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Lipid, Sterol and Fatty Acid Composition of Antarctic Krill (*Euphausia superba* Dana)

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ABSTRACT

The lipid classes, fatty acids of total and individual lipids and sterols of Antarctic krill (*Euphausia superba* Dana) from two areas of the Antarctic Ocean were analyzed by thin layer chromatography (TLC), gas liquid chromatography (GLC) and gas liquid chromatography/mass spectrometry (GLC/MS). Basic differences in the lipid composition of krill from the Scotia Sea (caught in Dec. 1977) and krill from the Gerlache Strait (caught in Mar. 1981) were not observed. The main lipid classes found were: phosphatidylcholine (PC) (33-36%), phosphatidylethanolamine (PE) (5-6%), triacylglycerol (TG) (33-40%), free fatty acids (FFA) (8-16%) and sterols (1.4-1.7%). Wax esters and sterol esters were present only in traces. More than 50 fatty acids could be identified using GLC/MS, the major ones being 14:0, 16:0, 16:1(n-7), 18:1(n-9), 18:1(n-7), 20:5(n-3) and 22:6(n-3). Phytanic acid was found in a concentration of 3% of total fatty acids. Short, medium-chain and hydroxy fatty acids ($C \leq 10$) were not detectable. The sterol fraction consisted of cholesterol, desmosterol and 22-dehydrocholesterol.
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INTRODUCTION

Krill (*Euphausia superba* Dana) lives exclusively in cold Antarctic waters and is the central link in the Antarctic food web. Its general chemical and biochemical composition has been the subject of several investigations (1). A number of contributions also have dealt with the lipid content and lipid composition of this pelagic euphausiid. Lipid contents between 1% and 6% have been published (2), and remarkably differing data have been reported for lipid composition (3-12). The main lipid classes found by almost all investigators were phosphoglycerolipids, triacylglycerols (TG), free fatty acids (FFA) and free sterols. The dominating fatty acids reported were 16:0 among saturated fatty acids and 18:1, 20:5 and 22:6 among unsaturated and polyunsaturated fatty acids. This investigation has been carried out to give thorough and complete analyses of lipid classes, fatty acids and sterols, supported by mass spectrometry (MS).

MATERIALS AND METHODS

Sample Collection and Preparation

Antarctic krill were collected from the Scotia Sea on December 16, 1977 at 57° 47′ S; 42° 43′ W (13) and from the Gerlache Strait on March 12, 1981 at 64° 33.7′ S; 62° 32′ W (14) during the second (1977/78) and third (1980/81) Antarctic expeditions of the Federal Republic of Germany with FMS "Julius Fock" and FRV "Walther Herwig," respectively, using a 1219 mesh pelagic Krill net.

Krill samples of 5 kg were quick-frozen and stored at −35 C until analyzed. Subsamples prepared from the core of the 5 kg samples were homogenized in a mortar under liquid nitrogen, and lipid extraction was performed according to Folch et al. (15). Lipids were dissolved in dichloromethane: methanol 1:1 (v/v) and stored under a nitrogen atmosphere at −23 C.

Thin Layer Chromatography and Gas Liquid Chromatography

Crude lipids were separated into classes by TLC on HPTLC-plates (E. Merck, Darmstadt) developed with n-hexane:diethylether:glacial acetic acid 60:40:1 (v/v) for neutral lipids, and with dichloromethane:methanol:glacial acetic acid 60:30:10 (v/v) or dichloromethane:methanol:aqueous ammonia 60:20:5 (v/v) for polar lipids. Lipid classes were visualized by exposure to iodine vapor or by charring with 50% sulphuric acid. After 2 dimensional TLC using the above mentioned solvents, identification was achieved by comparison with standard mixtures and lipid class specific stainings (16). After the silica gel was scraped off, the eluted acylglycerols were quantified by an enzymatic test for esterified glycerol (E. Merck, Darmstadt), and phosphoglycerides by phosphorus determination (17). FFA and sterols were determined by GLC using heptadecanoic acid and stigmasterol, respectively, as internal standards.

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Fatty acid methyl ester (FAME) of total lipids and individual lipid classes were prepared with 14% boron trifluoride in methanol (18), and fatty acid benzyl esters (FABE) according to Klemm et al. (19). Trimethylsilylation of sterols was carried out as described by Ballantine et al. (20). FAME and FABE were purified by TLC prior to GLC analysis. Separations and identifications were carried out on a polar wall coated (WCOT) open-tubular glass column (25 m) coated with SILAR 10 C (Packard instruments), temperature programmed from 110 C to 210 C (3 C/min) and on a 50 m fused silica column (WCOT) coated with CP SIL 5, temperature programmed from 100 C to 320 C (3 C/min) using a Packard 428 gas chromatograph equipped with a FID and a HP 3371 integrator. Helium was used as carrier gas at a flow of 1 ml/min with a split ratio of 100:1. The presence of plasmalogens and alkylglycerols was tested subsequent to hydrolysis using the procedure of Pugh et al. (21).

