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Article

Identification of Lipid Components in the Abdominal Muscle of Fall-Caught *Crangon crangon* from a Coastal Area of the Baltic Sea

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O principal objetivo deste trabalho foi determinar as classes de lipídios principais e as composições de ácidos graxos (FFAs) em triacilglicerols (TAGs) e fosfolipídios de camarões adultos *Crangon crangon* em zonas costeiras do Golfo de Gdansk (Mar Báltico, Polônia). Cromatografia líquida de alta eficiência com detector de epalhamento de luz laser (HPLC-LLSD) foi utilizada na separação de classes de lipídios. A composição de FFAs foi determinada por cromatografia gasosa-espectrometria de massas (GC-MS) e espectrometria de massas por tempo de voo com dessorção de matriz assistida por laser (MALDI-TOF-MS). As seguintes classes de lipídios foram separadas usando HPLC-LLSD: TAGs, FFAs, esteróis (ST) e lipídios polares (PL). As análises por HPLC-LLSD mostraram a prevalência de esteróis, não identificados por MALDI-TOF-MS. Esteróis representaram 2,5 mg g^{-1} de lipídeos totais com base nesta análise. TAGs e FFAs foram em seguida as classes de lipídios mais abundantes, contabilizando 1,9 e 2,0 mg g^{-1} de lipídeos totais, respectivamente.

The main aim of this work was to determine the principal lipid classes and compositions of fatty acids (FFAs) in triacylglycerols (TAGs) and phospholipids in adult shrimps *Crangon crangon* from coastal areas of the Gulf of Gdańsk (Baltic Sea, Poland). High performance liquid chromatography with a laser light-scattering detector (HPLC-LLSD) was used to separate the lipid classes. The FFA composition was determined with gas chromatography-mass spectrometry (GC-MS) and matrix-assisted laser-desorption time-of-flight mass spectrometry (MALDI-TOF-MS). The following lipid classes were separated using HPLC-LLSD: TAGs, FFAs, sterols (ST) and polar lipids (PL). The HPLC-LLSD analysis showed the dominance of sterols, which were missing from MALDI-TOF-MS, sterols made up 2.5 mg g^{-1} of the total lipids on the basis of this analysis. TAGs and FFAs were the next most abundant lipid classes, accounting 1.9 mg g^{-1} and 2.0 mg g^{-1} of the total lipids, respectively.

Keywords: *Crangon crangon*, lipid components, HPLC-LLSD, GC-MS, MALDI-TOF

Introduction

Lipids include fatty acids, their derivatives, and substances biosynthetically or functionally related to these compounds.[1] They form dynamic bilayer membranes and are the main source of energy.[2-4] Fatty acids are basic structural components of complex lipids, such as phospholipids and glycolipids. Their derivatives are hormones and intercellular informers, and they interface with proteins as their lipophilic modifiers. Fatty acids can be saturated, monounsaturated and polyunsaturated.[5] Those FFAs that cannot be synthesized by mammals are called essential fatty acids (EFAs), they are of great importance in living organisms.[6,7] Plants and marine organisms contain polyunsaturated, long-chained omega-3 fatty acids (n-3 PUFA).[6] In 1940s, Sinclair[8] described the connection between a diet rich in polyunsaturated fatty acids from sea fish and low mortality from cardiovascular causes in a population of Eskimos. The next important contributions were the studies of Bang and Dyerberg among the Greenland Inuits.[8] The authors demonstrated the ability of PUFA to reduce serum levels of triacylglycerols, chylomicrons and low density-lipoprotein (LDL) cholesterol.[9] Omega-3 FFAs are responsible for the development and proper functioning of the central nervous system by reducing depressions and anorexia nervosa.[6] They are useful in treating Alzheimer's disease in mice, in the prevention of cardiovascular

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disease and cancer.[10-12] The daily demand for omega-3 acids is about 1.0-1.5 g, but some sources recommend up to 2 g for adult men.[13] The diet of North Americans, which is very rich in n-6 PUFA, supplies only 0.15% of the daily demand for omega-3 acids (about 130 mg day^{-1}).[6,11] The dietary ratio of n-6:n-3 is 8:1, whereas it should be not higher than 4:1 or 2:1.[6,11] In 1991, the consumption of n-6 PUFA was 10 to 25 times higher than that of n-3 PUFA.[14] High levels of omega-6 FAs are a cause of stress, depression and various illnesses. Moreover, they limit the modification of n-3 α-linolenic acid (ALA) to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[6] Absorbing large amounts of saturated fatty acids (SFA) is also very harmful, causing hypercholesterolemia, coronary heart disease (CHD) and mortality.[15] In 2001/2002, 65.7% of Americans were classified as clinically overweight or obese because the SFA to PUFA ratio was between 11:1 and 4:1. The proper ratio of SFA to PUFA is 3:1 or less, fish and shellfish are rich in n-3 PUFA and reduce SFA and cholesterol contents.[15] Unfortunately, humans cannot synthesize DHA and EPA fatty acids, so they have to absorb them with food or obtain them from the conversion of α-linolenic acid (ALA).[7,11]

The primary sources of EFA (particularly EPA and DHA) are fish, fish oils mackerel 2500 mg 100 g^{-1}) and seafood (shrimp 300 mg 100 g, the same EFA content as in cod).[11] Therefore, there exists a possibility of using krill oil as an alternative or substitute for fish oil.[16] Atlantic krill has much more EPA *per* g than standard fish oil capsules (240 mg g^{-1} EPA in krill as against only 180 mg g^{-1} in standard fish oil).[6] *Crangon crangon* (Linnaeus 1758) is a small shrimp (maximum length 89 mm) belonging to the *Crangonidae* family. Shrimps inhabit the sandy or muddy bottoms of shallow coastal waters down to 130 m. They are common in the Eastern Atlantic, the Atlantic coast of Morocco, in the Mediterranean and Black Seas.[17] The shrimp is caught on a large scale (40322 tons $year^{-1}$ in the North Atlantic and 113 tons $year^{-1}$ in the Mediterranean Sea), according to surveys from 2005.[18]

The primary aim of the current research was to determine the free fatty acid profile, as well as the FA profiles in triacylglycerols and phospholipids in the abdominal muscle of fall-caught *Crangon crangon*. The approximate quantitative analysis of lipid classes was done by HPLC-LLSD.

