DOI 10.1007/s10600-018-2308-0
Chemistry of Natural Compounds, Vol. 54, No. 2, March, 2018

LIPIDS, VINYL ALCOHOLS, AND FATTY ACIDS FROM SOFT TISSUES OF THE BIVALVE MOLLUSK *Mactra chinensis*

O. V. Tabakaeva* and A. V. Tabakaev

The compositions of lipids, vinyl alcohols, and fatty acids from total lipids of the soft tissues of the bivalve mollusk Mactra chinensis *were analyzed. Bound lipids were found to be dominant in all parts and made up >83.3% of the lipid mass predominantly in adductor. The total contents of dimethylacetals in various mollusk parts were 3.3–11.9% of the total FAs predominantly in motor muscle and viscera. The lipids contained 39 FAs of various structures with different quantitative contents and localizations of which 6 were saturated; 14, unsaturated, and 19, polyunsaturated. Adductor and muscle contained 37 FAs; viscera, 38 FAs.*

Keywords: lipids, vinyl alcohols, fatty acids, bivalve mollusk.

Bivalve mollusks are some of the most broadly distributed, populous, and massive groups of marine animals with bodies covered by a calciferous bivalve shell. This remarkable hydrobiont group has attracted attention since ancient times and was used as food. New commercial species of burying bivalve mollusks, i.e., clams such as *Spisula sachalinensis*, *Anadara broughtonii*, and *Mactra chinensis* have drawn special attention.

Mactra chinensis is a bivalve mollusk inhabiting the Sea of Japan (near the shores of Primorsky Krai from Pos′et Bay to Ol′ga Bay and western Sakhalin), the Okhotsk Sea (near the shores of southern and western Sakhalin), and the South Kuril basin that burrows into sandy sediment to depths of 0.5–13 m at water temperatures up to 23.5°C and salinity 29.0–30.3. It has a strong triangular-oval convex shell with valves up to 127 × 103 mm. Harvesting occurs from May to November. The body mass of the raw mollusk is 25–30% of the total mass [1].

The lipid compositions of marine organisms, including mollusks, are under active investigation [2] because they are known to include unique fatty acids (FAs) atypical of terrestrial organisms.

Long-chain fatty alcohols that are incorporated into neutral and P-containing lipids with an ether bond (alkyl lipids) and aldehydes found in plasmalogens are hydrophobic lipid components in addition to FAs. Plasmalogens, or phosphoglyceracetals, received their name from the plasma aldehyde reaction, which is used in histochemistry as an aldehyde test. Plasmalogens and phosphatides have the same structure. However, although hydrolysis of phosphatides releases FAs, acid hydrolysis of plasmalogens forms higher aliphatic aldehydes and diacylglycerins; alkaline hydrolysis, alkenyl ethers of glycerin and higher FAs. Plasmalogens are usually cleaved at the vinyl-ether bond to release aldehydes so that the plasmalogen aldehyde composition can be studied. Methods were developed for specific hydrolysis by AcOH (90%), Cl_3CCO_2H, salts of Hg and other heavy metals. Glycerophospholipids with an alken-1-yl ether group that were derivatives of higher fatty aldehydes were detected in tissues and organs of all animals regardless of their level of organization. Plasmalogens are also present in rather high concentrations in the human body, where they make up ~22% of total phospholipids [3].

The goal of the present work was to investigate the composition of lipids from soft tissues of the Far-East bivalve mollusk *M. chinensis*. The studied soft tissues of *M. chinensis* gathered in Okunevaya and Spokoinaya Bays of Primorsky Krai in 2015–2106 included motor muscle (so-called "foot"), adductor (closing) muscle, mantle, and viscera. Earlier, the lipid content was 0.4–1.1% with the most in viscera and the least in mantle [4].

Table 1 presents results for the separation of total lipids into individual classes, i.e., free (FL) and bound lipids (BL), unsaponifiable substances (US), neutral lipids (NL), sterols, glyceroglycolipids, phospholipids (PL), cholesterol and its esters, and FAs. The classes were distributed very differently in soft tissues of *M. chinensis*.

Far-Eastern Federal University, School of Biomedicine, Vladivostok, e-mail: yankovskaya68@mail.ru. Translated from *Khimiya Prirodnykh Soedinenii*, No. 2, March–April, 2018, pp. 187–190. Original article submitted August 14, 2017.

