Food Chemistry 216 (2017) 347–354

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Zwitterionic hydrophilic interaction solid-phase extraction and multi-dimensional mass spectrometry for shotgun lipidomic study of *Hypophthalmichthys nobilis*

Renyao Jin [a,1], Linqiu Li [b,1], Junli Feng [a], Zhiyuan Dai [a], Yao-Wen Huang [c], Qing Shen [a,*]

[a] Institute of Seafood, Zhejiang Gongshang University, Hangzhou, China
[b] School of Public Health, Guangdong Medical University, Dongguan, China
[c] Department of Food Science and Technology, University of Georgia, Athens, GA, United States

ARTICLE INFO

Article history:
Received 10 June 2016
Received in revised form 15 August 2016
Accepted 22 August 2016
Available online 24 August 2016

Keywords:
Zwitterionic hydrophilic interaction liquid chromatography
Solid-phase extraction
Multi-dimensional mass spectrometry
Shotgun lipidomics
Hypophthalmichthys nobilis

ABSTRACT

Zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) material was used as solid-phase extraction sorbent for purification of phospholipids from *Hypophthalmichthys nobilis*. The conditions were optimized to be pH 6, flow rate 2.0 mL·min^{-1}, loading breakthrough volume $\leqslant$ 5 mL, and eluting solvent 5 mL. Afterwards, the extracts were analyzed by multi-dimensional mass spectrometry (MDMS) based shotgun lipidomics; 20 species of phosphatidylcholine (PC), 22 species of phosphatidylethanoamine (PE), 15 species of phosphatidylserine (PS), and 5 species of phosphatidylinositol (PI) were identified, with content 224.1, 124.1, 27.4, and 34.7 μg·g^{-1}, respectively. The MDMS method was validated in terms of linearity (0.9963–0.9988), LOD (3.7 ng·mL^{-1}), LOQ (9.8 ng·mL^{-1}), intra-day precision (<3.64%), inter-day precision (<5.31%), and recovery (78.8–85.6%). ZIC-HILIC and MDMS shotgun lipidomics are efficient for studying phospholipids in *H. nobilis*.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

In China, the total yield of freshwater fishery was 26.35 million tons in 2013, accounting for 42.7% of the total aquaculture yield. Bighead carp (*Hypophthalmichthys nobilis*, *H. nobilis*) is a popular low-value freshwater fish, which has been introduced into more than 70 other countries in Europe, United States, and the Middle East (Liu, Liang, Regenstein, & Zhou, 2012). The gross product of the four major Chinese carps, comprising bighead carp, black carp, grass carp, and silver carp, exceeds 60% of the total freshwater fishery yield (Duan, Zhang, & Tang, 2004). Bighead carp is considered a super food, high in protein and phospholipids, low in fat, which has the beneficial functions of reducing the levels of blood fat and cholesterol, lowering the risk of cardiovascular disease, and improving memory (Hong, Zhou, Wu, Luo, & Shen, 2014). Previous studies mainly focused on the aquaculture techniques, but chemical composition and nutritional characterization of bighead carp is still limited (Cooke, Hill, & Meyer, 2009).

Phospholipids are the main structural constituents of biological membranes and increasingly considered as nutrients with putative health benefits and positive nutritional functions (Chen & Subbaiah, 2013; Pacettia et al., 2015). The molecular types of phospholipids vary due to a glycerol backbone with the headgroup at the sn-3 position and the fatty acyl chains (FACs) at the sn-1 and/or sn-2 position (Boselli, Pacetti, Lucci, & Frega, 2012). For the analysis of phospholipids in bighead carp, thin-layer chromatography (TLC) and optical methods have been shown to provide a quantitative measure of phospholipid classes with low resolution. Recent developments in MS technology enabled the comprehensive characterization of phospholipids, fostering the nutritional function of lipids and lipid-related biomarker exploration (Calvano, van der Werf, Sabbatini, & Palmisano, 2015; Calvano, Zambonin, & Palmisano, 2011; Chen, Belikova, & Subbaiah, 2012; Shen, Dai, Huang, & Cheung, 2016). Liquid chromatography coupled to MS is capable of identification and quantification of phospholipid species, but this technique is usually time-consuming in sample preparation and analysis

Abbreviations: PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PI, Phosphatidylinositol; PS, Phosphatidylserine; MS, Mass spectrometry; MDMS, Multi-dimensional mass spectrometry; FAC, Fatty acyl chain; ZIC, Zwitterionic; HILIC, Hydrophilic interaction liquid chromatography; PIS, Product ion scan; NLS, Neutral loss scan; SPE, Solid-phase extraction; LOD, Limit of detection; LOQ, Limit of quantification; SM, sphingomyelin; S/N, signal-to-noise; MCA, Multiple-channel acquisition.

* Corresponding author at: Institute of Seafood, Zhejiang Gongshang University, Hangzhou, Zhejiang Province 310035, China.
E-mail addresses: leonqshen@163.com, sq@zjgsu.edu.cn (Q. Shen).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.foodchem.2016.08.074
0308-8146/© 2016 Elsevier Ltd. All rights reserved.

(Shen & Cheung, 2014). In contrast, multi-dimensional mass spectrometry (MDMS)-based shotgun lipidomics can fast profile phospholipids by directly infusing lipid extracts and precursor ion scan (PIS) and neutral loss scan (NLS) detection.

