Analysis and Identification of Golden pompano (Trachinotus blochii) Head Phospholipid Molecular Species by Liquid Chromatography-Mass Spectrometry

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Abstract: In this study, we first isolate phospholipid (PL) from Golden pompano head (GPH), and elucidate its structure. Gas chromatography (GC) was used to assess the GPH-PL fatty acid composition, Fourier transform infrared spectroscopy (FTIR) and ultraviolet absorption spectrometry (UV) were used for the qualitative analysis of GPH-PL, and LC-MS analysis was used to determine the major PL species. The results show that the contents of the various molecular species of GPH-PL were generally in the order phosphatidylcholine (PC) > sphingomyelin (SM) > lysophosphatidylcholine (LPC) > phosphatidylethanolamine (PE). The main molecular PC species are 16:0/18:2, 13:0/23:2, 27:2/9:0, 16:0/18:1, 12:0/22:2, 18:0/18:1, 18:0/24:1, and 18:1/24:0. The major SM species are 16:1/16:0, 16:0/18:1, 16:0/18:2, 16:0/26:2, and 18:1/24:1. The major LPC species are 18:1 and 16:0. The major PE species are 18:0/18:1 and 16:0/22:6. The total eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents in the GPH-PLs were 18.39%, and the content of DHA in the PL fraction was 16.47%. These results suggest that PLs from GPH is rich in polyunsaturated fatty acids (PUFA), which have good activity in anti-inflammation, anti-tumor, anti-osteoporosis and other aspects, and have important development prospects in the future.

Key words: Golden pompano head, phospholipids, Fourier transform infrared spectroscopy, ultraviolet absorption spectrometry, fatty acid composition, liquid chromatography-mass spectrometry

1 Introduction

Phospholipids (PLs), a class of lipids that contain phosphorus, are polar lipids and can be roughly divided into two types: glycerophosphatides and sphingomyelins (SMs). PLs are amphiphilic molecules with a hydrophilic head and hydrophobic tail. The hydrophilic head is composed of substituents such as amines, bases or alcohols bound to a phosphate, and the hydrophobic tail is composed of a fatty acid chain. Therefore, it is both hydrophilic and hydrophobic. The hydrophilic head and hydrophobic tail are close to each other, and together with proteins and cholesterol, they constitute the lipid bilayer structure. This lipid bilayer structure is commonly referred to as the cell membrane. PLs are classified based on their linker, and the major PLs include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and SM. PLs are not only important in maintaining normal physiological functions, such as normal cell membrane fluidity, but also play a role in normal cell apoptosis and signal transmission. PLs can improve immune function by activating macrophages and enhancing the body’s ability to resist diseases. At the same time, the biological activity and function of PLs have attracted extensive attention. PLs are very important in human biology, and the regular consumption of PL-rich substances can promote the metabolism of fat and improve brain function as well as assist in the prevention and treatment of cardiovascular, cerebrovascular, hepatic, and neurological diseases; diabetes; Alzheimer’s disease and other diseases. Studies using milk SM, synthetic SM and synthetic dihydrosphingomyelin have demonstrated that SM protects CF1 mice from 1,2-dimethylhydrazine-induced colon cancer. This result suggests that the mechanism by which dietary sphingolipids reduce colon carcinogenesis may involve the metabolism of complex sphingolipids, such as SM, to the bioactive sphingoid bases, which are important in signal transduction and cell regulation through the inhibition of cell growth, the induction of cell differentiation and the stimulation of apoptosis. Therefore, in the processing of Golden pompano, PLs could be extracted from the fish head, which is gener-
ated as byproduct of processing, and could be further separated and purified for use in medicine and healthcare to produce high value-added products. Chen and Chen[10] used TLC combined with spectrophotometry to analyze and determine the PLs in the roe of yellow crocod; the relative contents of PC, PE, PI, SM and lysophosphatidylcholine (LPC) were 69.38%, 4.14%, 8.52%, 7.24% and 10.72%, respectively. Rao et al.[9] studied the PL composition of the freshwater fish katla and maricarra dace, in which PC accounts for approximately 55%-60% of the PLs, PE accounts for approximately 37%-39%, and PI accounts for approximately 3%-6%. The types and contents of PLs in animals are related to the species, cell types, cell locations and nutrient intake. PLs obtained from marine organisms contain more polyunsaturated fats than those from terrestrial organisms; the contents of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are notably higher in marine organisms. Fatty acids are important metabolic energy-storage compounds for fish growth and development. Fatty acids are also important endogenous nutrients in fish. In most fish, the most abundant saturated fatty acids is usually C16:0, and some studies have suggest that C16:0 and C18:1 fatty acids are the main energy sources for fish metabolism. Yang et al.[10] measured the total lipid (TL), PL and fatty acid contents in the ovaries of filefish, a type of saltwater fish, and found that the PLs accounted for 23.01%-33.03% of the TL. Among the PLs, the sum of EPA and DHA in the PL fatty acids was up to 20.48%. DHA and EPA are n-3 PUFAs, which can maintain brain and retinal functions, delay brain aging, prevent cardiovascular diseases, inhibit tumor growth and have anti-inflammatory and anti-anaphylactic effects. Some studies have found that PLs combined with n-3 PUFAs (EPA PL and DHA PL) possess unique biological activities, such as enhanced membrane permeability, better oxidation stability and enhanced erythrocyte deformation, due to the characteristics of both the PL and n-3 PUFA.

