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Lipid and Fatty Acid Composition of the Antarctic Krill *Euphausia superba*

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Abstract: Total lipid content, lipid class and fatty acid composition of the Antarctic krill *Euphausia superba* collected from the water of King George Island, Antarctica during austral summer of 1997-1998, were investigated. The overall lipid content of *E. superba* was 72 mg/g dry mass similar to the reported values for most temperate species. The neutral lipid of *E. superba* was 29% of the total lipid and that of phospholipid was 71%. The majority of neutral lipid was triacylglycerols (31.6% of neutral lipids) while phosphatidyl choline (44% of phospholipid) was the most abundunt in phospholipids. The quantitative composition of the fatty acid in *E. superba* show consisting mostly of the saturated 16:0 (16.6% - 22.1%), along with the polyunsaturated 20:5(n-3) (20.6% - 22.1%) and 22:6(n-3) (14.9% - 16.9%) acids.

Key words: Antarctic krill (*Euphausia superba*), Polyunsaturated fatty acids (PUFA), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid).

1. Introduction

It has been reported that the n-3 series polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) are effective for the prevention and cure of thrombosis, atheroscleresis, and subsequent blood circulation disease by lowering plasma cholesterol and triglycerols (Kelly 1991). These fatty acids are also known to have a high nutritional value in the diet of many economically important mariculture species, increasing the overall health and growth as well as the disease resistance in scallops and salmonoid fish (Bell and Tocher 1989). PUFAs are contained in the fish oil of blue backed fish including herring and mackerel, however the primary producer of these fatty acids in fish is known to be marine microalgae, accumulated in the fish body through the food chain (Cohen 1986). Fish oil has a long history as a principle source for EPA production in the industry and health-care field. Still, there are some difficulties in the purification of EPA because of the highly complicated fatty acid composition of fish oil. In addition, the content of PUFAs other than EPA is very high, and a large amount of unwanted autooxidation by-products during transportation and storage of caught fish, also add to the problem. To alleviate the problem of getting EPA, there have been some attempts to produce EPA from EPA producing microalgae by a fermentation process instead of fish oil, but the cultivation of microalgae requires strictly controlled growth conditions such as light, oxygen and carbon dioxide levels, which can be economically inefficient. Recently, EPA producing bacteria were isolated from the intestine of blue backed fish (Yazawa *et al.* 1988; Cho and Mo 1999) providing another alternative way to obtain EPA.

Krill are any member of the crustacean suborder

Euphausiacea or of the genus Euphausia within that suborder. The name is sometimes also used to refer to *Euphausia superba*, an Antarctic type species. The Euphausiacea are shrimplike pelagic marine animals. Description of about 85 species have been reported and most of them have bioluminescent organs (photophores) underside of the body, making them visible at night. They are of great importance in certain regions of the sea as food for various fishes, birds, and whales, particularly blue whales and finback whales. Krill occur in vast swarms that may gather near the ocean surface or at depths greater than 2,000 m. The body of mature *E. superba* is about 5 cm long and translucent, with reddish brown blotches. The larvae pass through nine stages of development. Males mature in about 22 months and females in about 25 months. During a spawning period of about five and a half months the eggs are shed at a depth of about 225 m. The krill larvae gradually move toward the surface as they develop, feeding on microscopic organisms. From January to April swarms of *E. superba* in the Antarctic Ocean may contain as much as 20 kg of these animals per cubic metre. Because of their vast numbers and nutritive qualities, krill have been regarded as a potential food source for man (The Encyclopedia of Britanica 1997).

Krill (*Euphausia superba*) obtains food by filter feeding phytoplanktons such as dinoflagellate or diatoms. The use of krill as human food is not very high, however their estimated biomass is about few thousands million tons and considered as new food for human in the future. The research concerning krill as a food source is concentrated on their nutritional value as protein sources. Nevertheless considering the fact that krill consumes microphytoplanktons as their main food source, their lipids can be a significant source for polyunsaturated fatty acids such as EPA (eicosapentaenoic acid) or DHA (docosahexaenoic acid) which are reported to be main fatty acid groups in microalgae. In this paper, results of lipids and fatty acid composition of krill collected in January 1998 from the water of Weaver Peninsula cross the Marian Cove near the King Sejong Station are reported.