Experimental

Biological material

Adults of *Crangon crangon* were collected in November 2008 in the coastal area of the Gulf of Gdańsk (Sobieszewo Island). *C. crangon* were decapitated and the muscles from shrimps were dissected and frozen at −80 ºC in glass tubes. Tissues were weighed and were divided into two samples.

The total mass of the biological sample (3476.7 mg) was divided into two samples: the first sample was extracted in dichloromethane (mass 2452.7 mg) and the second sample was extracted in a mixture of chloroform-methanol, Folch's method (mass 1024.0 mg). Tested tissues of *C. crangon* from first part of the analytical studies were obtained from 10 muscles of *C. crangon*. Four muscles of *C. crangon* were used to the second extraction. To determine the variability of the results, they were compared with the student's *t*-test: significant at $p < 0.05$.

Chemicals and reagents

Dichloromethane, methanol, acetone, *n*-hexane, acetonitrile (all HPLC grade), ethanol 99.8% and isooctane analytically pure were obtained from Chempur (Piekary Śląskie, Poland), 2,5-dihydroxybenzoic acid (DHB, HPLC grade) from Sigma-Aldrich (Poznań, Poland), and NaCl 0.9% from Fresenius-Kabi (Kutno, Poland). Sodium hydrate pills were purchased from POCH SA (Gliwice, Poland) and BSTFA+TMCS (99:1), (BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide) and TMCS (trimethylchlorosilane)) was supplied by Supelco (Bellefonte, USA). Saturated branched-chain fatty acid 18-methyl-eicosanoate was the internal standard and was obtained from Sigma-Aldrich (Poznań, Poland).

Dichloromethane extraction, HPLC and GC-MS analyses

Muscle lipids were extracted directly after the isolation of the abdominal muscle from the biological material. The first sample (10 adult stages, total weight of 2452.7 mg) was extracted in 20 mL of dichloromethane and homogenized for 20 min using a pestle and mortar. The solvent extract was concentrated by rotary evaporation and then evaporated to dryness in a stream of nitrogen. The lipid extract was separated into classes of compounds using HPLC-LLSD and a normal-phase 250 × 4.6 mm analytical column packed with Econosil Silica (Alltech, particle size 5 μm). The mobile phase consisted of *n*-hexane (solvent A) and dichloromethane containing 15% acetone (solvent B). The gradient was programmed linearly from A to B within 35 min. The subsamples of TAGs and PLs obtained were hydrolyzed with a solution of KOH in methanol (0.5 mol dm^{-3}, 3 h at 70 ºC). Trimethylsilyl derivatives (TMSi) of FFAs and sterols were obtained by the addition of 100 μL of a BSTFA:TMCS mixture (99:1) to 1 mg of each sample and heating for 1 h at 100 °C. Fatty acid methyl esters from the

hydrolysis of TAGs and PLs, as well as TMSi derivatives of sterols and free fatty acids, were then GC-MS analyzed on a Finnigan Mat SSQ 710 mass spectrometer coupled to a Hewlett Packard 5890 gas chromatograph. Compounds were separated in a 30 m × 0.25 mm i.d., HP-5 capillary column (film thickness 0.25 μm). The column temperature was programmed from 60 to 300 °C at a rate of 4 °C min^{-1}. The injector temperature was 300 °C, and the carrier gas was helium. During the whole process of sample preparation to analysis, before and after separation of each class of lipid, before the derivatization to trimethylsilyl and methyl esters of fatty acids, extracts were stored at a temperature of −3 °C since there exists a very high probability of oxidation of analyzed polyunsaturated fatty acids.

Folch's method of extraction and MALDI-TOF analyses

The second sample (4 adult stages with a total weight of 1024.0 mg) was extracted in a mixture of chloroform-methanol (2:1, v/v) according to Folch *et al.*[19] After that, the solvent was washed with 0.9% aqueous NaCl. MALDI-TOF-MS coupled to a reflectron (Bruker, Bremen) was used to identify the extract components. Spectra were recorded in positive ion mode ($[M + H]^+$, $[M + Na]^+$ and $[M + K]^+$) with an accelerating voltage of 19 kV and a reflective voltage of 20 kV. The MALDI-TOF-MS was equipped with a pulsed N_2 laser with wavelength λ at 337 nm and a delay time of 1500 ns. The mass range in the MALDI-TOF mass spectra was from 200 to 2000 amu. The lipid samples were dissolved in ethanol and mixed with saturated matrix solution, 2,5-dihydroxybenzoic acid (DHB). After that 1 μL of the solution was deposited on a sample plate and dried in a stream of air.