Golden Pompano, also known as Trachinotus blochii. It is one of the most important marine fish in China and Southeast Asian countries. Golden pompano production in China was approximately 100 thousand tones in 2017. Due to its high nutritional value, such as 19.65% protein, 10.38% lipids and 1.2% minerals, it has become popular among consumers in recent years[11]. With the increasing demand for Golden pompano, many by-products (fish head, skin, bone and viscera) are produced during processing. Fish head is one of the main by-products, and components of a skull and a small amount of flesh. The chief nutrient constituents of Golden pompano head (GPH) are protein and fat. Phosphorus, calcium, iron, and vitamins are also contained in GPH, especially a great deal of available PLs. Currently, the productions of feed fishmeal, calcium chelate or other calcium supplement products, seasonings, gelatin or polypeptides, etc, are the most used form of GPH[12]. However, most of the GPH are treated as waste, which cause waste of resources and environmental pollution. Therefore, the evaluation of GPH should be emphasized from the perspectives of both resource and economic benefits. In recent years, studies on PLs have mainly focused on their physiological activities, and there are few reports on the determination of PL structures in domesticated or wild organisms. In this study, the structures of the PLs in GPH were determined, and the PLs in the fish head were identified. This report lays a scientific theoretical foundation for the extraction and analysis of functional lipid components and for the use of these materials in functional foods, medicine and cosmetics development.

2 Experimental
2.1 Experimental procedures
Golden pompano (length: 22.5 ± 2.9 cm; height: 12.09 ± 1.54 cm) was obtained between Dec 2017 and Jan 2018 from xiang tai fishery in Haikou City, Hainan Province, China, and was then immediately transported to the laboratory on ice. TLC plates (Silica MF-254, Agela Technologies, CHN) were used for analytical TLC separations. Standard fatty acid methyl esters (C1-C24) were procured from Sigma-Aldrich (St. Louis, USA). Other chemicals and reagents used in this study were of analytical grade and were acquired commercially.

2.2 Lipid extraction and separation
A certain amount of GPH was crushed with an ultrafine pulverizer. Analytical-grade 95% ethanol was used for extraction at under 25°C at a m/v ratio of 1:10 for 1.5 h (×3). The crude ethanol extract was filtered through a Büchner funnel, and the solid residue was re-extracted (×2). The resulting extract was concentrated on a rotary evaporator at 45°C. The crude ethanol extract obtained by rotary evaporation was mixed with chloroform/methanol/H2O in a centrifuge tube in a ratio of 8:4:3 by volume. The mixture was vortexed for 1 min and then centrifuged at 4000 rpm for 10 min. The lower layer was collected, and 0.2x its volume of 0.9% (m/v) sodium chloride solution was added. This mixture was vortexed for 1 min and then centrifuged at 4000 rpm for 10 min. The organic phase (lower layer) was collected after centrifugation, and the organic phase was concentrated by rotary evaporation at 45°C[13]. Then, the TL were dissolved in a small volume of chloroform, and the TL of GPH were separated into the different lipid classes by silica gel column chromatography. The column was sequentially eluted with chloroform, acetone and methanol. Ultimately, three fractions (Fr I, Fr II, and Fr III) were obtained[14-16].
2.3 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra were acquired over a spectral window of 4000-500 cm⁻¹ using an FTIR spectrometer (Tensor27, Bruker Optics, Germany). To form the tablet, approximately 2 mg of Fr III and approximately 300 mg of KBr were placed in an agate mortar. The compounds were thoroughly ground under infrared light to mix them well, and then the mixture was pressed into a transparent sheet. The tablet was transferred to the sample holder, and interval scanning was conducted over wavenumbers ranging from 4000 to 500 cm⁻¹ to obtain the infrared absorption spectra. The functional groups and molecular structure were inferred based on the infrared absorption spectrum absorption peaks and wavenumbers.

