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Direct infusion mass spectrometric identification of molecular species of glycerophospholipid in three species of edible whelk from Yellow Sea

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ABSTRACT

Whelk has been exploited commercially as a delicacy for a long time. Although previous studies have suggested that whelk 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 the PL have not been reported yet. In this study, more than 220 molecular species of glycerophospholipid (GP) belonging to eight classes including glycerophosphocholine, glycerophosphoethanolamine, glycerophosphoserine, glycerophosphoinositol, lysoglycerophosphocholine, lysoglycerophosphoethanolamine, lysoglycerophosphoserine and lysoglycerophosphoinositol in three species of edible whelks (*Buccinium yokomaruae*, *Neptunea arthritica cumingi* Cosse and *Volutharpa ampullaceal*) were identified for the first time by using direct infusion tandem mass spectrometric method. Most of the predominant GP molecular species contained n-3 LC-PUFA, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Meanwhile, the whelk lipids contained a high proportion of PL (32.92–55.55% of total lipids) and PUFA (30.45–41.42% of total FA). Among PL, phosphatidylcholine (44.18–65.49 mol%) was dominant.

1. Introduction

Whelk is one of the abundant marine edible univalves, with the production of almost 334,000 tonnes in 2015 (http://www.fao.org/fishery/statistics/en). Most of the population is mainly concentrated in the Pacific and the Indian Ocean waters (Robertson, 2003). Whelks are currently fished throughout Asia, Europe, Central and South America, and then they are primarily exported to Europe, Japan, China and Korea where their meat is considered as a delicacy (Woodcock & Benkendorff, 2008). Whelks are predatory marine gastropods that are highly favoured for their high protein meat (Xavier Ramesh & Ayyakkannu, 1992). The nutritional value of whelks is high resulting from their high content of protein and are rich in certain physiologically and pharmacologically active substances such as polypeptides, polysaccharides and long chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFA) (Kideys & Hartnoll, 1991; Miletic, Miric, Lalic, & Sobajic, 1991; Saito & Hashimoto, 2010).

Among the major types of whelk components, lipids, with an approximate content of 10% (on a dry weight basis) have attracted increasing attention (Beach, Quilliam, & Hellou, 2009; Miletic et al., 1991). Triacylglycerols (TAG) and phospholipids (PL) are the two major lipid classes in whelk (Carrasco, Phillips, & Sewell, 2016; Oudejans & Van der Horst, 1974; Zarai et al., 2011). Normally, PL account for about 40% of total lipids, nearly equal to TAG (Zarai et al., 2011). Whelk is also rich in PUFA, which normally accounts for more than 40% of total fatty acids (FA). (Christie, Brechany, & Stefanov, 1988; Lee & Lim, 2005; Tang, Wei, Huang, Zou, & Yue, 2016). Moreover, previous studies have also indicated that the PL in whelk contain a high percentage of PUFA, in particular eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Saito & Hashimoto, 2010; Stuart, Gillis, & Ballantyne, 1998).

It is well accepted that n-3 LC-PUFA, especially EPA and DHA, play very important roles in many aspects of human health, in particular in reducing the risk of cardiovascular disease, inflammation, hypertension, allergies, immune and renal disorders (Le Grandois et al., 2009). Therefore, it is widely recommended that a daily consumption of 250–1000 mg of EPA/DHA provides health benefits (Kuratko & Salem,

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Table 1
Different class compositions (%) of the lipids recovered from three species of whelks.

Samples	PL	TAG	DAG	MAG	FFA	CHO
1	32.92 ± 0.51c	58.08 ± 0.59a	0.68 ± 0.06b	0.48 ± 0.06c	2.73 ± 0.29c	4.42 ± 0.24a
2	55.55 ± 0.06a	34.07 ± 0.34c	1.40 ± 0.08a	0.77 ± 0.03a	5.70 ± 0.34a	2.54 ± 0.15b
3	51.46 ± 0.17b	41.97 ± 0.34b	1.30 ± 0.02a	0.69 ± 0.02b	4.05 ± 0.09b	1.00 ± 0.02c

[a–c]Values in the same column with different lower-case letters are significantly different at $P < .05$. Samples 1–3 are lipids recovered from *Buccinium yokomaruae, Neptunea arthritica cumingi* Cosse and *Volutharpa ampullaceal* respectively. *Abbreviations are:* PL, phospholipid; TAG, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol; FFA, free fatty acid; CHO, cholesterol.