0009-3130/18/5402-0223 ©2018 Springer Science+Business Media, LLC

TABLE 1. Compositions of Lipids from Soft Tissues of Bivalve Mollusk *M. chinensis*

Parameter, %	Mantle	Adductor	Motor muscle	Viscera
Free lipids (of lipid mass)	16.5 ± 0.52	10.5 ± 0.41	11.9 ± 0.44	14.7 ± 0.60
Unsaponifiable substances (of lipid mass)	23.6 ± 1.10	27.0 ± 1.21	13.3 ± 0.52	22.4 ± 0.89
Bound lipids (of lipid mass)	83.3 ± 4.12	89.3 ± 4.13	87.9 ± 4.07	85.1 ± 4.20
Neutral lipids (of BL mass)	15.7 ± 0.70	9.9 ± 0.44	11.3 ± 0.47	14.1 ± 0.63
Sterols (of BL mass)	1.9 ± 0.04	4.6 ± 0.21	6.8 ± 0.24	2.7 ± 0.10
Glyceroglycolipids (of BL mass)	2.4 ± 0.52	7.2 ± 0.30	8.4 ± 0.35	3.7 ± 0.14
Phospholipids (of BL mass)	39.0 ± 1.87	36.1 ± 1.80	42.7 ± 2.03	49.2 ± 2.30
Cholesterol (of BL mass)	18.4 ± 0.70	25.5 ± 1.16	20.9 ± 1.00	14.6 ± 0.67
Cholesterol esters (of BL mass)	11.2 ± 0.35	16.3 ± 0.70	8.9 ± 0.41	14.7 ± 0.60

TABLE 2. Compositions of Vinyl Alcohols of Total Lipids from Soft Tissues of Bivalve Mollusk *M. chinensis*, % GC

Fatty acid	Mantle	Adductor	Motor muscle	Viscera
DMA *i*-16:0	0.1 ± 0.05	–	0.9 ± 0.04	0.6 ± 0.02
DMA 16:0	0.3 ± 0.01	0.5 ± 0.02	1.1 ± 0.05	1.5 ± 0.07
DMA *i*-17:0	1.0 ± 0.05	0.3 ± 0.01	1.9 ± 0.09	2.8 ± 0.14
DMA 17:0	0.7 ± 0.03	0.2 ± 0.01	1.7 ± 0.08	1.8 ± 0.09
DMA *i*-18:0	0.5 ± 0.02	–	1.5 ± 0.07	0.9 ± 0.04
DMA 18:0	0.6 ± 0.03	0.1 ± 0.05	1.3 ± 0.06	2.0 ± 0.10
DMA 18:1	0.9 ± 0.04	0.4 ± 0.02	1.1 ± 0.05	1.7 ± 0.08
DMA 19:0	–	0.1 ± 0.05	0.4 ± 0.02	–
DMA 19:1	–	1.5 ± 0.07	–	0.9 ± 0.04
DMA 20:0	0.5 ± 0.02	0.2 ± 0.01	0.1 ± 0.05	–
Total	4.6 ± 0.22	3.3 ± 0.15	10.0 ± 0.50	11.9 ± 0.58

BL were the principal lipids in all soft tissues of *M. chinensis*, made up >83.3% of the lipid mass, and dominated in adductor. Quantitative differences in the BL contents in the soft tissues were insignificant and <7.2%. The contents of US were rather high, scattered from 13.3 to 27.0%, and dominant in adductor. Muscle had the minimal (13.3%) content of US. The difference compared with other parts was 1.68–2.03 times. The PL contents were 36.1–49.2% of the BL mass, which defined them as the main class. Viscera had the highest content of PL. The total contents of cholesterol and its esters was 29.3–41.8% of the BL mass with dominance in adductor. Adductor contained the maximum amount of cholesterol; viscera, the minimum. The maximum content of cholesterol esters was also found in adductor; the minimum, in muscle. NL were third in content in BL of *M. chinensis* at 9.9–15.7% of the BL mass and dominance in mantle. Adductor had the lowest content (9.9%); muscle and viscera, intermediate amounts. Glyceroglycolipids reached a maximum in muscle that was 3.5 times the minimum content in mantle. Sterols were the least represented of the class (1.9–6.8% of BL mass). A comparison of the contents of the various lipid classes in soft tissues of *M. chinensis* showed that mantle led the other parts in FL and NL contents; adductor, US, BL, cholesterol and its esters; motor muscle, sterols and glyceroglycolipids; viscera, phospholipids.

Table 2 presents results from methanolysis of total lipids from various parts of *M. chinensis* to produce a mixture of dimethylacetals (DMA), among which nine vinyl-alcohol derivatives were identified.