2.4 Fatty acid methyl ester (FAME) preparation and fatty acids composition analysis by Gas Chromatography (GC)

The FAMEs were prepared as follows[18]. The fatty acids were characterized following conversion to the corresponding FAMES. The saponification of 5 mg of Fr III was achieved by the reaction with 2 mL of methanolic NaOH (0.5 mol/L) at 65°C for 30 min. Then, 2 mL of 14% boron trifluoride-methanol was added for FAME derivatization. The mixture was heated at 60°C for 3 min. Then, until the mixture was cooled to room temperature, 2 mL of n-hexane (HPLC grade, >95%) was added, and the solution was mixed. After shaking and settling, the samples were centrifuged at 3000 rpm for 5 min, and the resulting FAMEs (upper layer) were concentrated under flowing nitrogen gas.

The FAME samples were analyzed by GC (Agilent 7890A) equipped with a fused silica capillary column (CP7489, 100 m × 0.25 mm × 0.2 μm, CP-Sil 88, Chrompack; Agilent, USA) and an FID[19]. The temperature increase program was 86 min in total: the oven temperature was increased to 175°C at 13°C/min with a hold time of 27 min at 175°C, and then the temperature was increased to 215°C at 4°C/min and maintained at 215°C for 35 min. The Fr III fatty acid components were identified by comparison to the standard mass spectrograms, and the relative quantity of each chemical constituent was determined by the peak area normalization method.

2.5 Identification and analysis by Ultraviolet (UV) Absorption Spectroscopy

A solution of Fr III in methanol was prepared at 0.2 mg/mL, and the UV spectrum was obtained over a wavelength ranging from 190 to 700 nm.

2.6 Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

Liquid chromatography-mass spectrometry (LC-MS, Thermo, Ultimate 3000 LC, Orbitrap Elite) was used as the analysis platform. The LC separations were carried out on a reversed-phase 100×2.1 mm Hypersil GOLD C₁₈ column with 1.9 μm particles, and the column was kept at 45°C. Mobile phase A was acetonitrile/water (60/40, v/v) with 0.1% formic acid and 10 mmol/L ammonium acetate. Mobile phase B was acetonitrile/water (10/90, v/v) with 0.1% formic acid and 10 mmol/L ammonium acetate. The injection volume was 4 μL, and the automatic injector temperature was 4°C.

The mass spectrometry conditions were as follows. For ESI+: oven temperature, 300°C; sheath gas flow rate, 45 arb; aux gas flow rate, 15 arb; sweep gas flow rate, 1 arb; spray voltage, 3.0 kV (ESI+: 3.2 kV); capillary temp, 350°C; S-lens RF level, 30%. For ESI−: oven temp 300°C; sheath gas flow rate, 45 arb; aux gas flow rate, 15 arb; sweep gas flow rate, 1 arb; spray voltage, 3.2 kV; capillary temp, 350°C; S-lens RF level, 60%. The obtained data were extracted and preprocessed with lipid search software (Thermo, Lipid Search). The data, including Lipid Ion, class, fatty acid chains (Fatty Acid, FA1, FA2, and FA3), CalcMz, IonFormula, retention time (RT) and peak intensity, were then normalized and edited into a two-dimensional data matrix by Excel 2010 software.

3 Results

3.1 Silica gel column chromatograph

The TL in the GPH samples were 2.28±0.61% on a wet weight basis. The TLs were separated by silica gel column chromatography based on the differences in lipid polarity and adsorption and desorption capacities in the two phases (stationary phase and mobile phase). Since the polar lipids are tightly bound to the silica gel, when the TL are passed through the silica gel column, the nonpolar lipids (NLs) pass directly through the column and appear with the chloroform solvent front. The moderate polar lipids (GLs) were eluted with acetone, and the polar PLs were eluted with methanol (Fig. 1). The yields from the three fractions (Fr I, Fr II, and Fr III) were 58.51%, 5.93%, and 14.60%, respectively. The proportions of the lipid classes are presented in Table 1; the NLs were most abundant (58.51%) in the TL, and GL and PL constituted small proportions based on gravimetric analysis.

3.2 FTIR Analysis of the PLs

Appropriate amounts of Fr III were extracted and rapidly ground with KBr, and the structure of the GPH-PLs was analyzed by FTIR spectroscopy. As shown in Fig. 2, the vibrational band at 3242 cm⁻¹ was one of the hydrogen bonds, the band at 1737 cm⁻¹ was the absorption peak of the –C = O- stretching vibration, the band at 1648 cm⁻¹ was from the benzene ring stretching vibrations, the bands at 2925
cm\(^{-1}\) and 1463 cm\(^{-1}\) were the characteristic vibrations of \(-\text{CH}_2-\) and \(-\text{CH}_3-\), the band at 1230 cm\(^{-1}\) was the absorption peak of the \(-\text{PO}_2-\) stretching vibration, the band at 1085 cm\(^{-1}\) was the absorption peak of the \(-\text{C-O-PO}_2-\) stretching vibrations, the band at 970 cm\(^{-1}\) was the characteristic vibration frequency of C-C-N, and the band at 720.59 cm\(^{-1}\) was the absorption peak of long chains composed of 4 -\text{CH}_2- units. Thus, this sample, which can be qualitatively extracted, is the PLs, and the PL components should include PC and PE.