2. Materials and Method

Chemicals

Analytical grade of n-hexane, acetyl chloride, chloroform, methanol, ethanol, diethyl ether, and other solvents used for lipid manipulation were purchased from Merck Co (Germany). BHT (butyl hydroxy toluene), aliphatic fatty acids standards, long chain aldehydes, and phospholipid standard such as phosphatidyl choline, phosphatidyl ethanol amine, phosphatidyl serine were purchased from Sigma Chemical Co (USA). All other chemicals and solvents used were also analytical grade.

Total lipid and lipid class analysis

Live krill (*Euphausia superba*) samples were collected from the shore water of Weaver Peninsula, King George Island during the austral summer expedition of 1997-1998 (Fig. 1). Collected krill were transported to King Sejong Station and stored frozen at -20 ℃, then kept frozen in dry-ice for transportation to Korea Ocean Research & Development Institute (KORDI) for analysis. About a 20 g krill sample was freeze-dried and determined its dry weight, then the samples were extracted with 30 volume of Folch solution (chloroform:MeOH = 2:1, v/v) for 3 times (Folch *et al.* 1957). All extracts were combined and then back-extracted with 0.88% KCl solution to remove non-lipid materials. The sample was dried with anhydrous Na_2SO_4, and excess solvent was removed with rotary evaporator under vacuum in pre-weighed pear flask to determine the weight of extractable total lipid.

Total extracted lipid was separated into neutral lipid and phospholipid classes with silica gel column chro-

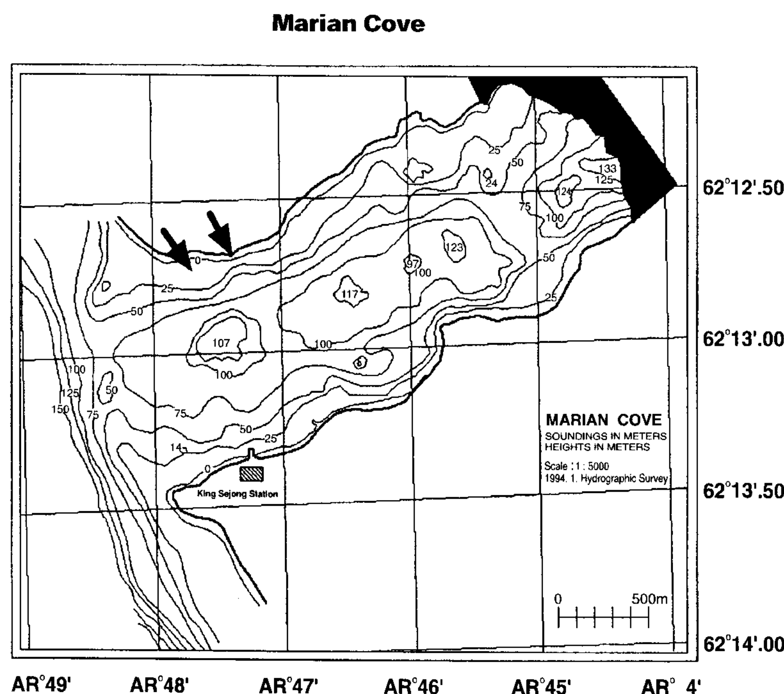

Fig. 1. Sampling site map of krill. Collection sites were marked with arrows.

matography. Total lipid (about 10 mg) was dissolved in chloroform and loaded onto silica gel column (0.6 × 8.0 cm), washed with 10 bed volume of chloroform (flow rate : 2 ml/min) to elute neutral lipids and then washed with 10 bed volume of methanol to elute phospholipids. Each lipid fractions were concentrated with rotary evaporator and the weight were determined gravimetrically.

Lipid composition was analyzed with TLC-FID (Thin Layer Chromatography with Flame Ionization Detector, Iatron Co., Japan) with a solvent system hexane:diethyl ether:formic acid (80:20:0.2, v/v) for separating neutral lipids, and chloroform:MeOH:water:AcOH (65:30:5:1, v/v) for phospholipids. About a 50 μg lipid mixture was loaded onto Chromarod-3 TLC rod (Iatron Co., Japan) and developed first with neutral lipid solvent and partially scanned with FID to obtain the composition of neutral lipid. After the scanning the chromarod was developed again in a phospholipid solvent system and second scanning for phospholipid analysis. The integrated area was calibrated with known amount of standard lipids, such as triacyl glycerol, diacyl glycerol, phosphatidyl choline, phosphatidyl ethanolamine and so on and amount of each lipid was calculated.