The success of lipidomics study depends on not only the advanced analytical instruments, but also the completeness of extraction of phospholipids. Traditional methods for the recovery of all major lipid classes are on the basis of chloroform/methanol extraction, such as Folch, Bligh and Dyer, and Sloane Stanley methods. However, a specific method for the purification of phospholipid is still missing. The ZIC-HILIC stationary phase comprises a sulfobetaine type zwitterionic functionality covalently attached to porous silica, which carries both positive and negative charges on the surface. The functional moieties contain two oppositely charged groups in close proximity at a stoichiometric ratio; the electrostatic interaction between the charged groups on the stationary phase and oppositely charged analytes is weaker compared to normal ion exchangers (Jiang, Fischer, Girmay, & Irgum, 2006). Such weak electrostatic interaction allows the use of diluted buffer, and is favorable for mass spectrometry (Xuan et al., 2006). These qualities make ZIC stationary phases exceptionally well suited for HILIC separations. Rezanka et al. successfully separated diacyl- and plasmalogen phospholipids of anaerobic beer-spoilage bacterial genus *Pectinatus* on a ZIC-HILIC column (Řezanka, Siristova, Matoulková, & Sigler, 2011). Zheng et al. profiled the phospholipids in *Leishmania donovani* using ZIC-HILIC in combination with Fourier transform MS (Zheng et al., 2010). ZIC material can also be used as an SPE sorbent for the purification or enrichment of peramivir, paralytic shellfish poisoning toxins, and glycopeptides (Elizabeth, Lesley, Jean-Pierre, Sergey, & Elena, 2008; Lindegardh et al., 2008; Neue, Mormann, Peter-Katalini, & Pohlentz, 2011; Shen, Yang, & Cheung, 2015). However, application of ZIC-HILIC-based SPE for lipidomics study has not been reported so far.

In this study, a ZIC-HILIC SPE protocol was developed for shotgun profiling of phospholipids from bighead carp samples.

2. Materials and methods

2.1. Chemicals and regents

Phospholipid standards, including 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (PC 14:0/14:0), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (PE 15:0/15:0), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoinositol (PI 16:0/16:0), and 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (PS 14:0/14:0) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). These internal standards were selected because they represent ⩽1% of endogenous cellular lipid mass. HPLC-grade chloroform, acetonitrile, and methanol were purchased from Fisher Scientific (Waltham, MA). ZIC-HILIC SPE cartridge (50 μm, 60 Å, 3 mL, 100 mg) was obtained from Merck SeQuant, (Sweden). Water (resistivity of 18 MΩ·cm) was purified using a Milli-Q system (Millipore, Billerica, MA). The other chemicals and solvents used during the experiments were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Sample preparation

Fresh bighead carp (*H. nobilis*), with mean weight 1268 ± 85.3 g and mean length 42.6 ± 1.21 cm, were transported after capture to the Institute of Seafood, Zhejiang Gongshang University. In the laboratory, the bighead carps with no evident signs of parasites were pooled. After removing the fish scale (30.72 ± 2.13 g), internal organs (132.19 ± 9.10 g), heads (432.87 ± 37.54 g) and tails (115.72 ± 24.67 g), the ordinary muscle (458.26 ± 41.37 g) from both sides of the bighead carp was obtained using a mechanical deboner. The muscle sample was washed with cold distilled water, followed by grinding to uniformity (Micromill grinder, Shanghai Pingcheng Co., China) protected by nitrogen flow. Fresh surimi was obtained, which was used for lipid extraction.

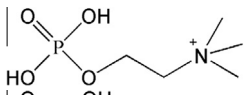
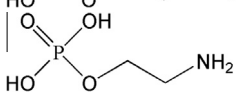
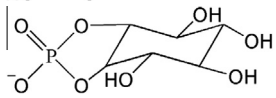
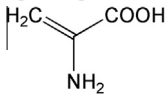
2.3. Phospholipid extraction

Crude lipids were extracted using a classic Folch method with modification. Briefly, 0.1 g fresh bighead surimi sample were accurately weighted, and placed in a 5 mL polytetrafluoroethylene (PTFE) tube; 2 mL of chloroform/methanol (2:1, v/v) mixture were added for extracting the lipids using an ultrasonic device for 15 min. After that, a portion of 2 mL ultrapure water was added to the PTFE tube, and shaken. After centrifugating at 9000*g* for 10 min, the solvent was separated into two phases: the lower phase was collected, while the upper phase was re-extracted for another two times according to the same procedure. Finally, the collected solvents were combined and concentrated by nitrogen blowing. During the experiments, all the extraction processes were conducted at low temperature (4 °C) to minimize the risk of oxidation of the unsaturated bonds or lipid hydrolysis.

2.4. SPE procedure

ZIC-HILIC SPE was conducted to obtain the purified phospholipids from crude lipid extracts. The pH of sample was adjusted to 6 using ammonium formate, then the sample was loaded on the SPE cartridge at a flow rate of 2.0 mL·min^{-1}. The loading

Table 1
MS/MS parameters for shotgun lipidomics of bighead carp sample.

Class	Formula	Fragment structure	Fragment type	Scan mode	DP (V)	CE (V)
PC	$[C_5H_{15}O_4NP]^+$		*m*/*z* 184	+PIS	100	40
PE	$C_2H_8O_4NP$		141 amu	+NLS	90	35
PI	$[C_6H_{10}O_5P]^-$		*m*/*z* 241	−PIS	110	30
PS	$C_3H_5O_2N$		87 amu	−NLS	100	40

Download English Version:

https://daneshyari.com/en/article/1183087

Download Persian Version:

https://daneshyari.com/article/1183087

Daneshyari.com