GLC/MS analysis of FAME and trimethylsilyl (TMS) sterols was performed on a HP 5985A quadrupole mass spectrometer, ionization energy 70 eV, ion source temperature 200 C, column: 25 m WCOT coated with CP SIL 5 (Chrompak), temperature programmed from 140 C to 280 C (4 C/min).

Individual FAME, FABE and TMS sterol peaks were identified by co-chromatography with standards, by comparison with calculated equivalent chain length (ECL) values (22) and by mass spectra. To ensure identification of unusual fatty acids, samples were hydrogenated and rechromatographed. For positional analysis, cleavage of PC and PE was performed with phospholipase A_2 from *Crotalus durissus terrificus* (Boehringer, Mannheim). After 24 hr incubation in diethylether and 0.1 M tris-buffer, the reaction mixture was separated by TLC into lysophospholipids and FFA.

RESULTS AND DISCUSSION

Lipid Content and Lipid Composition

The total lipid content and the lipid composition data of the 2 krill samples are given in Table 1. Although different lipid compositions have been published, there is general agreement as to the main lipid classes present in *Euphausia superba* (3-12). The krill caught in December 1977 has a lower fat content than the krill caught in March 1981. This increase in fat content during the catching season, which coincides with the sexual maturity (2) of krill, has been shown previously (14). Beginning with a low fat content of approx. 1% on a wet weight basis in November/December, the fat content increases to approx. 6% in March/April.

TABLE 1

Lipid Composition of Antarctic Krill (*Euphausia superba* Dana)

Sample	12/1977	3/1981
Total lipid content (% wet weight)	2.7 ± 0.2	6.2 ± 0.3
Phospholipids		
Phosphatidylcholine	35.6 ± 0.1	33.3 ± 0.5
Phosphatidylethanolamine	6.1 ± 0.4	5.2 ± 0.5
Lysophosphatidylcholine	1.5 ± 0.2	2.8 ± 0.4
Phosphatidylinositol	0.9 ± 0.1	1.1 ± 0.4
Cardiolipin	1.0 ± 0.4	1.6 ± 0.2
Phosphatidic acid	0.6 ± 0.4	
Neutral lipids		
Triacylglycerols	33.3 ± 0.5	40.4 ± 0.1
Free fatty acids[a]	16.1 ± 1.3	8.5 ± 1.0
Diacylglycerols	1.3 ± 0.1	3.6 ± 0.1
Sterols	1.7 ± 0.1	1.4 ± 0.1
Monoacylglycerols	0.4 ± 0.2	0.9 ± 0.1
Others[b]	0.9 ± 0.1	0.5 ± 0.1
Total	98.9	99.3

Data are expressed as wt % of total lipids and represent means ± standard deviation of 3 separate experiments.

[a]Probably mostly artifacts.

[b]Traces of lysophosphatidylethanolamine, phosphatidylserine, sphingomyelin, glycolipids, sterol esters, waxes and carotenoids were detected.

Euphausia superba is extremely rich in phospholipids (≥40% of total lipids) and TG (33 and 40% respectively of total lipids). While the relative content of phospholipids is similar in the 1977 and 1981 samples, the percentages of TG differ somewhat. This is in accordance with the previous results of our laboratories (23), which show that the relative phospholipid concentration will not change with varying total lipid contents. In other marine organisms an increase of total lipid content usually is caused by an increase of TG (24).

The sterol contents of 1.4% and 1.7% respectively of total lipids are in the range which has been reported (2,25) for Krill. These are very low values compared with those of Clarke (3), who found up to 16.9% sterols of total lipids in krill from South Georgia. This difference may be due to the methods. Clarke used densitometry (3) and our laboratory GLC.

In the 1977 sample the FFA content is about twice that of the 1981 sample. The high value could be caused by the longer storage time of the 1977 sample. A residual lipolytic activity against phospholipids exists even at temperatures of −30 C and below. Samples of

the same haul which were cooked on board immediately after hauling and stored under the same conditions showed a FFA content which was much lower, ranging from 1% to 3% of total lipids. This low FFA content of freshly caught krill also was confirmed by Ellingsen (11).

In addition, lysophosphatidylcholine, lysophosphatidylethanolamine, phosphatidylinositol phosphatidic acid, cardiolipin and mono- and diacylglycerols were detected, whereas phosphatidylserine, sphingomyelin, glycolipids, wax esters and sterol esters were present only in trace amounts. Wax esters were found by Bottino (8) in the euphausiid *Euphausia crystallorophias* but not in *Euphausia superba*. The composition of carotenoids was not investigated but had been analyzed by others (26-28).