### 3.3 Fatty acid composition of the PLs

The fatty acid composition of the PLs was determined based on the peak area percentages of in the GC FID data (Table 2). The fatty acid composition of the GPH-PLs included considerable quantities of hexadecanoic acid (25.35%). Octadecanoic acid constituted 9.60% of the GPH-PLs, and octadecenoic acid was approximately 19.75% of the GPH-PLs. EPA and DHA in the GPH-PL fractions were 1.92% and 16.47%, respectively. PUFAs (EPA and DHA) were found to be more concentrated in the PL fractions. EPA was less abundant than DHA. Significant concentrations of EPA and DHA in the PL fraction were also observed in other roe lipids studied previously. The concentration of EPA and DHA in the PL fraction of Antarctic krill was also determined, and in this case, the total content of EPA and DHA was approximately 22% -35% of the total fatty acids in the PL fraction. The roes of rohu and murrel showed EPA contents of 1.1 and 1.0% and DHA contents of 14.1 and 6.8%, respectively, among the fatty acids, in their PL fractions.

**Table 2** Fatty acid composition (%) of PL

| Fatty acid | PL | Fatty acid | PL |
|------------|----|------------|----|
| C14:0      | 0.49 ± 0.10 | C18:2n-6  | 14.59 ± 0.20 |
| C16:0      | 25.35 ± 0.37 | C20:2n-6  | 1.62 ± 0.01  |
| C17:0      | 0.71 ± 0.04  | C20:4n-6  | 1.51 ± 0.19  |
| C18:0      | 9.60 ± 0.19  | C20:5n-3  (EPA) | 1.92 ± 0.09 |
| C24:0      | 1.29 ± 0.12  | C22:5n-3  (DPA) | 1.63 ± 0.16 |
| C15:1      | 1.88 ± 0.13  | C22:6n-3  (DHA) | 16.47 ± 0.32 |
| tC16:1     | 1.26 ± 0.14  | Saturated | 37.45        |
| C17:1      | 0.78 ± 0.13  | Monounsaturated | 23.66    |
| tC18:1     | 17.53 ± 0.05 | Polysaturated | 38.04      |
| cC18:1     | 2.21 ± 0.41  | other      | 0.85         |
| C24: n-9   | 0.30 ± 0.02  | -           | -           |

*Values are the mean of triplicate analyses ± SD.

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**Fig. 1** TL was applied to a silica gel column and eluted using the following parameters: flow rate: 10, pressure max: 200, detection: 205, monitor: 210, initial waste: 0, start tube: 1. TL was applied to a silica gel column and eluted with chloroform (0-10 min), acetone (10-30 min), and methanol (30-60 min). Three fractions were obtained.

**Fig. 2** FTIR spectrum of PLs over the wavenumber window from 4000 cm\(^{-1}\) to 500 cm\(^{-1}\).

**Table 1** Composition of GPH Lipid classes

| Lipid classes | Chloroform fraction (NL) | Acetone fraction (GL) | Methanol fraction (PL) |
|---------------|--------------------------|-----------------------|------------------------|
| Composition/wt.% | 58.51 ± 4.85 | 5.93 ± 0.19 | 14.6 ± 3.23 |

*Values are the mean of triplicate analyses ± SD.*
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3.4 Full-wavelength UV spectrum of PLs

As shown in Fig. 3, the absorption spectrum of the PC standard has only two absorption peaks, one at 283 nm and one at 212 nm. The absorption spectra of the PLs extracted in this experiment are similar to that of the PC standards, confirming that FrⅢ contains PLs and that PC is present in the PLs.

3.5 LC-MS Analysis and identification of the PLs

The ionization intensity of the PLs was investigated in ESI+ and ESI- modes. ESI+ mode showed the ideal ionization intensity of the PLs, and no background interference was observed. The ionization intensity of PL in ESI-mode was weaker, and substantial background noise was observed. Therefore, the ESI+ was selected for subsequent analyses of the molecular species (Fig. 4).

Mass spectrometry was performed under the following conditions: for ESI+: oven temperature, 300°C; sheath gas flow rate, 45 arb; aux gas flow rate, 15 arb; sweep gas flow rate, 1arb; spray voltage, 3.0 kV (ESI: 3.2 kV); capillary temp, 350°C; S-lens RF level, 30%.

