77*(1), C107-114.

Sarafian, M. H., Gaudin, M., Lewis, M. R., Martin, F. P., Holmes, E., Nicholson, J. K., & Dumas, M. E. (2014). Objective set of criteria for optimization of sample preparation procedures for ultra-high throughput untargeted blood plasma lipid profiling by ultra performance liquid chromatography-mass spectrometry. *Analytical Chemistry, 86*(12), 5766-5774.

Shen, Q., Wang, Y., Gong, L., Guo, R., Dong, W., & Cheung, H. Y. (2012). Shotgun lipidomics strategy for fast analysis of phospholipids in fisheries waste and its potential in species differentiation. *Journal of Agricultural and Food Chemistry, 60*(37), 9384-9393.

Shirai, N., Higuchi, T., & Suzuki, H. (2006). Analysis of lipid classes and the fatty acid composition of the salted fish roe food products, Ikura, Tarako, Tobiko and Kazunoko. *Food Chemistry, 94*(1), 61-67.

386 Shirouchi, B., Nagao, K., Inoue, N., Ohkubo, T., Hibino, H., & Yanagita, T. (2007). Effect of
387 Dietary Omega 3 phosphatidylcholine on obesity-related disorders in obese Otsuka
388 Long-Evans Tokushima fatty rats. *Journal of Agricultural and Food Chemistry, 55*,
389 7170-7176.
390 Sterin, M., Cohen, S. J., & Ringel, I. (2004). Hormone sensitivity is reflected in the phospholipid
391 profiles of breast cancer cell lines. *Breast Cancer Research and Treatment, 87*, 1-11.
392 Suzumura, M. (2005). Phospholipids in marine environments: a review. *Talanta, 66*(2), 422-434.
393 Theaker, E. D., Abdi, M., Drucker, D. B., Boote, V., & Korachi, M. (1999). Fast atom
394 bombardment mass spectrometry of phospholipids of clinically important Candida
395 species. *Journal of Dental Research, 78*(5), 1069-1069.
396 Vance, J. E., & Steenbergen, R. (2005). Metabolism and functions of phosphatidylserine.
397 *Progress in Lipid Research, 44*(4), 207-234.
398 Wang, Q., Xue, C., Li, Z., & Xu, J. (2008). Analysis of DHA-rich phospholipids from egg of
399 squid Sthenoteuthis oualaniensis. *Journal of Food Composition and Analysis, 21*(4),
400 356-359.
401 Wang, Q., Xue, C.-H., & Li, Z.-J. (2008). Phosphatidylcholine levels and their fatty acid
402 compositions in squid egg: A comparison study with pollack roe and sturgeon caviar.
403 *Journal of Food Lipids, 15*(2), 222-230.
404 Wang, Y., & Zhang, H. (2011). Tracking phospholipid profiling of muscle from
405 Ctennopharyngodon idellus during storage by shotgun lipidomics. *Journal of Agricultural*
406 *and Food Chemistry, 59*(21), 11635-11642.
407 Wood, J. D., Nute, G. R., Richardson, R. I., Whittington, F. M., Southwood, O., Plastow, G.,
408 Mansbridge, R., da Costa, N., & Chang, K. C. (2004). Effects of breed, diet and muscle
409 on fat deposition and eating quality in pigs. *Meat Science, 67*(4), 651-667.
410 Yan, X., Li, H., Xu, J., & Zhou, C. (2010). Analysis of phospholipids in microalga Nitzschia
411 closterium by UPLC-Q-TOF-MS. *Chinese Journal of Oceanology and Limnology, 28*(1),

106-112.

Yuan, X. C., & Zhao, W. W. (2016). China Fishery Statistical Yearbook. Beijing, China.

Zhao, J., Li, J., Wang, J., & Lv, W. (2012). Applying different methods to evaluate the freshness of large yellow croacker (*Pseudosciaena crocea*) fillets during chilled storage. *Journal of Agricultural and Food Chemistry, 60*(45), 11387-11394.

Zhao, Y. Y., Wu, S. P., Liu, S., Zhang, Y., & Lin, R. C. (2014). Ultra-performance liquid chromatography-mass spectrometry as a sensitive and powerful technology in lipidomic applications. *Chemico-Biological Interactions, 220*, 181-192.

