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Characterization of lipids in three species of sea urchin

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ABSTRACT

Sea urchin gonad has been regarded as a "healthy" food. Although previous studies have suggested that sea urchin gonad might serve as a potential rich source of long chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFA) enriched phospholipid (PL), the molecular species profile of its PL has rarely been reported. In this study, about 200 molecular species of glycerophospholipid (GP), including glycerophosphocholine, glycerophosphoethanolamine, glycerophosphoserine, glycerophosphoinositol, lysoglycerophosphocholine and lysoglycerophosphoethanolamine, in gonads from three species of sea urchin (*Glyptocidaris crenularis*, *Strongylocentrotus intermedius* and *Strongylocentrotus nudus*) were characterized using tandem mass spectrometry. Most of the predominant GP molecular species contained PUFA, especially eicosapentaenoic acid (EPA). Meanwhile, the sea urchin lipids contained a high proportion of PL (39.45–50.30% of total lipids) and PUFA (34.47–46.56% of total FA). Among PL, phosphatidylcholine (67.88–72.58 mol%) was dominant. Considering the high level of PUFA enriched GP, sea urchin gonads provide great potential as health-promoting food for human consumption.

1. Introduction

Sea urchins are marine invertebrates of the phylum *Echinodermata* which live on the ocean floor. To date more than 800 species of sea urchins have been found. Gonads (also termed "uni" or "roe"), the edible portion of sea urchin, account for approximately 10% of their total weight (La Cruz-García, López-Hernández, González-Castro, Rodríguez-Bernaldo De Quirós, & Simal-Lozano, 2000). They are half-moon shaped, yellow-orange in colour, and chiefly composed of moisture, protein, carbohydrate and lipid (Dincer & Cakli, 2007). With distinctive aroma and good taste, sea urchin gonads are expensive and a delicacy in many countries, especially China and Japan.

Recently, sea urchin gonad has been regarded as a "healthy" food due to its positive effects on health arising from constituent lipids, proteins (polypeptides), polysaccharides, carotenoids, vitamins and minerals (Archana & Babu, 2016; Pozharitskaya et al., 2015). Generally, the lipid content of sea urchin gonad is > 20% on a dry weight basis (Archana & Babu, 2016; Zhu et al., 2010). Previous studies have reported that phospholipid (PL) is the principal lipid (more than 40% of total lipids) in sea urchin gonads (Kalogeropoulos, Mikellidi, Nomikos, & Chiou, 2012; Mita & Ueta, 1989). A study by Mita and Ueta (1989) indicated that the phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) isolated from sea urchins spermatozoa contained a high percentage of polyunsaturated fatty acids (PUFAs) (33.2–67.6% of total fatty acids), in particular arachidonic acid (AA; 20:4n-6) and eicosapentaenoic acid (EPA; 20:5n-3). Moreover, Kostetsky, Sanina, and Velansky (2014) characterized 26 species of glycerophosphocholine (GPCho) and 23 species of glycerophosphoethanolamine (GPEtn) from sea urchin (*Strongylocentrotus intermedius*) gonad. Among them, the molecular species containing long chain omega-3 PUFA (n-3 LC-PUFA) such as 20:5 and 22:6 were predominant. Therefore, sea urchin gonads might serve as a potential rich source of PL enriched PUFAs. However, to the best of our knowledge, there has been no information on simultaneous analysis of fatty acid composition, lipid class composition, PL class composition and glycerophospholipid (GP) molecular species in gonads from different species of sea urchins. Meanwhile, Kostetsky et al. (2014) published the only research findings on characterization of molecular species of two PL classes (GPCho and GPEtn) in sea urchin gonad. Therefore, to provide nutritional and functional information and pave the way for better utilization of sea urchin gonads, detailed characterization of the lipid profile, including the molecular species of more PL classes from different species of sea urchin, was deemed necessary.