Fatty acid analysis

For the preparation of free fatty acid, total lipid was saponified with 5% KOH solution in ethanol. After incubating in a sealed vial at 80 ℃ for 2 hours, water (5ml) and diethyl ether (5 ml) were added for extraction and the mixture was centrifuged for separating organic phase from aqueous phase. Ether layer was evaporated and the unsaponifiable materials were redissolved in ethanol. The remaining water layer was acidified with 1 N HCl to pH 2 and then extract with diethyl ether (3 × 5 ml) to obtain free fatty acids. The fatty acid fraction was transmethylated with 5% methanolic HCl for 1 hr at 60 ℃ and the constituted fatty acid methyl esters were extracted with n-hexane (3 ×5 ml) which were concentrated with a speed-vac

concentrator (Vision Co., Korea)

Fatty acid methyl ester was analyzed with gas chromatography (Hewlett-Packard, HP5890II) equipped with

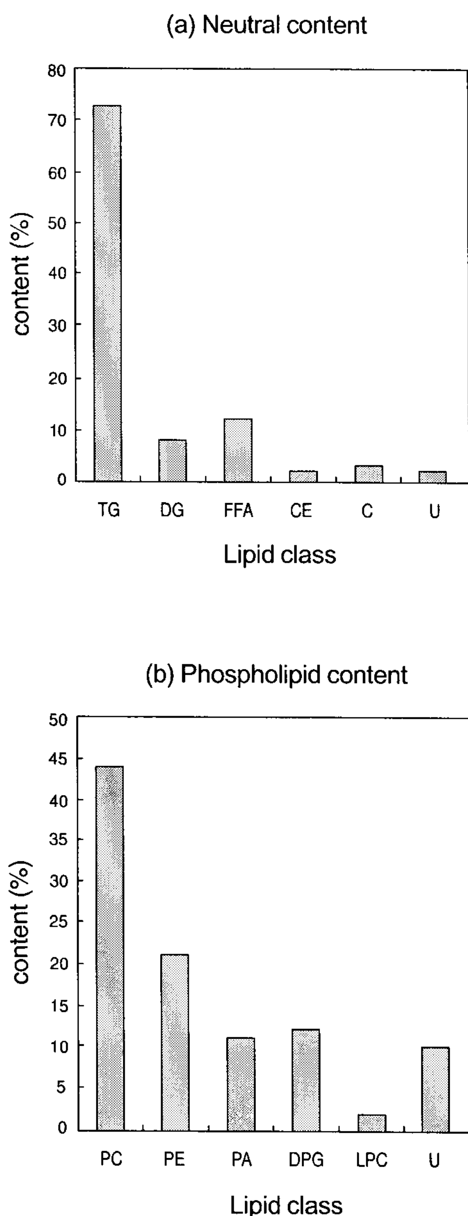

Fig. 2. The composition of (a) neutral lipid and (b) phospholipid of krill. TG: triacyl glycerol, DG: diacyl glycerol, C: cholesterol, CE: cholesterol ester, FFA: free fatty acid, PC: phosphatidyl choline, PE: phosphatidyl ethanol amine, PA: phosphatidic acid, DPG: diphosphogly ceride, LPC: lysophosphatidyl choline, UI: unidentified.

chromate data aquisition interface (Interface Co., Korea). Omegawax-320 capillary column (30 m × 0.32 mm) was used with oven temperature at 200 ℃. The temperatures of injector and detector (FID) were 250 ℃ and 260 ℃, respectively. Identification of fatty acids was performed by comparing to authentic standards as well as equivalent chain length (ECL) calculation (Christie 1989).

3. Results and Discussion

About a 2 kg sample of live krill, *E. superba* was collected in January 1998 near Weaver Peninsula in Marian Cove and processed for lipid content analysis. The length of each animal was about 3 - 4 cm long (mean = 34 mm, SD = 0.4 mm, n = 10) and about 1.8 - 2.5 gram (mean = 2.1 g, SD = 0.2 gram, n = 10) in weight. Considering adult krill can reach up to 5 - 6 cm long, this collection might represent an immature state. The dry weight was about 24% of wet weight. The amount of lipid extracted from krill was about 72 mg/g dry weight, and 29% of whole lipid was in the form of neutral lipid and 71% as phospholipids. The ratio of neutral lipids to phospholipids showed the nutritional status of organism and the fact that most of the lipid material in krill was in the form of phospholipids which are the usual constituent of cell membrane is unexpected considring the time of sampling, i.e. austral summer when the population of microalgae is the highest for the year. So it may be better assumed that krill is in good the nutritional condition of krill and energy could be stored in the form of neutral lipid.