Figure 4 shows the mass spectrometric data of GPH-PLs. Based on the information in Fig. 4, Chem Station software was used to analyze the molecular and ionic fragments. All the PLs could be quantitatively analyzed and compared to

![Graph showing full-wavelength UV spectrum of PLs.](image)

**Table 3** Content of each component in GPH-PLs by LC-MS analysis.

| PL                  | Name                   | Abbreviation | Content (μg/mg) | Total (μg/mg) |
|---------------------|------------------------|--------------|----------------|--------------|
| Glycerol phospholipid | Phosphatidylcholine    | PC           | 276.65         |              |
|                     | lysophosphatidylcholine| LPC          | 65.53          |              |
|                     | Phosphatidylethanolamine| PE          | 6.91           | 351.81       |
|                     | Lysophosphatidylethanolamine| LPE      | 2.21           |              |
|                     | Phosphatidylglycerin   | PG           | 0.30           |              |
|                     | Phosphatidyserine      | PS           | 0.20           |              |
| sphingomyelin       | sphingomyelin          | SM           | 121.65         | 121.65       |
| other               |                         | –            | –              | 12.63        |

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### Table 4  Results of molecular signature and molecular species analysis of GPH-PL.

| Retention time /min | Molecular formula | Fatty acid chains | PC Content (μg/mg) |
|---------------------|-------------------|-------------------|--------------------|
|                     |                   | Sn-1              | Sn-2              | CalcMz  | |
| 3.537123            | C_{39}H_{68}NPO₇ | 8:0p              | 10:0              | 522.36  | 4.70 |
| 5.826958            | C_{39}H_{68}NPO₇ | 6:0               | 16:0              | 594.41  | 1.54 |
| 13.104212           | C_{39}H_{68}NPO₇ | 22:6              | 23:5              | 894.60  | 1.85 |
| 13.119582           | C_{39}H_{68}NPO₇ | 16:0              | 18:2              | 758.57  | 1.14 |
| 13.47372            | C_{39}H_{68}NPO₇ | 22:6              | 22:6              | 878.57  | 5.47 |
| 14.966747           | C_{39}H_{68}NPO₇ | 19:1              | 22:6              | 846.60  | 2.91 |
| 15.264843           | C_{39}H_{68}NPO₇ | 16:1              | 24:7              | 830.57  | 8.46 |
| 16.0036             | C_{39}H_{68}NPO₇ | 16:1p             | 20:4              | 764.56  | 3.36 |
| 16.298858           | C_{39}H_{68}NPO₇ | 16:0p             | 24:7              | 816.59  | 1.21 |
| 16.83739            | C_{39}H_{68}NPO₇ | 19:1              | 20:4              | 822.60  | 2.38 |
| 17.494397           | C_{39}H_{68}NPO₇ | 16:0              | 14:0              | 706.54  | 3.29 |
| 17.564085           | C_{39}H_{68}NPO₇ | 16:0              | 16:1              | 732.55  | 2.47 |
| 17.636798           | C_{39}H_{68}NPO₇ | 16:0p             | 20:4              | 766.57  | 5.54 |
| 18.073353           | C_{39}H_{68}NPO₇ | 16:1              | 20:2              | 784.59  | 3.07 |
| 18.15971            | C_{39}H_{68}NPO₇ | 16:0              | 20:3              | 784.59  | 3.07 |
| 18.413912           | C_{39}H_{68}NPO₇ | 16:0              | 17:0              | 748.59  | 1.12 |
| 18.493662           | C_{39}H_{68}NPO₇ | 15:0              | 16:0              | 720.55  | 1.12 |
| 18.497468           | C_{39}H_{68}NPO₇ | 18:0p             | 22:6              | 818.61  | 2.96 |
| 18.572608           | C_{39}H_{68}NPO₇ | 25:0              | 8:0               | 748.59  | 1.12 |
| 18.722702           | C_{39}H_{68}NPO₇ | 16:1p             | 19:5              | 748.53  | 1.71 |
| 18.989912           | C_{39}H_{68}NPO₇ | 16:0e             | 20:4              | 768.59  | 2.93 |
| 18.993595           | C_{39}H_{68}NPO₇ | 16:0e             | 16:1              | 718.57  | 3.38 |
| 19.10851            | C_{39}H_{68}NPO₇ | 14:0e             | 16:0              | 692.56  | 1.85 |
| 19.254997           | C_{39}H_{68}NPO₇ | 17:1              | 17:1              | 758.57  | 1.69 |
| 19.456905           | C_{39}H_{68}NPO₇ | 16:0e             | 18:2              | 744.59  | 11.46 |
| 19.703095           | C_{39}H_{68}NPO₇ | 13:0              | 23:2              | 786.60  | 11.45 |
| 19.735982           | C_{39}H_{68}NPO₇ | 27:2              | 9:0               | 786.60  | 11.45 |
| 20.065527           | C_{39}H_{68}NPO₇ | 16:0p             | 17:0              | 732.59  | 1.19 |
| 20.384742           | C_{39}H_{68}NPO₇ | 18:0e             | 22:6              | 820.62  | 4.46 |
| 20.698213           | C_{39}H_{68}NPO₇ | 16:0e             | 18:1              | 746.61  | 19.54 |
| 20.891273           | C_{39}H_{68}NPO₇ | 12:0e             | 22:2              | 744.59  | 10.16 |
| 21.115683           | C_{39}H_{68}NPO₇ | 18:0              | 18:1              | 788.62  | 6.88 |
| 21.327242           | C_{39}H_{68}NPO₇ | 20:0              | 18:2              | 814.63  | 1.29 |
| 21.558643           | C_{39}H_{68}NPO₇ | 18:0e             | 18:2              | 772.62  | 6.20 |
| 21.878065           | C_{39}H_{68}NPO₇ | 16:0p             | 19:0              | 760.62  | 1.01 |
| 22.373675           | C_{39}H_{68}NPO₇ | 20:0p             | 16:1              | 772.62  | 2.63 |
| 22.392422           | C_{39}H_{68}NPO₇ | 16:0p             | 17:1              | 730.57  | 1.06 |
| 22.999777           | C_{39}H_{68}NPO₇ | 18:0e             | 18:1              | 774.64  | 19.45 |
| 23.003692           | C_{39}H_{68}NPO₇ | 18:1              | 24:2              | 868.68  | 1.40 |
| 23.685947           | C_{39}H_{68}NPO₇ | 18:0              | 24:2              | 870.69  | 7.45 |
| 24.750448           | C_{39}H_{68}NPO₇ | 20:0              | 24:2              | 898.73  | 1.85 |
| 25.067505           | C_{39}H_{68}NPO₇ | 18:0              | 24:1              | 872.71  | 13.75 |
| 25.079978           | C_{39}H_{68}NPO₇ | 18:0              | 22:1              | 844.68  | 3.68 |
| 25.302512           | C_{39}H_{68}NPO₇ | 16:0              | 24:0              | 846.69  | 1.77 |
| 25.709865           | C_{39}H_{68}NPO₇ | 35:2              | 9:0               | 898.73  | 1.85 |
| 25.869153           | C_{39}H_{68}NPO₇ | 18:1              | 24:0              | 872.71  | 13.75 |
| 28.132753           | C_{39}H_{68}NPO₇ | 16:0e             | 22:6              | 792.59  | 1.43 |
| 28.323111           | C_{39}H_{68}NPO₇ | 16:0              | 16:0              | 734.57  | 1.06 |