Zhang, J., Yang, W., Li, S., Yao, S., Qi, P., Yang, Z., Feng, Z., Hou, J., Cai, L., Yang, M., Wu, W., & Guo, D. A. (2016). An intelligentized strategy for endogenous small molecules characterization and quality evaluation of earthworm from two geographic origins by ultra-high performance HILIC/QTOF MS(E) and Progenesis QI. *Analytical and Bioanalytical Chemistry, 408*(14), 3881-3890.

Zhou, Q., Gao, B., Zhang, X., Xu, Y., Shi, H., & Yu, L. L. (2014). Chemical profiling of triacylglycerols and diacylglycerols in cow milk fat by ultra-performance convergence chromatography combined with a quadrupole time-of-flight mass spectrometry. *Food Chemistry, 143*, 199-204.

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.

Tables:

Figure graphics

Fig. 1

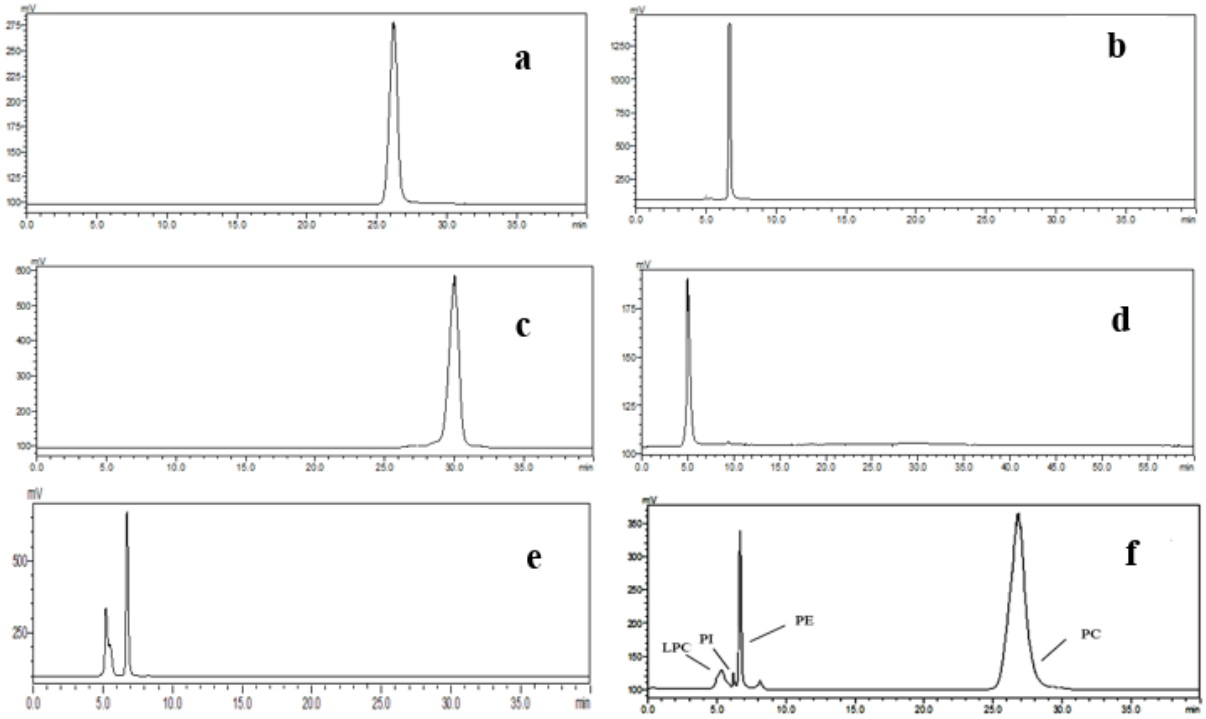


Fig. 2

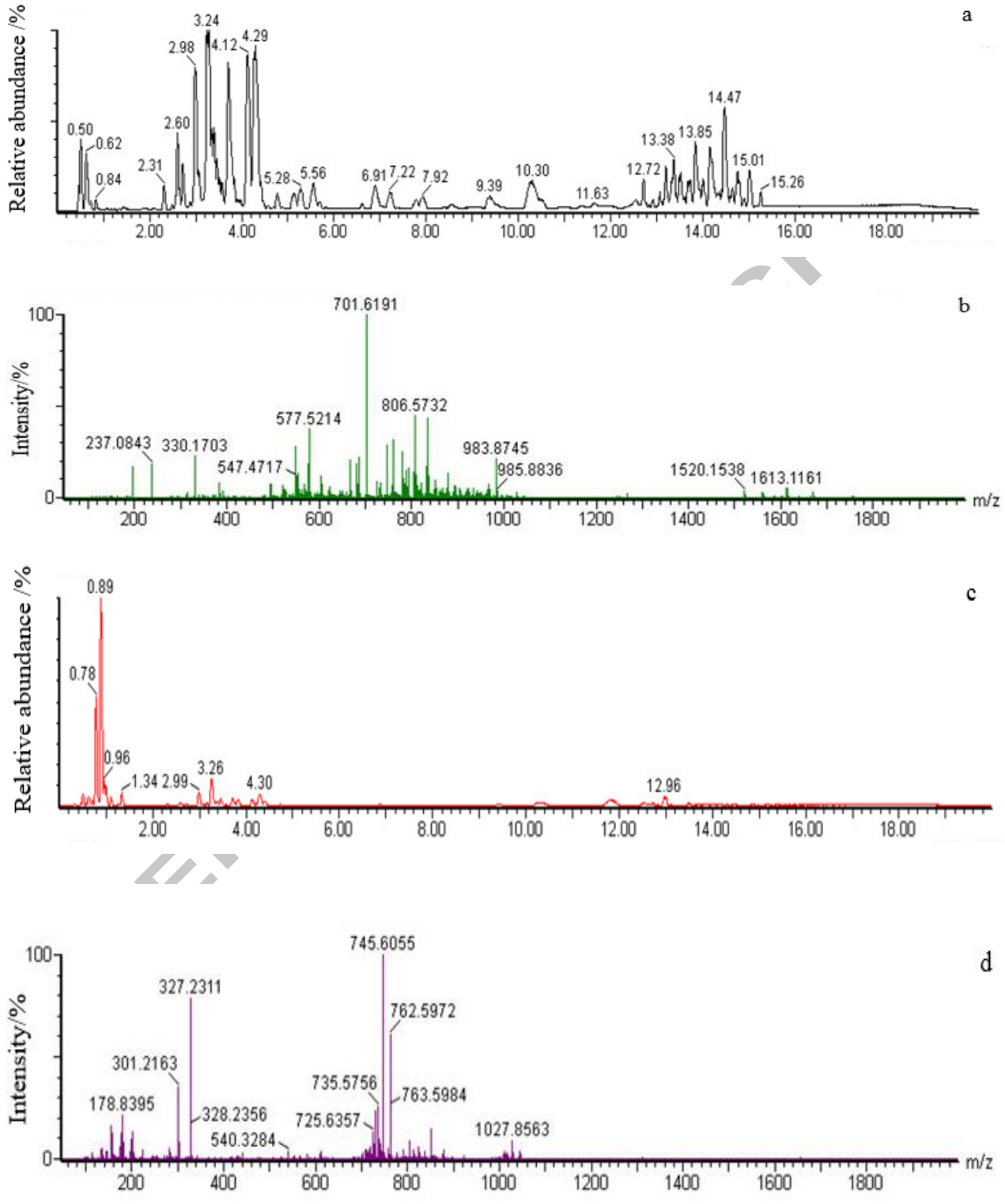

a
Relative abundance /%
3.24
2.98
4.12
4.29
0.50
0.62
2.60
2.31
0.84
5.28
5.56
6.91
7.22
7.92
9.39
10.30
11.63
12.72
13.38
13.85
14.47
15.01
15.26
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
b
Intensity/%
701.6191
806.5732
577.5214
237.0843
330.1703
547.4717
983.8745
985.8836
1520.1538
1613.1161
m/z
200
400
600
800
1000
1200
1400
1600
1800
c
Relative abundance /%
0.89
0.78
0.96
1.34
2.99
3.26
4.30
12.96
d
Intensity/%
745.6055
327.2311
762.5972
301.2163
735.5756
763.5984
178.8395
328.2356
725.6357
1027.8563
540.3284

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
∑EPA+DHA	41.90±1.97
∑SFP	33.07±1.65
∑MUFA	18.28±1.14
∑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.