Fatty Acid Composition of Total Lipids

The composition of the fatty acids of total lipids of *Euphausia superba* is similar to that of other marine crustaceans and some marine fishes (29) (Tables 2 and 3). The main fatty

TABLE 3

Branched Chain Fatty Acid Composition of Total Lipids of *Euphausia superba* Dana

Sample	M+	ECL	12/1977	3/1981
13:0 i	228	12.6	tr.	n.d.
14:0 i	242	13.6	0.05 ± 0.01	n.d.
15:0 i	256	14.6	0.19 ± 0.00	0.31 ± 0.15
15:0 ai	256	14.7	0.21 ± 0.01	0.24 ± 0.07
16:0 i	270	15.6	0.09 ± 0.03	0.10 ± 0.06
17:0 i	284	16.6	0.54 ± 0.05	0.20 ± 0.02
17:0 br[a]	284	16.4	tr.	0.09 ± 0.02
17:1 br	282	16.5	0.05 ± 0.03	0.11 ± 0.08
17:1 br	282	16.2	tr.	0.10 ± 0.05
18:0 i	298	17.6	tr.	0.10 ± 0.01
Phytanic[b] acid	326	17.7	2.82 ± 0.41	1.2 ± 0.43

Data are expressed as wt % of total fatty acids and represent means ± standard deviation of 3 separate experiments.

tr. = trace; n.d. = not detected; br. = branched; i = iso; ai = anteiso.

[a]Presumably 7-methylhexadecanoic acid.

[b]3,7,11,15-tetramethylhexadecanoic acid.

TABLE 2

Fatty Acid Composition of Total Lipids of *Euphausia superba* Dana

Sample	M+[a]	ECL[b]	12/1977	3/1981	Sample	M+[a]	ECL[b]	12/1977	3/1981
10:0	186	10.0	tr.	tr.	18:4(n-3)	290	17.4	0.67 ± 0.07	0.62 ± 0.49
11:0	200	11.0	tr.	tr.	19:0	312	19.0	tr.	0.11 ± 0.16
12:0	214	12.0	0.23 ± 0.06	0.22 ± 0.06	19:1	310	18.8	0.12 ± 0.04	0.20 ± 0.09
13:0	228	13.0	0.04 ± 0.01	0.07 ± 0.04	19:2	308	18.7	tr.	0.07 ± 0.05
14:0	242	14.0	11.33 ± 1.48	15.23 ± 2.31	20:0	326	20.0	0.04 ± 0.00	0.19 ± 0.14
14:1	240	13.8	tr.	0.19 ± 0.01	20:1(n-7)	324	19.8	0.40 ± 0.01	0.50 ± 0.09
15:0	256	15.0	0.34 ± 0.01	0.27 ± 0.05	20:1(n-9)	324	19.7	0.77 ± 0.04	1.35 ± 0.23
15:1	254	14.8	tr.	0.04 ± 0.03	20:2	322	19.6	tr.	0.08 ± 0.06
16:0	270	16.0	25.91 ± 2.33	31.79 ± 1.73	20:4(n-3)	318	19.5	0.46 ± 0.10	0.22 ± 0.06
16:1(n-7)	268	15.7	7.26 ± 0.35	7.37 ± 0.34	20:5(n-3)	316	19.3	12.71 ± 1.57	7.83 ± 1.27
16:1(n-?)	268	15.8	0.09 ± 0.13	0.30 ± 0.01	21:0	340	21.0	tr.	tr.
16:2(n-6)	266	15.6	0.82 ± 0.01	0.12 ± 0.06	21:5(n-3)	330	20.2	0.42 ± 0.03	0.30 ± 0.18
16:3	264	15.5	tr.	0.29 ± 0.01	22:0	354	22.0	0.14 ± 0.03	tr.
16:4(n-3)	262	15.4	0.74 ± 0.06	0.48 ± 0.14	22:1(n-7)	352	21.6	0.29 ± 0.17	0.41 ± 0.16
17:0	284	17.0	0.06 ± 0.02	0.17 ± 0.15	22:1(n-9)	352	21.5	0.51 ± 0.06	1.22 ± 0.33
17:1	282	16.7	tr.	0.41 ± 0.05	22:5(n-3)	344	21.2	0.54 ± 0.09	0.24 ± 0.11
17:1	282	16.8	tr.	0.12 ± 0.06	22:5	344	21.4	tr.	0.04 ± 0.03
18:0	298	18.0	1.21 ± 0.18	2.14 ± 0.23	22:6(n-3)	342	21.1	5.41 ± 0.51	2.60 ± 0.79
18:1(n-7)	296	17.8	8.32 ± 0.54	7.49 ± 0.79	23:1	366	22.5	tr.	0.11 ± 0.07
18:1(n-9)	296	17.7	10.13 ± 2.20	10.52 ± 0.90	24:0	382	24.0	tr.	tr.
18:1(n-?)	296	17.9	tr.	0.09 ± 0.05	24:1	380	23.6	tr.	0.15 ± 0.11
18:2(n-6)	294	17.6	1.58 ± 0.09	0.74 ± 0.38	25:0	396	25.0	tr.	tr.
18:3(n-3)	292	17.6	0.47 ± 0.02	0.33 ± 0.07					
18:3(n-6)	292	17.3	0.21 ± 0.06	0.57 ± 0.35	Others[c]	–	–	3.95	2.45

Data are expressed as wt % of total fatty acids and represent means ± standard deviation of 3 separate experiments.

tr. = trace.

[a]M+: molecular weight of fatty acid methyl ester as determined by GLC/MS.

[b]ECL: equivalent chain length, calculated by plotting chain length (as carbon number) versus retention time on CP SIL 5.

[c]Predominantly branched chain fatty acids as given in Table 3 in detail.

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