Results

HPLC-LLSD analysis

The total quantity of lipids extracted with dichloromethane was 19.2 mg (7.9 mg g^{-1} dry mass), which comprised less than 1% of the total fresh weight of the biological material (Table 1). Four fractions were obtained as a result of the HPLC-LLSD separations: TAGs (f1), FFAs (f2), sterols (f3) and PLs (f4) (Figure 1). Their presence was confirmed on the basis of the retention time of the compounds identified by lipid standards.

All fractions were then subjected to further GC-MS analysis. Sterols were the dominant class of lipids and accounted 2.5 mg g^{-1} of the total lipids. The extracts contained similar amounts of TAGs and FFAs, which made up 1.9 and 2.0 mg g^{-1} of the total mass of lipids, respectively. Fraction 4 (polar lipids) was the least abundant class (Table 1).

Table 1. Total mass of lipid class (mg g^{-1} dry mass) in the muscle of *Crangon crangon*, n: number of individuals (n = 10)

Fraction	Composition / %	Lipid content / mg dry mass	Lipid content / (mg g^{-1} dry mass)
f1: TAG	24.0	4.6	1.89 ± 0.09
f2: FFA	25.0	4.8	2.01 ± 0.10
f3: Sterol	32.3	6.2	2.47 ± 0.12
f4: PL	18.7	3.6	1.53 ± 0.07

TAG: triacylglycerol; FFA: free fatty acid; PL: polar lipid.

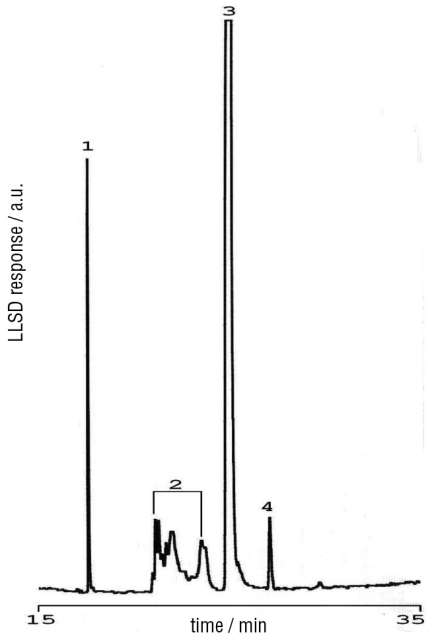

Figure 1. HPLC chromatogram of lipids extracted from *Crangon crangon*, 1: triacylglycerols (f1), 2: free fatty acids (f2), 3: sterols (f3), 4: polar lipids (f4). Each fraction was analyzed by GC-MS and the fractions were identified by mass spectra of GC-MS.

GC-MS analysis - fatty acid profiles

Table 2 summarizes the fatty acid profiles in neutral and polar lipids detected by GC-MS. Nine FAs ranging from C14 to C18 were obtained as a result of TAG (tFAs) (f1) hydrolysis, and 4 acids were detected after hydrolysis of the polar lipid fraction (pFAs) (f4). The free fatty acid FFAs, f2 fraction, contained a total of 22 FAs with 14-24 carbon atoms in the chain.

All compounds were identified as the corresponding TMSi derivatives. Figure 2 shows the total ion current (TIC) from the GC-MS analysis of the triacylglicerols (f1) (Figure 2a), free fatty acid fraction (f2) (Figure 2b) and polar lipids (f4) (Figure 2c). Ions at *m/z* 73 and 75 are typical of all TMSi derivatives. The main ions of FA are 117 and 145.

Table 2. Chemical composition of fatty acids in polar and neutral lipid fractions of abdominal muscle of adult *Crangon crangon* detected by GC-MS (μg g^{-1} dry mass), n: number of individuals (n = 10)

No.	*Fatty acid*	f1: Triacylglycerol	f2: Free fatty acid	f4: Polar lipid
1	14:0	72.2 ± 3.6	74.0 ± 3.1	–
2	15:0	32.3 ± 1.7	66.0 ± 2.7	–
3	16:1	96.9 ± 5.1	96.0 ± 3.8	202.5 ± 11.2
4	16:0	644.1 ± 32.1	652.0 ± 28.1	456.0 ± 23.1
5	17:1	26.6 ± 1.3	24.0 ± 1.1	–
6	17:0	19.0 ± 0.9	64.0 ± 2.6	–
6A[a]	18:2	39.9 ± 2.0	0	–
7	18:1	619.4 ± 31.1	410.0 ± 16.1	706.5 ± 35.3
8	18:0	349.6 ± 17.7	354.0 ± 13.9	135.0 ± 6.8
9	19:1	–	6.0 ± 0.3	–
10	19:0	–	8.0 ± 0.4	–
11	20:4	–	38.0 ± 1.7	–
12	20:5	–	40.0 ± 1.8	–
13	20:3	–	4.0 ± 0.2	–
14	20:1	–	20.0 ± 0.9	–
15	20:0	–	12.0 ± 0.5	–
16	20:2	–	18.0 ± 0.8	–
17	21:0	–	32.0 ± 1.3	–
18	22:1	–	16.0 ± 0.7	–
19	22:0	–	10.0 ± 0.5	–
20	23:0	–	2.0 ± 0.1	–
21	24:1	–	42.0 ± 1.8	–
22	24:0	–	12.0 ± 0.5	–

[a]6A: fatty acid 18:2 detected only in fraction 1.