Table 3

Class	m/z observed	Molecular formula	Ion (*m/z*)	Acyl chains (*sn*1/*sn*2)	Relative abundance (%)
PC	756.5546	$C_{40}H_{80}NO_8P$	32:0	10:0/22:0	0.48±0.01
	730.5388	$C_{40}H_{76}NO_8P$	32:2	14:1/18:1	0.44±0.31
	748.5860	$C_{41}H_{82}NO_8P$	33:0	10:0/23:0	0.20±0.00
	784.5865	$C_{42}H_{84}NO_8P$	34:0	10:0/24:0	0.32±0.02
	804.5750	$C_{42}H_{82}NO_8P$	34:1	15:0/19:1 & 16:0/18:1	19.43±0.47
	758.5706	$C_{42}H_{80}NO_8P$	34:2	12:0/22:2	2.52±0.05
	752.5230	$C_{42}H_{74}NO_8P$	34:5	14:0/20:5	0.18±0.02
	1554.1508	$C_{43}H_{84}NO_7P$	35:2	O-18:0/17:2	0.14±0.01
	814.6093	$C_{44}H_{90}NO_7P$	36:0	O-14:0/22:0	0.09±0.01
	796.6202	$C_{44}H_{88}NO_7P$	36:1	O-16:0/20:1 & 18:0/18:1	3.36±0.07
	786.6013	$C_{44}H_{84}NO_8P$	36:2	17:2/19:0	0.75±0.05
	768.5906	$C_{44}H_{82}NO_7P$	36:3	P-16:0/20:3	0.92±0.01
	790.5735	$C_{44}H_{82}NO_7P$	36:4	O-16:0/20:4	0.64±0.01
	764.5594	$C_{44}H_{78}NO_7P$	36:5	P-16:0/20:5 & 16:0/20:5	11.52±0.01
	778.5390	$C_{44}H_{76}NO_8P$	36:6	18:2/18:4	0.48±0.06
	794.5725	$C_{45}H_{80}NO_8P$	37:5	18:4/19:1	0.31±0.01
	818.6074	$C_{46}H_{86}NO_7P$	38:3	P-18:0/20:3	1.14±0.01
	794.6066	$C_{46}H_{84}NO_7P$	38:5	O-16:0/22:5 & 18:0/20:5	0.83±0.01
	792.5919	$C_{46}H_{82}NO_7P$	38:6	O-16:0/22:6 & P-16:0/22:6 & 16:0/22:6	13.33±0.05
	804.5548	$C_{46}H_{78}NO_8P$	38:7	22:6/16:1	2.41±0.16
	822.6038	$C_{47}H_{84}NO_8P$	39:5	20:4/19:1	0.14±0.01
	820.5883	$C_{47}H_{82}NO_8P$	39:6	17:0/22:6	1.59±0.04
	818.5704	$C_{47}H_{80}NO_8P$	39:7	22:6/17:1	1.49±0.07
	816.5551	$C_{47}H_{78}NO_8P$	39:8	22:6/17:2	0.05±0.01
	826.5391	$C_{48}H_{76}NO_8P$	40:10	18:4/22:6	0.15±0.02
	838.6332	$C_{48}H_{88}NO_8P$	40:4	18:0/22:4	0.10±0.01
	836.6177	$C_{48}H_{86}NO_8P$	40:5	18:0/22:5 & 18:3/22:2	1.73±0.07
	776.6157	$C_{43}H_{75}O_7P$	40:6	O-18:0/22:6 & 18:0/22:6	15.79±0.24
	832.5862	$C_{48}H_{82}NO_8P$	40:7	18:1/22:6	5.71±0.12
	848.6184	$C_{49}H_{86}NO_8P$	41:6	19:0/22:6	0.65±0.06
	854.5705	$C_{50}H_{80}NO_8P$	42:10	20:4/22:6	0.62±0.04
	852.5551	$C_{50}H_{78}NO_8P$	42:11	20:5/22:6	1.62±0.22
	864.6482	$C_{50}H_{90}NO_8P$	42:5	20:0/22:5	0.03±0.00
	862.6336	$C_{50}H_{88}NO_8P$	42:6	22:0/22:6	0.64±0.06
	860.6181	$C_{50}H_{86}NO_8P$	42:7	20:1/22:6	0.95±0.03
	880.5861	$C_{50}H_{84}NO_8P$	42:8	20:2/22:6	0.13±0.01
	878.5711	$C_{52}H_{80}NO_8P$	44:12	22:6/22:6	3.60±0.30
	916.6805	$C_{52}H_{96}NO_8P$	44:4	22:0/22:4	0.17±0.02
	890.6644	$C_{52}H_{92}NO_8P$	44:6	22:0/22:6 & 22:4/22:2	0.08±0.01
	888.6494	$C_{52}H_{90}NO_8P$	44:7	22:6/22:1	0.17±0.02
PE	1546.1638	$C_{42}H_{84}NO_8P$	37:0	15:0/22:0	0.21±0.03
	836.5237	$C_{49}H_{74}NO_8P$	44:12	22:6/22:6	0.71±0.05
	822.6024	$C_{47}H_{84}NO_8P$	42:5	20:5/22:0	0.48±0.04