Note: other PC with content < 1μg/mg
### Table 4

Continued.

| Retention time /min | Molecular formula | Fatty acid chains | LPC | Sn-1 | CalcMz | Content (μg/mg) |
|---------------------|-------------------|------------------|-----|------|--------|----------------|
| 2.5869              | C₂₃H₄₆NPO₇       | 14:0e            |     |      | 454.33 | 0.40           |
| 3.878048            | C₂₃H₄₄NPO₇       | 15:1             |     |      | 480.31 | 0.33           |
| 3.302467            | C₂₃H₄₈NPO₇       | 16:0p            |     |      | 480.34 | 0.51           |
| 4.153697            | C₂₃H₄₈NPO₇       | 16:0e            |     |      | 482.36 | 10.48          |
| 2.24201             | C₂₃H₄₆NPO₇       | 16:1             |     |      | 494.32 | 0.33           |
| 3.44012             | C₂₃H₄₈NPO₇       | 16:0             |     |      | 496.34 | 9.70           |
| 3.306228            | C₂₃H₄₆NPO₇       | 18:1p            |     |      | 506.36 | 0.62           |
| 5.880528            | C₂₃H₄₆NPO₇       | 18:0e            |     |      | 510.39 | 4.38           |
| 3.063775            | C₂₃H₄₆NPO₇       | 18:2             |     |      | 518.32 | 0.49           |
| 2.769103            | C₂₃H₄₆NPO₇       | 18:1             |     |      | 520.34 | 5.97           |
| 3.728638            | C₂₃H₄₆NPO₇       | 18:1             |     |      | 522.36 | 10.94          |
| 4.993597            | C₂₃H₄₆NPO₇       | 18:0             |     |      | 524.37 | 3.52           |
| 1.913183            | C₂₃H₄₆NPO₇       | 20:5             |     |      | 542.32 | 0.46           |
| 3.207387            | C₂₃H₄₆NPO₇       | 20:3             |     |      | 546.36 | 0.32           |
| 4.106765            | C₂₃H₄₆NPO₇       | 20:2             |     |      | 548.37 | 1.91           |
| 5.291787            | C₂₃H₄₆NPO₇       | 20:1             |     |      | 550.39 | 0.92           |
| 2.341907            | C₂₃H₄₆NPO₇       | 22:6             |     |      | 568.34 | 2.45           |
| 3.298727            | C₂₃H₄₆NPO₇       | 22:5             |     |      | 570.36 | 0.66           |
| 9.625493            | C₂₃H₄₆NPO₇       | 22:0             |     |      | 580.43 | 0.32           |
| 2.501702            | C₂₃H₄₆NPO₇       | 23:5             |     |      | 584.37 | 0.59           |
| 2.402072            | C₂₃H₄₆NPO₇       | 22:6             |     |      | 590.32 | 0.36           |
| 9.621688            | C₂₃H₄₆NPO₇       | 24:1             |     |      | 606.45 | 0.57           |
| 13.310222           | C₂₃H₄₆NPO₇       | 24:0             |     |      | 608.46 | 1.02           |
| 16.973817           | C₂₃H₄₆NPO₇       | 37:6             |     |      | 778.57 | 0.71           |
| 18.15568            | C₂₃H₄₆NPO₇       | 38:6             |     |      | 814.57 | 0.48           |
| other               |                  |                  |     |      |        | 7.09           |
| total               |                  |                  |     |      |        | 65.53          |