Table 2
Different phospholipid class compositions (mol%) of the lipids recovered from three species of whelks.

Samples	PC	PE	LPC	PI	PS	PG	PA
1	44.18 ± 0.02b	19.73 ± 0.01a	6.53 ± 0.01a	8.32 ± 0.02a	10.37 ± 0.02a	7.81 ± 0.01	3.07 ± 0.00
2	47.03 ± 0.02b	19.56 ± 0.02a	8.01 ± 0.01a	8.69 ± 0.01a	5.90 ± 0.01b	7.65 ± 0.01	3.14 ± 0.01
3	65.49 ± 0.01a	14.99 ± 0.01b	7.95 ± 0.00a	6.85 ± 0.01a	4.72 ± 0.00b	nd	nd

[a–c]Values in the same column with different lower-case letters are significantly different at $P < .05$. Samples 1–3 are lipids recovered from *Buccinium yokomaruae, Neptunea arthritica cumingi* Cosse and *Volutharpa ampullaceal*, respectively. *Abbreviations are:* PC, phosphatidylcholine; PE, phosphatidylethanolamine; LPC, lysophosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PG, phosphatidylglycerol; PA, phosphatidic acid.

Table 3
Different fatty acid compositions (%) of the lipids recovered from three species of whelks.

Fatty acid	1	2	3
C14:0	2.29 ± 0.12b	3.04 ± 0.19ab	3.55 ± 0.31a
C15:0	0.49 ± 0.03b	0.56 ± 0.07ab	0.66 ± 0.09a
C16:1n-7	4.24 ± 0.29b	6.44 ± 0.34a	7.05 ± 0.38a
C16:0	14.52 ± 1.16a	15.03 ± 0.79a	16.96 ± 0.15a
C17:1n-7	nd	0.23 ± 0.02b	0.32 ± 0.00a
C17:0	1.87 ± 0.31b	1.51 ± 0.14b	2.54 ± 0.36a
C18:4n-3	1.54 ± 0.27b	1.64 ± 0.05a	nd
C18:2n-6	1.04 ± 0.16b	1.62 ± 0.22a	1.86 ± 0.06a
C18:2n-5	nd	nd	0.94 ± 0.00
C18:1n-9	15.67 ± 0.10a	13.81 ± 0.31c	14.33 ± 0.07b
C18:0	5.15 ± 0.02b	5.17 ± 0.25b	6.53 ± 0.12a
C20:5n-3	11.65 ± 0.46b	9.38 ± 0.54c	13.62 ± 0.77a
C20:4n-6	3.01 ± 0.44a	2.58 ± 0.25a	1.41 ± 0.08b
C20:2n-9	0.98 ± 0.07a	0.44 ± 0.02b	0.53 ± 0.05b
C20:2n-7	nd	0.42 ± 0.01b	1.36 ± 0.12a
C20:1n-9	13.54 ± 0.44b	14.91 ± 0.47a	11.78 ± 0.35c
C20:1n-7	nd	4.12 ± 0.14b	5.36 ± 0.17a
C22:6n-3	12.43 ± 0.28a	7.17 ± 0.74b	6.54 ± 0.26b
C22:5n-3	3.37 ± 0.05a	3.68 ± 0.23a	2.52 ± 0.13b
C22:4n-6	0.94 ± 0.07a	0.37 ± 0.01b	0.44 ± 0.01b
C22:2n-9	2.83 ± 0.15b	3.47 ± 0.07a	1.68 ± 0.03c
C22:2n-6	3.48 ± 0.09b	3.82 ± 0.26a	nd
C22:1n-9	1.21 ± 0.04b	3.07 ± 0.33a	0.47 ± 0.02c
SFA	24.33 ± 0.15b	25.31 ± 0.09b	30.24 ± 0.23a
MUFA	34.24 ± 0.01b	40.18 ± 0.20a	39.04 ± 0.36a
PUFA	41.42 ± 0.20a	34.58 ± 0.16b	30.45 ± 0.11c

[a–c]Values in the same column with different lowercase letters are significantly different at $P < .05$. Samples 1–3 were lipids recovered from *Buccinium yokomaruae, Neptunea arthritica cumingi* Cosse and *Volutharpa ampullaceal* respectively, nd, not detected.