The dominant FAs in all samples were, 16:0 (f1: 644.1 ± 32.1 μg g^{-1}; f2: 652.0 ± 28.1 μg g^{-1}; f4: 456.0 ± 23.1 μg g^{-1}), 16:1 (f1: 96.9 ± 5.1 μg g^{-1}; f2: 96.0 ± 3.8 μg g^{-1}; f4: 202.5 ± 11.2 μg g^{-1}), 18:0 (f1: 349.6 ± 17.7 μg g^{-1}; f2: 354.0 ± 13.9 μg g^{-1}; f4: 135.0 ± 6.8 μg g^{-1}) and 18:1 (f1: 619.4 ± 31.1 μg g^{-1}; f2: 410.0 ± 16.1 μg g^{-1}; f4: 706.5 ± 35.3 μg g^{-1}). The total content of these four compounds was between 75.6 (f2) and 100.0% (f4) (see Table 2 for details). The other abundant FAs were, 14:0 (f1: 72.2 ± 3.6 μg g^{-1}; f2: 74.0 ± 3.1 μg g^{-1}), 15:0 (f1: 32.3 ± 1.7 μg g^{-1}; f2: 66.0 ± 2.7 μg g^{-1}), 17:0 (f2: 19.0 ± 0.9 μg g^{-1}), 18:2 (f1: 39.9 ± 2.0 μg g^{-1}, present only in this fraction) and EPA, which was detected only in fraction 2 and accounted 40.0 ± 1.8 μg g^{-1} of the total mass of FFAs.

GC-MS analysis - composition of sterols

HPLC-LLSD analysis revealed the dominance of sterols in the lipid composition. Only cholest-5-en-3β-ol (cholesterol) and 22-trans-24-norcholesta-5,22-dien-3β-ol (24-norcholestadienol) were detected in the sterol fraction on the basis of mass spectra obtained during GC-MS analysis (Figure 3), with cholesterol accounting for more than 95% of the total sterols.

MALDI-TOF analysis

To confirm the presence of phospholipids in a sample, a MALDI-TOF analysis was performed on the extracts obtained using Folch's extraction. As the current study focuses on the essential fatty acid content, it will be presented the results only for those phospholipids containing EPA or DHA combined with other FAs. The FFA profile was also studied to assess the usefulness of the method in FFA analysis. Other compounds were not analyzed by this technique.

MALDI-TOF mass spectra possibly revealed the presence of polar lipids, which were initially identified on the basis of the following ions: molecular ion $[M + H]^+$,