The n-3 LC-PUFA, in particular EPA and docosahexaenoic acid

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(DHA; 22:6n-3) have been found to possess a wide range of health benefits, such as improving heart disease related outcomes, promoting infant development, decreasing tumour growth and metastasis, inhibiting inflammation, platelet aggregation, hypertension and hyperlipidaemia, and favourably modifying insulin sensitivity (Anderson & Ma, 2009; Riediger, Othman, Suh, & Moghadasian, 2009). Therefore, there is a convergence of opinion that daily consumption of 250–1000 mg of EPA/DHA provides health benefits (Kuratko & Salem, 2013). The n-3 LC-PUFA derived from foods is mainly in the triacylglycerol (TAG) and PL forms. Recently, n-3 LC-PUFA in the PL form have captured increasing attention due to their superior bioavailability (Cook et al., 2016; Köhler, Sarkkinen, Tapola, Niskanen, & Bruheim, 2015; Yurko-Mauro et al., 2015), higher tissue-delivery capacity (Cansell, 2010; Graf et al., 2010; Liu et al., 2014; Rossmeisl et al., 2012), and better health promoting effects (Batetta et al., 2009; Ramprasath, Eyal, Zchut, & Jones, 2013; Sampalis et al., 2003; Ulven et al., 2011) compared to TAG-containing n-3 LC-PUFA. Therefore, PL-enriched lipids in sea urchin gonad may account for much of its nutritional and healthful functions.

Sea urchin *Glyptocidaris crenularis*, *Strongylocentrotus intermedius* and *Strongylocentrotus nudus* are the most common species widely distributed in East Asian Seas (Shang et al., 2014; Suh et al., 2014; Zhao, Zhou, Tian, Feng, & Chang, 2014). Hence, the aim of this study was to investigate the lipid content, fatty acid composition, lipid class composition, PL class composition and GP molecular species belonging to six classes, including GPCho, GPEtn, glycerophosphoserine (GPSer), glycerophosphoinositol (GPIns), lysoglycerophosphocholine (LGPCho) and lysoglycerophosphoethanolamine (LGPEtn) in *Glyptocidaris crenularis*, *Strongylocentrotus intermedius* and *Strongylocentrotus nudus*. This will help to understand the specific health benefits of lipids in sea urchin gonads, as well as to provide theoretical basis for utilization of sea urchin gonads as novel sources of functional foods and to fill the existing knowledge gap in the field.

2. Materials and methods

2.1. Materials

Three species of fresh sea urchins, namely *Glyptocidaris crenularis*, *Strongylocentrotus intermedius* and *Strongylocentrotus nudus,* were purchased from a local market in Dalian, Liaoning, China. After dissection, the fresh gonads were collected and lyophilized in a freeze-dryer (2KBTES-55, VirTis Co., Gardiner, NY, USA) for 72 h, subsequently crushed into a powder and stored at −30 °C until use. GP standards were purchased from Avanti Polar Lipids, INC. (Alabaster, AL, USA). Reagents, including methanol, acetonitrile, chloroform and ammonium formate, were HPLC-grade and purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA, USA). Deuterated chloroform ($CDCl_3$) and methanol (MeOD), triethyl phosphate (TEP), cesium carbonate ($CsCO_3$) and D_2O were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). All other reagents were of analytical grade and purchased from Kemiou Chemical Reagent Co., Ltd. (Tianjin, China).

2.2. Lipid extraction and sample preparation

Total lipids were extracted from samples using a modified version of the methyl *tert*-butyl ether (MTBE) method (Matyash, Liebisch, Kurzchalia, Shevchenko, & Schwudke, 2008). Briefly, 2.0 g of dried sea urchin gonad powder was completely mixed with 3 ml of methanol and 10 ml of MTBE in a conical flask. After stirring the mixture at 30 °C for 1 h, 2.5 ml of deionized water was added. Following adequate mixing, the mixture was centrifuged at 7800*g* for 10 min. The organic layer was subsequently transferred into a centrifuge tube and the precipitate was extracted again according to the aforementioned method. Finally, the organic layers were combined and dried using a stream of nitrogen at 35 °C, to obtain lipids from sea urchin gonads. The recovered lipids were then weighed and stored at −30 °C for further analysis within 2 weeks.