	814.6339	$C_{44}H_{85}O_8P$	41:1	19:0/22:1	0.12±0.02
	806.5709	$C_{46}H_{80}NO_8P$	41:6	22:6/19:0	60.84±0.89
	776.5655	$C_{45}H_{78}NO_7P$	40:6	P-18:0/22:6	0.94±0.07
	808.5870	$C_{44}H_{84}NO_8P$	39:2	17:2/22:0	16.33±0.53
	824.6182	$C_{45}H_{88}NO_8P$	40:1	18:0/22:1	0.11±0.02
	768.5553	$C_{43}H_{78}NO_8P$	38:4	18:3/20:1	0.29±0.02
	764.5237	$C_{43}H_{74}NO_8P$	38:6	P-16:0/22:6	5.53±0.28
	766.5438	$C_{43}H_{76}NO_8P$	38:5	P-18:1/20:4	1.00±0.05
	810.6025	$C_{44}H_{86}NO_8P$	39:1	17:0/22:1	4.96±0.28
	782.5705	$C_{42}H_{82}NO_8P$	37:1	15:0/22:1	8.47±0.11
PA	842.6641	$C_{46}H_{89}O_8P$	43:1	21:0/22:1	2.01±0.35
	816.6504	$C_{44}H_{87}O_8P$	41:0	19:0/22:0	10.97±0.77
	814.6339	$C_{44}H_{85}O_8P$	41:1	19:0/22:1	12.72±0.56
	776.6157	$C_{43}H_{75}O_7P$	40:6	O-18:0/22:6	4.76±0.12
	1556.0977	$C_{44}H_{79}O_8P$	41:4	20:4/21:0	8.18±0.29
	800.6169	$C_{43}H_{83}O_8P$	40:1	18:0/22:1	3.14±0.25
	802.6331	$C_{43}H_{85}O_8P$	40:0	18:0/22:0	3.58±0.33
	706.5387	$C_{38}H_{73}O_8P$	35:1	14:1/21:0	11.85±1.81
	772.5865	$C_{41}H_{79}O_8P$	38:1	16:0/22:1	18.73±0.50
	754.5389	$C_{40}H_{73}O_8P$	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	1.47±0.04
	818.6004	$C_{48}H_{88}NO_9P$	42:4	O-20:0/22:4	6.08±0.09
	1694.1742	$C_{46}H_{86}NO_9P$	40:3	O-20:0/20:3	1.51±0,18
	806.5701	$C_{46}H_{82}NO_9P$	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	$C_{44}H_{82}NO_9P$	38:3	O-18:0/20:3	12.00±0.28
	744.5544	$C_{41}H_{80}NO_9P$	35:1	O-16:0/19:1	2.68±0.10
	858.5996	$C_{45}H_{90}NO_9P$	39:0	O-18:0/21:0	1.89±0.03
	1544.0897	$C_{42}H_{78}NO_9P$	36:3	O-16:0/20:3	1.51±0.07
	828.5546	$C_{43}H_{84}NO_9P$	37:1	O-18:0/19:1	2.48±0.31
	1570.1002	$C_{42}H_{80}NO_9P$	36:2	O-16:0/20:2	1.05±0.07
	830.5698	$C_{43}H_{86}NO_9P$	37:0	O-16:0/21:0	6.10±0.44
	1598.1164	$C_{43}H_{82}NO_9P$	37:2	O-20:0/17:2	1.04±0.07
PG	1564.1147	$C_{42}H_{83}O_9P$	36:0	P-16:0/20:0	55.42±1.13
	1616.1414	$C_{44}H_{85}O_9P$	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	$C_{47}H_{91}N_2O_6P$	42:3	d18:2/24:1	21.62±0.26
	813.6856	$C_{47}H_{93}N_2O_6P$	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 sphingenine 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.