The total DMA contents in various mollusk parts were 3.3–11.9% of total FAs with predominance in muscle and viscera. Although a total of nine DMA were found, individual parts were characterized by the presence of seven (mantle, adductor, viscera) or eight alcohols (motor muscle). DMA of all soft tissues typically had mainly saturated homologs with 16–20 C atoms of both branched and straight-chain isomers. Saturated derivatives and monounsaturated linear C18:1 and C19:1 were detected. The main lipids in mantle were DMA 18:1 and *i*-17:0; adductor, 19:1; motor muscle, *i*-17:0, 17:0, and *i*-18:0; viscera, 17:0 and 18:0. The contents of DMA 19:0 and 20:0 were low (<0.5% of total FAs). DMA 19:0 was absent in mantle and viscera. DMA 20:0 was missing only in lipids from viscera. The lipid compositions of soft tissues of the Far-East bivalve mollusk *M. chinensis* indicated that they contained aldehyde-generating species localized predominantly in motor muscle and viscera.

TABLE 3. Fatty-Acid Compositions of Lipids from Soft Tissues of Bivalve Mollusk *M. chinensis*, % GC

Fatty acid	Mantle	Adductor	Motor muscle	Viscera
14:0	1.7 ± 0.07	2.3 ± 0.10	3.0 ± 0.14	3.2 ± 0.13
15:0	0.1 ± 0.05	0.9 ± 0.04	0.2 ± 0.01	0.5 ± 0.02
16:0	18.9 ± 0.80	12.9 ± 0.60	11.5 ± 0.48	14.3 ± 0.72
17:0	1.3 ± 0.06	0.9 ± 0.04	1.5 ± 0.06	0.4 ± 0.02
18:0	3.5 ± 0.13	5.2 ± 0.23	2.6 ± 0.12	6.5 ± 0.28
20:0	0.3 ± 0.01	0.2 ± 0.01	0.1 ± 0.005	0.1 ± 0.005
14:1	0.5 ± 0.02	0.9 ± 0.04	1.0 ± 0.05	1.8 ± 0.08
16:1(n-9)	0.2 ± 0.01	0.1 ± 0.005	0.5 ± 0.02	0.3 ± 0.01
16:1(n-7)	2.5 ± 0.10	3.3 ± 0.15	5.0 ± 0.21	3.2 ± 0.15
16:1(n-5)	0.1 ± 0.005	0.5 ± 0.02	0.3 ± 0.01	0.4 ± 0.01
17:1	0.3 ± 0.01	0.1 ± 0.005	0.2 ± 0.01	0.4 ± 0.02
18:1(n-11)	0.1 ± 0.005	0.2 ± 0.01	0.3 ± 0.01	0.1 ± 0.005
18:1(n-9)	6.0 ± 0.27	2.8 ± 0.10	4.7 ± 0.21	5.3 ± 0.25
18:1(n-7)	0.5 ± 0.02	0.9 ± 0.04	1.1 ± 0.05	0.6 ± 0.03
18:1(n-6)	0.1 ± 0.004	–	–	0.3 ± 0.01
20:1(n-11)	3.3 ± 0.14	4.6 ± 0.20	3.9 ± 0.18	7.0 ± 0.32
20:1(n-9)	1.1 ± 0.05	2.0 ± 0.10	0.7 ± 0.03	1.5 ± 0.07
20:1(n-7)	2.6 ± 0.11	5.1 ± 0.22	2.9 ± 0.13	3.7 ± 0.16
22:1(n-11)	0.3 ± 0.01	0.7 ± 0.03	0.2 ± 0.01	0.1 ± 0.005
22:1(n-9)	0.3 ± 0.01	0.1 ± 0.005	0.2 ± 0.01	0.1 ± 0.005
16:2(n-6)	0.4 ± 0.02	0.5 ± 0.02	0.1 ± 0.005	0.3 ± 0.01
18:2(n-4)	0.1 ± 0.005	–	–	–
18:2(n-6)	0.6 ± 0.03	0.4 ± 0.02	0.3 ± 0.01	1.0 ± 0.04
18:3(n-3)	0.3 ± 0.01	1.1 ± 0.05	1.7 ± 0.07	0.5 ± 0.02
18:3(n-6)	0.3 ± 0.01	0.6 ± 0.02	0.1 ± 0.005	0.2 ± 0.01
18:4(n-1)	0.5 ± 0.02	0.9 ± 0.04	1.5 ± 0.07	1.0 ± 0.04
18:4(n-3)	4.9 ± 0.24	3.0 ± 0.13	4.2 ± 0.19	6.6 ± 0.30
20:2(n-6)	0.7 ± 0.03	1.8 ± 0.08	2.0 ± 0.08	0.9 ± 0.04
20:2(5,11)	0.4 ± 0.02	0.1 ± 0.005	0.5 ± 0.02	0.1 ± 0.005
20:3(n-6)	0.3 ± 0.01	0.1 ± 0.005	0.3 ± 0.01	0.2 ± 0.01
20:3(n-9)	0.8 ± 0.04	0.5 ± 0.02	0.3 ± 0.01	0.2 ± 0.01
20:4(n-3)	1.7 ± 0.06	1.2 ± 0.05	0.3 ± 0.01	1.9 ± 0.08
20:4(n-6)	4.7 ± 0.21	5.5 ± 0.20	4.0 ± 0.17	2.4 ± 0.10
20:5(n-3)	18.0 ± 0.80	15.0 ± 0.72	16.9 ± 0.84	13.0 ± 0.65
21:5(n-3)	0.1 ± 0.005	0.5 ± 0.02	1.4 ± 0.05	1.0 ± 0.05
22:4(n-6)	3.9 ± 0.17	2.0 ± 0.10	1.9 ± 0.06	3.0 ± 0.13
22:5(n-6)	0.7 ± 0.03	1.3 ± 0.05	1.9 ± 0.07	0.8 ± 0.03
22:5(n-3)	1.9 ± 0.07	2.2 ± 0.10	3.7 ± 0.15	2.5 ± 0.10
22:6(n-3)	14.0 ± 0.70	15.9 ± 0.71	16.8 ± 0.80	13.1 ± 0.72
Total	98.0 ± 4.49	96.3 ± 4.90	97.8 ± 4.56	98.5 ± 4.81
SFA	25.8 ± 0.98	22.4 ± 1.01	18.9 ± 0.91	25.0 ± 1.12
MUFA	17.9 ± 0.77	21.3 ± 1.00	21.0 ± 1.02	24.8 ± 1.15
PUFA	54.3 ± 2.62	52.6 ± 2.39	57.9 ± 2.80	48.7 ± 2.31
ω3	40.8 ± 1.69	38.9 ± 1.80	45.0 ± 1.90	38.6 ± 1.90
ω6	11.7 ± 0.45	12.2 ± 0.52	10.6 ± 0.50	9.1 ± 0.41
ω7	5.6 ± 0.20	9.3 ± 0.41	9.0 ± 0.40	7.5 ± 0.31
ω9	8.4 ± 0.37	5.5 ± 0.22	6.4 ± 0.30	7.4 ± 0.32
ω11	3.7 ± 0.18	5.5 ± 0.23	4.4 ± 0.19	7.2 ± 0.33