Among the neutral lipids, triacylglycerol (75%) was the most abundant followed by cholesterol (12%) and diacyl glycerol (8%). Triacylglycerol has been known as the major storage lipid in many marine and terrestrial organisms, although wax esters occur frequently as the major neutral lipid in many polar zooplankton (see the review by Clarke 1983). In this study, wax ester was found little amount among Antarctic krill in early January. Free fatty

acids were also detected (3%) and less amount of cholesterol esters were detected (2%). The storage lipids may increase in the end of summer season, albeit, the lipid metabolism is not considered to be the major mechanism for energy production during long term starvation as is usually the case in most other zooplankton. Two main phospholipids were phosphatidyl choline (PC; 44% of total phospholipid) and phosphatidyl ethanolamine (PE; 21%). The content of phosphatidic acid (PA) and diphosphoglyceride (DPG) were 11% and 12% of total phospholipid, respectively. A small amount of lysophosphatidyl choline (2%) was also detected. Lysophospholipids such as lysophosphatidyl choline and lysophosphatidyl ethanolamine are usually not the typical lipid component, insteads they are produced soon after death from the hydrolysis of corresponding phospholipids by enzyme, lipase or phospholipase or from the autohydrolysis during the storage. There are reports suggesting some phospholipase can be active even in subzero temperatures. The finding of free fatty acids in neutral lipids fraction may indicate such hydrolysis of phospholipids.

Table 1. The fatty acid composition of krill (*Euphausia superba*) collected during austral summer of 1997-1998.

Major fatty acids	Content of fatty acid (%)		
	total Kril powder	Neutral lipid	Phospholipid
14:0 (Myristic acid)	5.44	6.85	5.32
16:0 (Palmitic acid)	19.95	16.59	22.12
16:1 (n-7, Palmitoleic acid)	4.51	3.35	3.12
18:0 (Stearic acid)	12.37	1.34	1.03
18:1 (n-9, Oleic acid)	4.07	8.28	5.44
18:1 (n-7)	5.25	5.83	5.54
18:2 (n-6, Linoleic acid)	1.69	2.06	1.99
18:3 (n-3, Linolenic acid)	0.71	3.48	2.89
18:4 (n-3, ODTA)	1.10	7.01	5.19
20:5 (n-3, EPA)	16.14	22.14	20.59
22:6 (n-3, DHA)	14.09	14.86	16.86
other fatty acids	14.68	8.21	9.91
Saturated fatty acid	37.76	24.78	28.47
Polyunsaturated fatty acid	33.73	47.49	45.53
P/S ratio	0.89	1.92	1.60
	85.32	91.79	90.09

As a preliminary analysis of fatty acid composition of *E. superba*, a commercial krill powder sample purchased from a local market in Chile was analyzed for its fatty acid composition (Table 1). The concentration of EPA and DHA were 16.1 and 14.1% of total fatty acid, respectively, which is high in content, a good source of n-3 polyunsaturated fatty acids. Other major fatty acids were palmitic acid (19.6%) and stearic acid (12.4%) followed by 5.3% of 18:1(n-7) and 5.4% of 14:0. The content of monounsaturated fatty acids (18:1 and 16:1) was 13.6%. Total saturated fatty acid was 37.8% of total fatty acid and n-3 polyunsaturated fatty acid was 33.7% showing P/S ratio (polyunsaturated fatty acid/saturated fatty acid) of 0.89, a relatively high value. Unidentified fatty acid concentration in the krill powder was very high (14.7%) which seemed to be autooxidized fatty acid derivatives due to the prolonged storage time of krill powder in ambient temperature, or it may be due to unknown ingradients which may have been added during manufacturing process. Moreover, polyunsaturated fatty acids are very labile to autooxidation and in this sample the amount of 18:3 (n-3) was very low (0.7%).