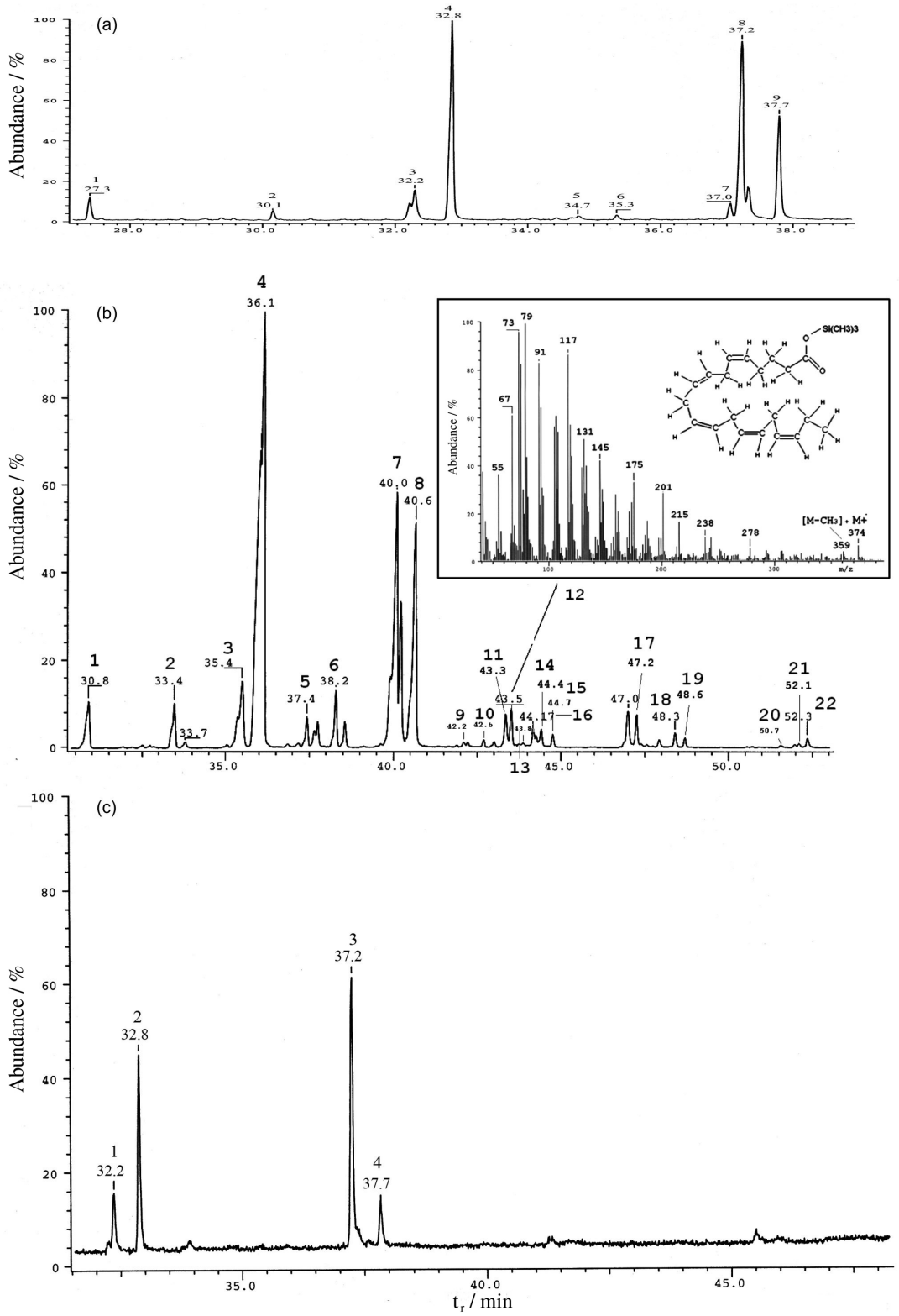

Figure 2. Total ion current (TIC) of: (a) fatty acid methyl esters obtained by hydrolysis of triacylglycerols extracted from *Crangon crangon.* 1 - $C_{14:0}$, 2 - $C_{15:0}$, 3 - $C_{16:1}$, 4 - $C_{16:0}$, 5 - $C_{17:1}$, 6 - $C_{17:0}$, 7 - $C_{18:2}$, 8 - $C_{18:1}$, 9 - $C_{18:0}$; (b) free fatty acids extracted from *Crangon crangon.* 1 - $C_{14:0}$, 2 - $C_{15:0}$, 3 - $C_{16:1}$, 4 - $C_{16:0}$, 5 - $C_{17:1}$, 6 - $C_{17:0}$, 7 - $C_{18:1}$, 8 - $C_{18:0}$, 9 - $C_{19:1}$, 10 - $C_{19:0}$, 11 - $C_{20:4}$, 12 - $C_{20:5}$, 13 - $C_{20:3}$, 14 - $C_{20:1}$, 15 - $C_{20:0}$, 16 - $C_{20:2}$, 17 - $C_{21:0}$, 18 - $C_{22:1}$, 19 - $C_{22:0}$, 20 - $C_{23:0}$, 21 - $C_{24:1}$, 22 - $C_{24:0}$. Inset: mass spectrum of $C_{20:5}$ isolated from the extract. Characteristic ions for TMSi of $C_{20:5}$: *m/z* 73 which corresponds to TMSi, *m/z* 117 TMSiOOC, *m/z* 131 TMSiOOCCH$_2$, *m/z* 145 TMSiOOCC$_2$H$_4$, *m/z* 201 TMSiOOCC$_6$H$_{16}$, *m/z* 215 TMSiOOCC$_7$H$_{14}$, *m/z* 238 M-C$_{10}$H$_{16}$, *m/z* 278 M-C$_7$H$_{12}$, *m/z* 359 M-CH$_3$, *m/z* 374 molecular ion; and (c) fatty acid methyl esters obtained by hydrolysis of polar lipids extracted from *Crangon crangon.* 1 - $C_{16:1}$, 2 - $C_{16:0}$, 3 - $C_{18:1}$, 4 - $C_{18:0}$.