Table 3 lists the FA compositions of lipids from soft tissues of *M. chinensis*.

Lipids of *M. chinensis* contained 39 FAs of various structures with different quantitative contents and localizations. Of these, 6 were saturated (SFA); 14, unsaturated; and 19, polyunsaturated (PUFA). Adductor and muscle had 37 FAs; viscera, 38 FAs.

SFAs made up 18.9–25.8% with the minimal content in muscle and maximal, in mantle. This class was second in content for all studied mollusk parts except muscle. The major SFAs were 16:0 (11.5–18.9% of total FAs with dominance in mantle); 18:0 (2.6–6.5% of total FAs with dominance in viscera); 14:0 (1.7–3.2% of total FAs with dominance in viscera). The total monounsaturated FA (MUFA) contents in the various mollusk parts was 17.9–24.8% with dominance in viscera. This class was the least represented quantitatively for mantle, adductor, and viscera although their contents were comparable with that of SFA in adductor and viscera. Adductor and muscle did not contain 18:1(n-6) FA. The main MUFAs with respect to content were 18:1(n-9) (2.8–6.0% of total acids with dominance in mantle); 20:1(n-11) (3.3–7.0% of total acids with dominance in viscera); 16:1(n-7) (2.5–5.0% of total acids with dominance in muscle); and 20:1(n-7) (2.6–5.1% of total acids with dominance in adductor). The contents of the others were substantially less.

PUFAs were the leading representative of the FA class in lipids of all mollusk parts. Adductor and muscle contained one PUFA less than mantle and viscera. The total PUFA contents were 48.7–57.9% of total FAs with dominance in muscle. The main PUFAs were eicosapentaenoic (13.0–18.0%) and docosahexaenoic acids (13.1–16.8%). Eicosapentaenoic FA reached a maximum in mantle that was 7–38% more than in other parts. Docosahexaenoic FA had the highest content in muscle and was 6–28% more than in other parts.