Note: other LPC with content < 0.3 μg/mg

| Retention time /min | Molecular formula | Fatty acid chains | PE | Sn-1 | Sn-2 | CalcMz | Content (μg/mg) |
|---------------------|-------------------|------------------|----|------|------|--------|----------------|
| 3.68679             | C₂₃H₄₆NPO₇       | 8:0p             |    | 10:0 |      | 480.31 | 0.23           |
| 5.0894              | C₂₃H₄₆NPO₇       | 8:0e             |    | 10:0 |      | 482.32 | 0.54           |
| 19.798158           | C₂₃H₄₆NPO₇       | 16:0p            |    | 18:2 |      | 700.53 | 0.21           |
| 21.274725           | C₂₃H₄₆NPO₇       | 18:0p            |    | 16:1 |      | 702.54 | 0.61           |
| 21.041105           | C₂₃H₄₆NPO₇       | 16:0p            |    | 18:2 |      | 702.54 | 0.61           |
| 21.196133           | C₂₃H₄₆NPO₇       | 18:0p            |    | 18:2 |      | 728.56 | 0.27           |
| 22.161943           | C₂₃H₄₆NPO₇       | 18:0p            |    | 18:1 |      | 730.57 | 1.06           |
| 18.632227           | C₂₃H₄₆NPO₇       | 16:0p            |    | 22:6 |      | 748.53 | 1.71           |
| 20.380975           | C₂₃H₄₆NPO₇       | 18:0p            |    | 22:6 |      | 776.56 | 0.58           |
| 13.761127           | C₂₃H₄₆NPO₇       | 22:6             |    | 22:6 |      | 836.52 | 0.23           |
| other               |                  |                  |    |      |      |        | 0.86           |
| total               |                  |                  |    |      |      |        | 6.91           |

Note: other PE with content < 0.2 μg/mg

| Retention time /min | Molecular formula | Fatty acid chains | LPE | Sn-1 | CalcMz | Content (μg/mg) |
|---------------------|-------------------|------------------|-----|------|--------|----------------|
| 4.341748            | C₂₃H₄₆NPO₇       | 18:1p            |    |      | 464.31 | 0.44           |
| 2.86655             | C₂₃H₄₆NPO₇       | 18:2             |    |      | 478.29 | 0.24           |
| 5.83866             | C₂₃H₄₆NPO₇       | 18:0p            |    |      | 488.31 | 0.11           |
| 2.740402            | C₂₃H₄₆NPO₇       | 22:6             |    |      | 526.29 | 0.78           |

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standard substance with known concentrations. As shown in Table 3, there are four main PL components in GPH-PL: PC, PE, SM and LPC. PC (276.65 μg/mg) was the most abundant, followed by SM (121.65 μg/mg). The LPC (65.53 μg/mg) content was moderate, the PE and LPE contents were low (6.91 μg/mg and 2.21 μg/mg, respectively), and the PI and PS contents were very small (0.30 μg/mg and 0.20 μg/mg, respectively). As shown in Table 4, the types of fatty acids attached to the carbon ends of each PL molecule were as follows: PC molecules mainly have C16:0,
C16:1, C18:0, C18:1, C20:4, C24:1, and C24:2; SM molecules mainly have C16:0, C18:1, C18:4, C24:1, and C26:2; PE molecules mainly have C16:0 and C18:0; and LPC molecules mainly have C16:0, C18:0, and C18:1.