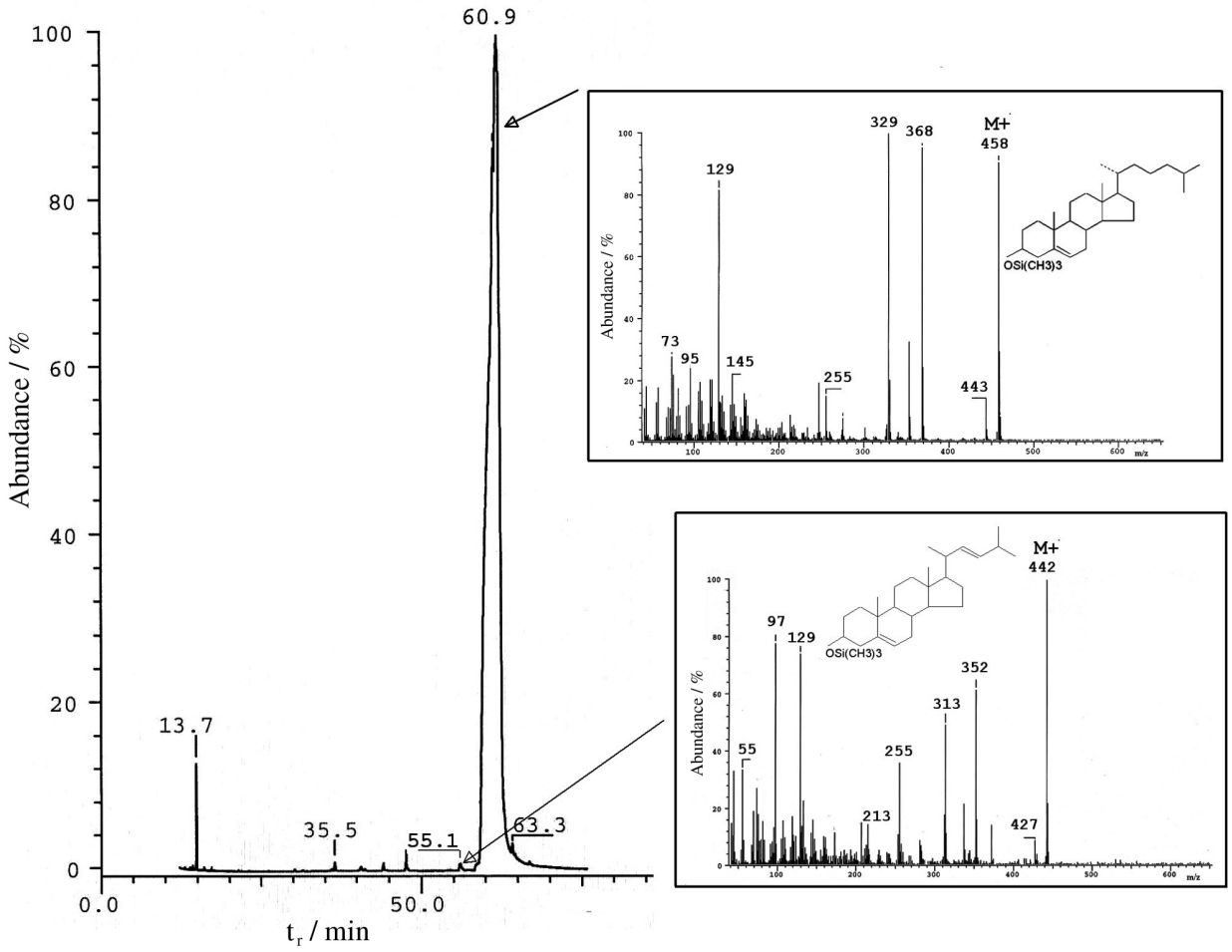

Figure 3. Total ion current (TIC) of sterols extracted from *Crangon crangon.* Inset: mass spectra of 24-norcholestadienol (trimethylsilyl ether, retention time 55.1 min) and cholesterol (trimethylsilyl ether, retention time 60.9 min) isolated from the extract. Characteristic ions for cholesterol: *m/z* 129, which corresponds to C_1,C_3 + TMSiO, *m/z* 255 M-(side chain + TMSiOH), *m/z* 329 M-(C_1,C_3 + TMSiO), *m/z* 353 M-(CH_3 + TMSiOH), *m/z* 368 M-TMSiOH, *m/z* 443 M-CH_3, *m/z* 458 molecular ion. Characteristic ions for 24-norcholestadienol: *m/z* 129 which corresponds to C_1,C_3+TMSiO, *m/z* 213 M-(side chain + C_3H_6 + TMSiOH), *m/z* 255 M-(side chain + TMSiOH), *m/z* 313 M-(C_1,C_3 + TMSiO), *m/z* 352 M-TMSiOH, *m/z* 427 M-CH_3, *m/z* 442 molecular ion.

cationated ions $[M + Na]^+$, and $[M + K]^+$ and the headgroups of the phospholipid (Table 3 and Figure 4).

Only 9 free fatty acids were identified in the extracts using MALDI-TOF-MS (Table 4, Figure 5). Through this table, the intention was to show the similarity or difference of certain fatty acids identified by using GC-MS techniques to MALDI-TOF-MS.

The dominant FAs detected by this method were fatty acids with 16 (*m/z* 257, 16:0) and 18 (*m/z* 285, 18:0) carbon atoms. Perhaps the reason is the overlapping of ions of the sample with ions of the matrix. Other saturated acids were present in very small quantities. Polyunsaturated fatty acids (EPA and DHA) were almost absent in free form. However, monounsaturated fatty acids (MUFA) 18:1 and 24:1 were detected. The FFA profile obtained by MALDI-TOF-MS differed from the one obtained by GC-MS analysis probably because MALDI-TOF-MS is not a method suitable for analyzing compounds with such a low molecular mass.

Discussion

Lipid classes

The class composition of lipids, with high levels of sterols, triacylglycerols, free fatty acids and phospholipids, was characteristic of *Decapoda*.[20] The polar lipid fraction was a dominant lipid class in the abdominal muscle of *Crangon crangon* according to all previously published data.[4,21-23] Phospholipids, which are relatively more polar and easy to emulsify, are better and a major source of EFA when compared to neutral lipids.[21,24,25] They are also essential in each organism lifecycle, form the membrane structure and are present in the fat bodies located in the hemocoel.[20] Phospholipids are the primary constituents of the algal diet of shrimps and krill.[26,27] Our results of MALDI-TOF-MS analyses of *Crangon crangon* lipids obtained by Folch's extraction are in good agreement with

Table 3. Measured mass-to-charge ratio (*m/z*) polar lipids in *Crangon crangon* determined by MALDI-TOF-MS

	Headgroup	$[M + H]^+$	$[M + Na]^+$ or $[M + K]^{+*}$
PC 10:0/20:5	184	696	718
PC 12:0/20:5	184	724	746
PC 16:0/20:5	184	780	802
PE13:0/20:5	142	696	718
PE15:0/20:5	142	724	746
PE18:3/20:5	142	760	–
PE19:0/20:5	142	780	802
PE20:5/20:5	142	–	806
PE13:0/22:6	142	–	760*
PE19:0/22:6	142	806	828
PS16:1/20:5	186	780	802
PS10:0/22:6	186	724	746
PS16:1/22:6	186	806	828
PG18:4/20:5	153, 173	789	811 and 827*
PG16:3/22:6	153, 173	789	811 and 827*

previous reports and confirm the predominance of polar lipids over the other lipid classes (Table 3). However, when a less polar solvent (dichloromethane) was used for the extraction, polar lipids were less abundant than any other lipid class detected.