The dilute lipid samples used for further analysis were prepared daily, by diluting them using a mixture of methanol and chloroform (1:2, v/v). Before injection for HPLC-MS/MS analysis, the sample solutions were filtered through a 0.22 μm microporous membrane. In order to semi-quantify the same compound in different samples, five GP internal standards, namely phosphatidyl GPCho 12:0/12:0, GPEtn 12:0/12:0 (internal standard for both GPEtn and LGPEtn), GPSer 12:0/12:0, GPIns 8:0/8:0 and LGPCho 10:0 were added to the samples in order to reach a concentration of 0.60, 0.15, 0.875, 0.10 and 0.15 μg/ml, respectively.

2.3. Lipid class composition analysis

Lipid class compositions were determined and analysed using an Iatroscan MK-6S thin layer chromatography-flame ionization detection (TLC-FID) analyzer (Iatron Inc., Tokyo, Japan), according to the procedures described in a previous study (Yin et al., 2015).

2.4. Fatty acid composition analysis

Fatty acid compositions were determined using an Agilent 7890A GC-5975C MSD (Palo Alto, CA, USA) equipped with an HP-5-MS capillary column (30 m × 0.25 mm, 0.25 μm) (Palo Alto, CA, USA), according to the procedures described elsewhere (Yin et al., 2015).

2.5. Quantitation of PL classes by ^{31}P NMR

Sample preparation was performed according to Burri, Hoem, Monakhova, and Diehl (2016) with some modification. Briefly, 500 mg of lipid samples were dissolved in 1 ml $CDCl_3$/MeOD (2:1, v/v). The addition of 1 ml of 0.2 M $CsCO_3$-EDTA solution in D_2O (pH 7.2–7.6) led to the separation of two layers. Organic and aqueous phases were separated after intense vortexing and centrifugation (7800*g* for 10 min), and then the lower organic phase was collected into an NMR sample tube and subsequently used for ^{31}P NMR spectrometry. ^{31}P NMR analyses were conducted on an Avance III 400 MHz Bruker NMR spectrometer (9.4 T) using 5 mm tubes and a quadruple nuclear probe. The number of transients was 4096 per spectrum acquisition to achieve an optimal signal-to-noise ratio. ^{31}P NMR spectra were acquired with a 9 s inter-pulse delay, 6488 Hz spectral width, 90 pulse angle (9.5l s), 32 K data points and 1 H decoupling (Waltz 16, decoupling power, 19 dB; pulse width, 13 s). Lock and shimming were performed on $CDCl_3$. PL class quantification data by ^{31}P NMR was processed using MestReNova 6.1.1 software. PL class quantification was conducted by comparing the peak area with TEP after integration, normalization was then carried out using the corresponding ratio.

2.6. HPLC–MS analysis

The HPLC–MS/MS analysis of lipid samples was performed on a Shimadzu LC-20AVP system (Shimadzu Co. Tokyo, Japan) which was coupled in-line with a hybrid API 4000 Qtrap (AB Sciex, Foster City, CA, USA) quadrupole-linear ion trap (QqLIT) mass spectrometer, as described previously (Yin et al., 2016). The eluent was solvent A [ACN/H_2O/100 mM NH_4HCO_2 (pH 3.2) = 95:2.5:2.5, v/v/v] and solvent B [H_2O/100 mM NH_4HCO_2 (pH 3.2) = 97.5:2.5, v/v] at a flow rate of 0.2 ml/min. The gradient program was conducted as follows: 0–30 min, 0–20% B; 30–31 min, 20–50% B; 31–45 min, 50% B. The lipid sample concentration for analysis of GPCho, GPEtn/LGPEtn, GPSer, GPIns and LGPCho was 30, 400, 1000, 1000 and 1000 μg/ml, respectively, and the corresponding injection volume was 5, 15, 10, 15 and 15 μl, respectively.

The HPLC system was coupled in-line with a hybrid API 4000 Qtrap (AB Sciex, Foster City, CA, USA) quadrupole-linear ion trap (QqLIT)