4 Discussion

There was a large difference in the PL species distribution between the different classes of PLs. The major species in the GPH-PCs were 16:0/18:1 and 18:0/18:1. In addition, the GPH-PCs also had high contents of 18:1/24:0 and 18:0/24:1 species. PC was the only PL class that was found to contain 14:0 fatty acids (16:0/14:0). PC also contained some 22:6 fatty acids. The main 22:6-containing species were 18:0/22:6 and 22:6/22:6. The molecular species are quite similar to those in the PCs from Atlantic salmon head and kidney. However, the content of 18:0/18:1 species in the GPH-PCs was high, and the 18:1/24:0 and 18:0/24:1 species are unique to the PC of GPH as 22:6 fatty acid and 22:6/22:6 species are not found in the Atlantic salmon head and kidney PCs, which may be related to the geographic source of the fish diet. The combination of PC to DHA not only shows their respective functions and nutritional properties, but also recent studies have shown that, except respective functions and nutritional properties of PC and DHA, the DHA combined to PC is more conducive to human digestion and absorption, and stronger physiological activity than that as a monomer fatty acid or in the form of its triglycerides. Moreover, DHA-PC has potential research value because PC has many activities such as improving fat metabolism and enhancing immunity. In the current study, GPH has potential research value for the high content of DHA-PC. Like most mammal-derived foods (beef, milk and poultry, for example), GPH contains high amounts of SM, which are composed of headgroup components (phosphocholine) and ceramide backbones (d18:1 D4, d18:0 and t18:0), with amide-linked fatty acids 16-30 carbon atoms in length. The lipid backbones of GPH-SM are mainly sphingosine (d18:1 t4, with smaller amounts of sphinganine and homologues with other chain lengths) with 18:1, 26:2 and 24:1 derivatives as the major fatty acids. The relationship between SM in food and human health has become the focus of current research. SM, obtained from natural food, have potential anti-cancer effects, has been found to prevent the formation of colon cancer and arteriosclerosis to some extent. By studying the regulation and action mechanism of SM signaling pathways, the pathogenesis of various diseases can be further clarified. Compared to the milk (39-119 mg/L), the content of SM in GPH (404.95 mg/kg) was significantly higher. So as to provide new ideas for the prevention and treatment of diseases. SM in GPH riches in SM, and thus can be used as raw materials for further research on their functions and nutrition. There were fewer PE species identified compared to the other PL classes. The major molecular species in PE were the 16:0/22:6 species. In addition, PE also contained a large amount of other 22:6 fatty acid-containing species, namely, 18:0/22:6 and 22:6/22:6 derivatives. Similar to PC, PE also contained a large amount of 16:0/18:1 and 16:0/18:2 species.

GPH-PLs contain a variety of fatty acid types and a large amount of unsaturated fatty acid chains; the PC molecules are mainly 16:0e/18:2, 13:0/23:2, 27:2/9:0, 16:0e/18:1, 12:0e/22:2, 18:0e/18:1, 18:0/24:1, and 18:1/24:0 derivatives and the SM molecules are mainly d16:1/16:0, d16:0/18:1, d18:1/24:1, and d16:0/26:2. Large quantities of DHA and EPA were also detected, and n-3 polyunsaturated fatty acid (PUFA) is present in large quantities in PC. The types and contents of fatty acid chains were basically consistent with the GC results. Therefore, the pretreatment method used herein was simple and effective, and no conventional chromatographic separation process was needed in this analysis and detection method; reliable results could be obtained by direct injection. This method thus provides a basis for the quantitative analysis of lipids in aquatic products such as *Golden pompano*. In this study, the method of phospholipid molecular species structure analysis provided a theoretical basis for the analysis of phospholipid functional activity of GPH. Based on the above analysis of the GPH-PL composition, further studies of GPH-PLs are necessary.

5 Conclusion

The molecular components of PLs can be directly separated and identified by LC-MS. There are four main PL components in GPH; PC, SM, PE and LPC. The results show that the contents of these species in GPH-PL were generally in the order PC > SM > LPC > PE. GPH-PL contains more unsaturated fatty acids, especially EPA and DHA. Therefore, GPH-PL is not only an excellent source of PLs but also a good supplement for EPA and DHA in the human diet. In addition, GPH-PLs are rich in SM, which play an important biological role in regulating the growth, differentiation, proliferation and apoptosis of various cell physiological or pathological conditions and have good nutritional and medicinal properties, which should be fully utilized.

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Conflict of Interest
The authors declare that they have no conflict of interest.

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