MALDI-TOF technique was developed to deal with compounds of high molecular mass, thus it is often the method of choice in the analysis of phospholipids.[28] The PL fractions are prevalent in muscles, whereas in the hepatopancreas, also a source of lipids during food deprivation, neutral lipids predominate. The reason for this is that in muscles the polar lipid fraction is the first to be consumed, whereas in the hepatopancreas, all the compounds are metabolized to the same degree.[22] As PUFAs are rich sources of energy, special attention was paid to PLs containing EPA or DHA. The PUFA level rises with decreasing water temperature in much the same way as during starvation, which could explain the large numbers of phospholipids with these acids in the structure, identified in our investigation.[22]

There is a slight prevalence of SFA over PUFA in the composition of the polar fraction in the cold season because benthic organisms take these acids from dead and living organisms as well as from plant material. Linoleic acid (LA) and linolenic acid (LNA) are converted into PUFA as a result of elongation and desaturation.[29] Polyunsaturated fatty acids prevail in the polar fraction during the remainder of the year because they are essential elements of every living cell, important components of cellular membranes, a source of energy and precursors of many biologically active compounds.[30]

Sterols were a dominant class of lipids according to the results of HPLC-LLSD analyses. They accounted for 32.3% of all lipids isolated with dichloromethane, which is not surprising since they are the second most

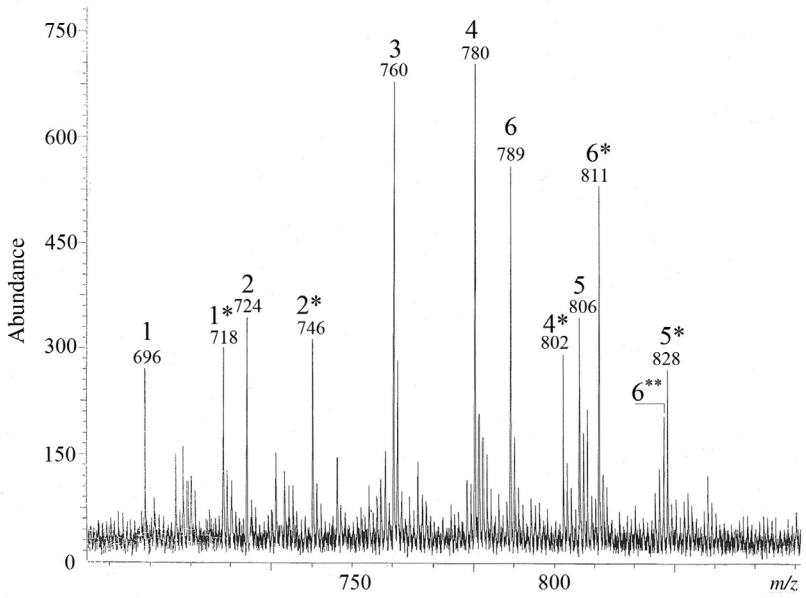

Figure 4. MALDI-TOF-MS mass spectrum of phospolipids in the muscle of *Crangon crangon*. 1 - PC 10:0/20:5 $[M + H]^+$, PE13:0/20:5 $[M + H]^+$, 1* - PC 10:0/20:5 $[M + Na]^+$, PE13:0/20:5 $[M + Na]^+$, 2 - PC 12:0/20:5 $[M + H]^+$, PE15:0/20:5 $[M + H]^+$, PS10:0/22:6 $[M + H]^+$, 2* - PC 12:0/20:5 $[M + Na]^+$, PE15:0/20:5 $[M + Na]^+$, PS10:0/22:6 $[M + Na]^+$, 3 - PE18:3/20:5 $[M + H]^+$, PE13:0/22:6 $[M + K]^+$, 4 - PC 16:0/20:5 $[M + H]^+$, PE19:0/20:5 $[M + H]^+$, PS16:1/20:5 $[M + H]^+$, 4* - PC 16:0/20:5 $[M + Na]^+$, PE19:0/20:5 $[M + Na]^+$, PS16:1/20:5 $[M + Na]^+$,5: PE19:0/22:6 $[M + H]^+$, PS16:1/22:66 $[M + H]^+$, 5* - PE19:0/22:6 $[M + Na]^+$, PS16:1/22:66 $[M + Na]^+$, 6 - PG18:4/20:5 $[M + H]^+$, PG16:3/22:6 $[M + H]^+$, 6* - PG18:4/20:5 $[M + Na]^+$, PG16:3/22:6 $[M + Na]^+$, 6** - PG18:4/20:5 $[M + K]^+$, PG16:3/22:6 $[M + K]^+$.

Table 4. Chemical composition of free fatty acids from abdominal muscle of *Crangon crangon* detected by MALDI-TOF-MS

$[M + H]^+$	Free fatty acid
229	14:0
243	15:0
257	16:0
281	18:2
283	18:1
285	18:0
331	22:5
359	24:5
369	24:1

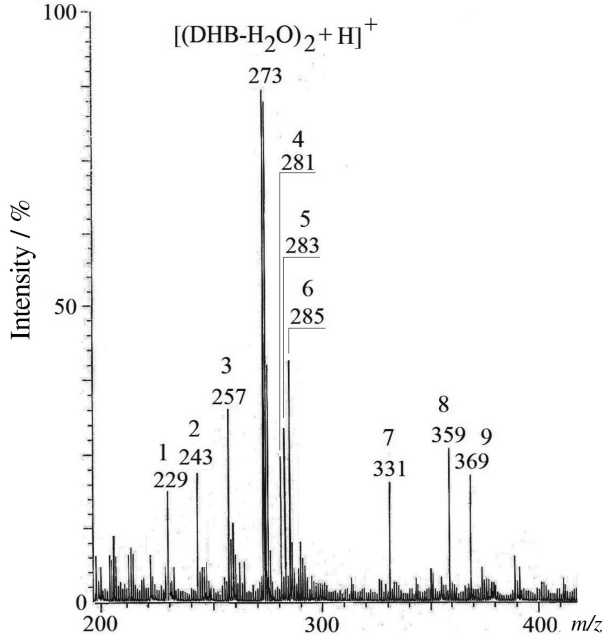

Figure 5. MALDI-TOF-MS mass spectrum of fatty acids in the muscle of *Crangon crangon*. 1 - $C_{14:0}$, 2 - $C_{15:0}$, 3 - $C_{16:0}$, 4 - $C_{18:2}$, 5 - $C_{18:1}$, 6 - $C_{18:0}$, 7 - $C_{22:5}$, 8 - $C_{24:5}$, 9 - $C_{24:1}$.