For a fresh krill sample, fatty acid composition of phospholipid class and neutral lipid class were investigated separately. The fatty acid composition showed similar patterns in both cases. The major fatty acids were EPA (22.1%), palmitic acid (16.6%) and DHA (14.9%) for neutral lipids and palmitic acid (22.1%), EPA (20.6%) and DHA (16.9%) for phospholipids. This indicated that the distribution of n-3 series fatty acid was probably in random and no specific function were performed in cell membranes. The major source of the n-3 polyunsaturated fatty acid might be their food, such as phytoplanktons rich in n-3 fatty acids. The P/S ratio of fresh krill was comparatively high, i.e. 1.92 in neutral lipid and 1.60 in phospholipid suggesting that krill is very promising source of n-3 series fatty acids. The difference in fatty acid composition between fresh krill and krill powder was the content of stearic acid (12.4% in krill powder while 1.3 and 1.0% in

neutral and phospholipid in fresh krill, respectively). The difference may have been influenced by the time of krill collection, i.e. season, physical state or by addition of another lipid source into krill powder during industrial manufacturing. Another important fatty acid in fresh krill was relatively high in content (5.2% and 7.0% in phospholipid and neutral lipid, respectively) of octadecatetraenoic acid (ODTA, 18:4, n-3) which is regarded as an intermediate in biosynthesis of EPA from linolenic acid (18:3, n-3). Also, linolenic acid (18:3, n-3) was higher than the krill powder sample (2.9 % and 3.5 % in phospholipid and neutral lipid, respectively, compared to 0.7 % of krill powder).

PUFAs concentration in filter-feeding marine animals are known to vary with phytoplankton composition in water column (see the review by Sargent 1976), because the majority of naturally occurring PUFAs, especially, 20:5(n-3) and 22:6(n-3) can only be synthesized by phytoplankton (Sargent 1976) and bacteria. The fatty acid composition of *E. superba* was also reflected dietary input of microalgal food organisms. During the period of krill collecting (January of 1998), nanoflagellates (>2 - <20 μm), *Cryptomonas* spp. (Cryptophyceae) was the predominant species (60% of microalgal carbon biomass, Ahn *et al.*, in press) in the water column at the *E. superba* sampling site. *Cryptomonas* spp. belong to Cryptophyceae which were reported to be rich in 16:0, 18:3(n-3) and 20:5(n-3) (Sargent 1976).

The importance of EPA and other n-3 series fatty acids in diet was raised by the pioneering work of Dyberg *et al.* (1978). They found the content of cholesterol and triglyceride in blood were 233 mg and 57 mg in average Eskimo while 273 mg and 129 mg in Danes, and the P/S ratio of 0.8 in the daily food of Eskimo while only 0.2 in that of Danes which were reflected the occurrence of disease in blood circulation system. The polyunsaturated fatty acids like EPA and DHA are quite ubiquitous in marine organisms. Although the exact function of these fatty acids is not elucidated clearly, the primary role in marine organisms is to maintain the fluidity of cell membrane in cold temperature. The physiological function of EPA has been studied by many research groups reporting the possible function as an antagonistic metabolite in the formation of eicosanoids such as prostaglandins, tromboxans, and leukotrienes.

The most available source of n-3 fatty acid such as EPA and DHA is blue backed fish such as mackerel. The content of EPA and DHA are relatively high (10 - 30% of total fatty acid) and such fish are comparatively inexpensive. However, the very complicated fatty acid composition of fish oil made it very difficult to obtain pure n-3 fatty acid higher than 95% purity. EPA production from marine microorganism is another source, several research groups had already reported EPA producing marine bacteria (DeLong and Yayanos 1986; Yazawa *et al.* 1988; Nichols *et al.* 1993; Cho and Mo 1999) and microalgae (Seto *et al.* 1984) as future candidates for industrial production of EPA. Moreover, EPA is produced as the sole PUFA in these bacteria for some unknown reason and the amount of other PUFA is almost negligible, which make these bacteria a promising source of PUFA through biotechnological process such as fermentation. More importantly, genetic engineering using these bacteria are promising because of much smaller genome size of bacteria than eukaryotic microalgae permitting easier genetic manipulation.

The fatty acid composition of krill was relatively simpler than many of blue-backed fish, thus easier purification of EPA and DHA from the total fatty acid of krill. Better medicinal effect of pure EPA than crude fish oil for blood viscosity and red cell deformability (Singer *et al.* 1984; Terano *et al.* 1983) and the approval of EPA as a medicine for hypercholesteremia disease by FDA (USA) at 1997 have increased the demand of pure EPA. The use of krill as a source of EPA accompanies several obstacles. Although the estimated amount of krill is in the range of a few billion tons, the capture and storage of krill is another problem. The fast decay of krill and the long distance from Antarctic

sea should be overcome. After solving these problems in capturing and manufacturing, krill can be a promising source of n-3 polyunsaturated fatty acids as well as protein in near future.

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