An analysis of lipid FAs from *M. chinensis* showed that they belonged mainly to the families ω3, ω6, ω7, ω9, and ω11. FAs of the ω11 family had the lowest contents in all parts at 3.7–7.2% with dominance in viscera. The contents of FAs of the ω9 family varied in the range 5.5–8.4% with dominance in mantle. FAs of the ω7 family made up 5.6–9.3% with dominance in adductor. FAs of the ω3 and ω6 families had high biological activities, which were scientifically proven and utilized in nutritional science [5]. FAs of the ω3 family occurred in significant amounts and were the most represented class. Their total contents were 38.6–45% with dominance in muscle. The main FAs of this family were eicosapentaenoic, docosapentaenoic, stearidonic, and docosahexaenoic acids. FAs of the ω6 family appeared in significantly lower amounts, 9.1–12.2% with dominance in adductor. The main FAs of the ω6 family were arachidonic, docosatetraenoic, and docosapentaenoic.

EXPERIMENTAL

GC was performed on a GC-14B gas chromatograph (Shimadzu, Japan) with a flame-ionization detector and capillary quartz column (0.25 mm × 30 m) with Supelcowax 10^{TM} stationary phase (Supelco, USA). The conditions were injector temperature, 220°C; detector, 220°C; column, 190°C. The carrier-gas (high-purity He) flow rate was 40 mL/min. Chromatographic peak areas were calculated. Results were processed on a PC using a Chromatopac C-R4A data processor (Shimadzu, Japan) and the MLCW program (Ampersand, Russia).

Structures of DMA of aldehydes and methyl esters of FAs were determined by GC-MS on a GCVS-QP2010 Ultra gas-chromatograph–mass-spectrometer (Shimadzu, Japan) with an ultrafast quadrupole mass-selective detector and an HP-5ms column (0.25 mm × 30 m). The temperature regime was 125°C for 1 min rising to 250°C at 5°C/min. The ionization energy was 70 eV. The carrier-gas (He) flow rate was 1 mL/min. The ion-source temperature was 250°C.

Lipids were extracted by the Folch method [6]. FL were extracted from previously ground material by multiple standings with extraction benzine. The US contents obtained from alkaline hydrolysis were determined in the FL and isolated as before [7]. BL were extracted from the mass remaining after evaporation of the benzine by standings (5×) with $CHCl_3$–MeOH (2:1). The $CHCl_3$–MeOH extract was purified of non-lipid components using aqueous $CaCl_2$ solution (0.05%). The contents of NL, glyceroglycolipids, and phospholipids in the BL were determined by column chromatography over silica gel with elution by benzine, $CHCl_3$, and MeOH. The quantitative contents of cholesterol esters were determined by the hydroxamate method; cholesterol, by a color reaction [8]; sterols, by the Liebermann–Burchard reaction [9] and measurement of the optical density at 656 nm on a UV-1800 spectrophotometer (Shimadzu, Japan). A calibration curve was constructed using a standard cholesterol solution.

FA methyl esters were produced by transesterification using the Carreau and Dubacq method [10] and purified by TLC using hexane–Et_2O (95:5) followed by elution from the silica gel by hexane. The eluate was concentrated to the minimal volume and analyzed by GC [11].

ACKNOWLEDGMENT

The work was sponsored by the Russian Science Foundation (Project No. 14-50-00034).

REFERENCES

1. S. V. Yavnov, *Izv. TINRO*, **159**, 123 (2009).
2. O. V. Tabakaeva and A. V. Tabakaev, *Chem. Nat. Compd.*, **51**, 946 (2015).
3. N. A. Preobrazhenskii and R. P. Evstigneeva, *Chemistry of Biologically Active Natural Compounds* [in Russian], Khimiya, Moscow, 1976, 456 pp.
4. O. V. Tabakaeva and A. V. Tabakaev, *Chem. Nat. Compd.*, **52**, 966 (2016).
5. B. A. Shenderov, *Klin. Pitan.*, **4**, 14 (2004).
6. J. Folch, M. Lees, and G. H. Sloan-Syanley, *J. Biol. Chem.*, **226**, 497 (1957).
7. *Handbook of Study Methods, Technical Control, and Production Accounting in the Oil-Fat Industry* [in Russian], Vol. 1, Book 2, 1967, 815 pp.
8. F. M. Engelbrecht, F. Mori, and I. T. Anderson, *S. Afr. Med. J.*, **48**, 250 (1974).
9. O. D. Kushmanova and G. M. Ivchenko, *Handbook for Laboratory Studies in Biological Chemistry* [in Russian], Meditsina, Moscow, 1983, 185 pp.
10. L. Caron, J. P. Dubacq, C. Bercaloff, and H. Jupin, *Plant Cell Physiol.*, **26**, 131 (1985).
11. J. P. Novak, *Quantitative Analysis by Gas Chromatography*, Marcel Dekker, New York, 1975.