2013). The food sources of n-3 LC-PUFA are mainly distributed in TAG and PL. Recently, n-3 LC-PUFA in the PL form has captured increasing attention due to their higher bioavailability (Cook et al., 2016; Yurko-Mauro et al., 2015) and tissue-delivery capacity (Cansell, 2010; Liu et al., 2014; Rossmeisl et al., 2012). Therefore, n-3 LC-PUFA-enriched PL in whelk may account for much of its nutritional and healthful functions.

The fate of FA at *sn*-1/*sn*-2 position of oral PL *in vivo* is different. In the intestinal lumen, PL is cleaved into 1-lysophospholipid and free fatty acid (FFA) by activated pancreatic phospholipase A_2 in the presence of trypsin, calcium ions and bile salts (Iqbal & Hussain, 2009; Phan & Tso, 2001). The hydrolysed FFA from *sn*-2 position is re-synthesized into TAG in intestinal epithelial cell, thereby being transferred into the lymph and the blood, whereas 1-lysophospholipid is mainly re-synthesized into PL in intestinal epithelial cell, and then being transferred into the lymph and the blood (Cohn, Wat, Kamili, & Tandy, 2008). Therefore, the determination of molecular species of glycerophospholipid (GP) would be essential for further reveal the heath-beneficial effects of PL-containing PUFA *in vivo*. However, to the best of our knowledge, characterization of the molecular species of the GP in whelks has not been achieved.

Whelks *Buccinium yokomaruae*, *Neptunea arthritica cumingi* Cosse and *Volutharpa ampullaceal* are the main species of edible whelks and are potential candidate species for aquaculture and functional foods (Tang et al., 2016). For the first time, an efficient shotgun lipidomics strategy was established in the present study to determine the molecular species of GP belonging to eight classes including glycerophosphocholine (GPCho), glycerophosphoethanolamine (GPEtn), lycerophosphoserine (GPSer), glycerophosphoinositol (GPIns), lysoglycerophosphocholine (LGPCho), lysoglycerophosphoethanolamine (LGPEtn), lysoglycerophosphoserine (LGPSer) and lysoglycerophosphoinositol (LGPIns) in the three aforementioned whelks. For lipidomics research of PLs, current mass spectrometer (MS) based lipidomics method involves steps of lipid extraction, liquid chromatography (LC) separation (for LC–MS methods), and MS (or MS/MS) analysis (Wolf & Quinn, 2008). Currently, direct infusion-MS (or MS/MS) (shotgun) and LC–MS (or MS/MS) methods are reported in the scientific literature in almost equal ratio for lipidomics research. In this study, the shotgun lipidomics approaches that use direct infusion MS were used to determine GP molecular species due to their relative simplicity of operation and fast analysis. Meanwhile, the lipid content, lipid classes, PL classes and FA compositions were also determined. This will help to understand the specific health benefits of lipids from various whelks, as well as to provide theoretical basis for utilization of whelk as a novel source of functional food.

2. Materials and methods

2.1. Materials

Three species of fresh whelks including *Buccinium yokomaruae*, *Neptunea arthritica cumingi* Cosse and *Volutharpa ampullaceal* that captured from Yellow Sea were purchased from a local market in Dalian, Liaoning, China. The average body weights for specimens of *Neptunea arthritica cumingi* Cosse and *Volutharpa ampullaceal* were about 5.0 g, while that for *Buccinium yokomaruae* was 7.0 g. In total, bout 120