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Study on the fatty acid compositions of deep-sea isopod *Bathynomus doederleinii*

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Introduction

Deep-sea isopod *Bathynomus doederleinii* (Fig.1) is a crustacean inhabiting deep sea and commonly found at the sea around Japan. Deep-sea isopod is usually caught as a bycatch of basket trap fishing, and discarded at the sea because it has no commercial value. Therefore, to utilize the deep-sea isopod as a food resource, its basic nutritional information of the muscle and whole body was examined in our previous study. In the previous study, both muscle and whole body of the deep-sea isopod contained higher amount of crude total lipids than other edible crustacean's meat did. Marine organism is generally known as a major source of poly unsaturated fatty acids (PUFA), such as 20:5n-3 and 22:6n-3. However, no investigation exists on the lipids and fatty acids of deep-sea isopod. Thus, in the present study, the lipid class and its fatty acid composition of the deep-sea isopod were clarified.

Materials and methods

Sample preparation
Deep-sea isopod was caught in the East China Sea by basket trap fishing. All samples were transported to our laboratory under ice-storage condition. Muscle and stomach contents were separated from the whole body and put into the vials with the mixture of chloroform and methanol (2:1, vol/vol). The whole body was freeze-dried and put into the vials by the same manner with described in the above. The samples were enclosed with a nitrogen gas and kept at -30°C until use.

Lipid extraction and classification
Crude total lipids of muscle, stomach contents and whole body were extracted according to Folch *et al*. procedure [1]. A silica gel column was used for separating the crude total lipids of muscle and whole body into classes according to Osako *et al*. procedure [2] with slight modification. The first elute (chloroform/n-hexane, 2:3, vol/vol) was collected as steryl ester (SE). This was followed with: chloroform eluting the triacylglycerols (TAG); chloroform/ether (35:1, vol/vol) eluting the sterols; chloroform/ether (9:1, vol/vol) eluting the diacylglycerols; chloroform/methanol (9:1, vol/vol) eluting the free fatty acids; chloroform/methanol (1:1, vol/vol) eluting the phosphatidylethanolamine (PE); and chloroform/methanol (1:20, vol/vol) to elute the phosphatidylcholine (PC). Individual lipids from each lipid class were identified with authentic samples by comparison of *Rf* values using thin layer chromatography.

Preparation of fatty acid methyl ester and gas chromatography (GC) analysis
TAG, PE and PC thus obtained were boiled with methanol containing 1% of hydrochloric acid under reflux for 3h to prepare the fatty acid methyl ester (FAME). The FAME were purified using silica gel column by elution with chloroform/ether (10:1, vol/vol). Individual FAME was quantified by using GC-FID (GC-2014, Shimadzu Seisakusho Co., LTD, Kyoto, Japan) equipped with a capillary column (SUPELCOWAX^{TM}10, 30m×0.25mm×0.25μm film thickness, Supelco Japan Co., LTD, Tokyo, Japan). The temperature of the injector, column, and detector were maintained at 250°C, 205°C and 260°C, respectively. Helium was used as carrier gas. The total flow ratio was kept at 11.6 mL/min.

Preparation of 4,4-dimethyloxazoline derivatives and GC-mass spectrometry (GC-MS) analysis
To qualify the FAME, 4,4-dimethyloxazoline derivatives (DMOX) were prepared from the FAME of crude total lipids for muscle and whole body according to Ito *et.al* procedure [3]. An excess amount of 2-amino-2-methyl-propanol was added to a small amount of FAME, and a nitrogen gas was enclosed with the samples. The samples were heated at 180℃ for 18 hours to prepare DMOX. After extracting with *n*-hexane, DMOX were purified using silica gel column by elution with chloroform/ethanol (38:11, vol/vol). DMOX were qualified by using GC-MS (GC-MS

QP-2010 Ultra, Shimadzu Seisakusho Co., LTD, Kyoto, Japan). The temperature of the injector and column were maintained at 250°C and 205°C, respectively. Helium was used as carrier gas. The total flow ratio was kept at 11.6 mL/min.

Results

TAG was the dominant lipid class in the crude total lipids of muscle and whole body of the deep-sea isopod, and it contained high amount of monounsaturated fatty acids (MUFA), such as 18:1n-9, 16:1n-7 and 20:1n-9. The lipids in whole body contained high amount of phospholipids, including PE and PC. The phospholipids contained higher levels of polyunsaturated fatty acids (PUFA), including 20:4n-6, 20:5n-3 and 22:6n-3 than TAG did. The stomach contents of the deep-sea isopod were liquid and showed various color such as yellow, red and black depending on each individual. The fatty acids of the stomach contents mainly contained 16:0, 18:1n-9, 20:5n-3 and 22:6n-3.

Fig. 1. Deep-sea isopod (*Bathynomus Doederleinii*).

Discussion

Compared to other crustacean such as squilla species [4], skeleton shrimp [5], decapods [6], and Antarctic krill [7], muscle and whole body of deep-sea isopod contained high amount of crude total lipid. In both of muscle and whole body, TAG was mainly contained in the lipid class. This suggest that TAG is dominant storage lipids in the muscle and the whole body of deep-sea isopod. The content of 18:1n-9 was markedly higher than that of other crustaceans [4-7]. The stomach contents also showed high amount of 18:1n-9 and n-3 PUFA, including 20:5n-3 and 22:6n-3, than that of n-6 PUFA. In general, the lipid contents and fatty acids profile of marine organisms are affected by their diet, and recent research reported that herbivorous fish species contain high amount of n-6 PUFA, and their stomach contents also include high amount of n-6 PUFA [8]. From these results, it is suggested that deep-sea isopod is carnivorous animal and their high content of 18:1n-9 and n-3 PUFA is originated from their diet. These results suggest that deep-sea isopod is MUFA and PUFA-rich marine species and can be utilized as these lipids resource.

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