common lipid class in shrimps, as confirmed by other authors.[22] Dietary food is an essential source of sterols for growth and survival in crustaceans, as they lack the ability to synthesize these compounds from scratch. A high level of cholesterol is required for the biosynthesis of sex and molting hormones, as well as for the formation of cell membranes and intercellular structures in crustaceans.[31,32] Cholesterol accounted for more than 95% of the total sterols, which is in good agreement with results previously reported Kanazawa.[31] Nonetheless, apart from cholesterol it was identified only 24-norcholestadienol. The composition of the sterol fraction is affected by the time of year when the material was collected, depth in the sea, geographical location and dietary sources.[33,31]

Triacylglycerols and free fatty acids from abdominal muscles were detected in comparable amounts (Table 1), only slightly lower than those of sterols. The FA composition may be affected by the extraction method used to isolate lipids from marine invertebrate tissues.[34] TAGs are reservoirs of energy in marine invertebrates and they can be used during short periods of starvation.[25] FFAs are used when an animal is forced to survive several months with less food available. Such use of energy sources is characteristic of marine crustaceans living both in deep seas and in freshwater.[35] Moreover, PLs can be converted to TAGs if they are needed as an energy source.[21]

Fatty acid profiles

Crustaceans are rich in essential fatty acids, mainly EPA and DHA, which are the major stores of energy in marine ecosystems.[29] Despite the small size of *Crangon crangon* (up to 9 cm), catches have grown from 37223 tons in 1999 to 44853 tons in 2005.[36] As expected, the major fatty acids were compounds 16:1, 16:0, 18:1 and 18:0, which is in good agreement with reports on the lipids in shrimps and prawns.[4,21-23,27,29,37,38] A similar composition of fatty acids was also determined during the analysis of the diet of herring, for which shrimps are one of the basic sources of food.[39] Our group also identified several fatty acids reported only by a few authors, those where compounds C13 and C24 (Table 4).[21,40]

The most significant difference between the results presented here and previous reports is the lack of DHA and the very low content of EPA (Table 2). These acids were usually dominant in the FA profile together with C16 and C18 compounds; however, lower levels of EPA and DHA during the fall were reported by Kasai and Sakai.[23] The EPA content in both neutral lipids and phospholipids of *Pandalus kessleri* was lower than in other seasons.[23] Similarly, the DHA content in *Mysis alymyra* lipids was at much lower levels than in other crustaceans.[27] Kasai and Sakai[23] also demonstrated the same seasonal changes between males and females in the relative contribution of EFA to the overall lipid content. Like MUFA, these acids are essential for benthic organisms; crustaceans can use them as primary sources of energy.[4,29] This could explain the highest levels of fatty acids 16:1 and 18:1 detected in *Crangon crangon* FA profiles during the fall. Differences in SFA, MUFA and PUFA content depend strongly on salinity. Low levels of polyunsaturated fatty acids and high contents of saturated acids have been noted in freshwater and in brackish water. Since PUFA can influence the osmoregulatory mechanism by altering the permeability of cell membranes, their levels increase

with salinity. The levels of these acids are also determined by diet.[29,41] The amounts of EPA and DHA increase when the content of C16 fatty acids is reduced, and *vice versa*.[38] Benthic species consume plant detritus, rich in saturated fatty acids, particularly palmitic (16:0) and stearic (18:0) acids.[29] The low PUFA values during November suggest that food deprivation significantly reduces total lipid levels and that these levels are correlated with values reported in the same season for zooplankton and dinoflagellates.[29,42] Sekino *et al.*[43] described higher levels of fatty acids with 16 and 18 carbon atoms from freshwater zooplankton species. Those species could be an additional source of saturated fatty acids for benthic crustaceans living in coastal marine areas. Moreover, of all the highly unsaturated fatty acids (HUFA), PUFA are the first to be reduced in muscle tissues of invertebrates during food deprivation.[22] They are also consumed in juvenile stages as compounds essential for the development and transformations of the organism.[29] This could explain why it was observed low levels or even a lack of EPA and DHA in free form in the lipids of Baltic shrimps caught in the fall.

Seasonal changes in the lipid content and the FA profile depend on weather conditions, climate, seawater temperature and season, in the fall, a low lipid content was reported.[4,23,38] The development of organisms and changes in nutritional conditions as well as the locality inhabited can reduce amounts of DHA and EPA.[4] Higher levels of EFA are needed in the larval stages, as they are used for somatic growth and neural tissue development.[7] The same metamorphosis to later stages requires much more energy.[38] The EFA intake increases with increasing body weight and respiration, and decreases with growing metabolic activity.[42]

The fatty acid profile in lipids from the abdominal muscle of *Crangon crangon* in the fall is in agreement with expectations and the results published by other researchers.[23] Consumption of shellfish is rapidly increasing, not only because they are tasty, but also because of the wealth of essential fatty acids they contain.[44] Shrimps are not caught in the Baltic Sea, but worldwide the catch is large (44853 tons in 2005) and still growing, which explains the importance of studies concerning the nutritional value of this species.[36]

Conclusion

The method analysis was successfully applied in the identification of lipid components in the abdominal muscle of fall-caught *Crangon crangon* from a coastal area of the Baltic Sea. This is the first time that the accurate chemical composition of the lipids in *Crangon crangon* was recognized.

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