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GAS CHROMATOGRAPHIC STUDIES ON THE SEASONAL CHANGES IN THE FATTY ACID COMPOSITION OF THE COPEPOD (CRUSTACEA) PLANKTON

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In earlier investigations we found that the fat of crustacean plankton has much higher iodine values in winter, than in summer (FARKAS and HERODEK, 1964). That time the fatty acid composition was analysed still by paper chromatography. From such a complicated mixture after developing on paper fatty acids differing in chain length and in degree of unsaturation get to the same spots. However by hydrogenating the samples before chromatography, it was possible to check the seasonal changes at least in the distribution according to the chain length. This way a definite increase in C_{20}, C_{22} fatty acid content of copepod crustaceans by decreasing water temperature was demonstrated. We supposed, that the bulk of these long-chain fatty acids consisted of poly-unsaturated-ones. Later by gas chromatography these long, highly unsaturated fatty acids were really demonstrable from several crustacean species (HERODEK and FARKAS, 1967).

In present work seasonal changes of the fatty acid composition are followed up by gas-liquid chromatography, in order to determine exactly which fatty acids are involved in the temperature adaptation of crustaceans.

Material and methods

The crustaceans were sampled with a No. 6 plankton net from Belső-tó pond in the peninsula of Tihany. The animals were transferred alive in water to the laboratory. The samples were examined under microscope, and used only if besides Cyclopids *(Cyclops vicinus, Acanthocyclops* sp.) other species were not present in more than one per cent. Fresh weight of the collected material was several grams. The animals were blotted and grounded by anhydrous Na_2SO_4. The samples were extracted three times with 30 ml petroleum ether/g sample under reflux in N_2 atmosphere. The lipid content of the pooled solvents was determined gravimetrically, after evaporating an aliquot. Accordingly another aliquot containing 40 mg lipid was taken, evaporated under N_2, and the lipid redissolved in hexane. Transmethylation was carried out with absolute methanol containing 5 per cent cc. HCl. Five ml of this hydrochloric acid-methanol mixture was added to the 40 mg lipid dissolved in 1 ml hexane, and the mixture was sealed into test tubes under N_2. The tubes were kept at 80°

Table 1.

Seasonal changes in the

Date of sampling	Water temperature t °C	14 : 0	14 : 1	15 : 0	15 : 1	16 : 0	16 : 1	16 : 2	16 : 3	18 : 0	18 : 1
1967. X. 11.	17	2.5	1.8	1.1	—	14.0	15.5	—	—	6.1	9.3
X. 25.	12	2.0	0.7	0.8	—	11.7	12.0	—	—	5.7	7.9
XII. 6.	4	1.9	1.0	1.7	—	8.4	10.8	—	—	3.8	7.9
1968. II. 27.	3	1.4	1.0	0.9	—	7.8	4.2	3.4	—	2.2	4.5
III. 13.	2	1.6	0.6	0.5	1.3	5.9	1.7	—	—	1.1	4.5
IV. 3.	14	2.5	1.7	0.4	0.9	10.8	7.8	3.8	2.2	6.6	8.4
VII. 6.	24	1.8	1.4	1.7	0.8	11.0	8.3	2.0	1.5	6.0	13.7

C for 4 hours. After cooling down the upper phase containing the methyl ester was separated.

The analysis was carried out by a Chrom III. IKZ (Laboratorni Pristroje CSSR) gas chromatograph. The equipment operated with flame ionization detector. Column length was 3 m, inner diameter 6 mm. It was filled with 20% ethylene glycolsuccinate on 80—100 mesh Chromosorb W. Column temperature was 184° C. Carrier gas was N_2, its flow rate 100 ml/min. Standard fatty acids (NIH) and mixtures of known fatty acid composition were used for peak identification. Peak areas were determined by triangulation. Correction factors for individual fatty acids were determined by means of known fatty acid mixtures of similar composition as the samples. With these factors the fatty acid composition was calculated in weight per cent.

Results

Altogether 25 different fatty acids were detected in the crustaceans *(Table 1)*. Some of them were not present in all samples in measurable quantities. There were 11 fatty acids surpassing at least in some months the 5 per cent of the total quantity of fatty acids. Each of the fatty acid 16 : 0, 16 : 1, 18 : 1, 18 : 4, 20 : 5 and 20 : 6 represented in some months more than 10 per cent. Only by 22 : 6 was the 20 per cent exceeded in winter. The longer the chain, the more prominent are the unsaturated acids. Among fatty acids with 14 carbon atoms the saturated myristic acid predominates. Among those with 16 carbon atoms 16 : 0 and 16 : 1 show about the same values. All C_{18} fatty acids were detected in quantities above 5 per cent, but the most unsaturated acid was already found in the highest amount. Among the C_{20} acids 20 : 4 and even more 20 : 5 dominate. Finally among the longest, 22 carbon atom long fatty acids only the most unsaturated acid was found above 5 per cent, but this 22 : 6 acid is the most abundant of all acids in the winter.

Investigations started with samples collected from still warm water, and changes in the fatty acid composition were followed during the cooling down of the water and its warming up in the next year. Accordingly data show in the middle of the *Table* the fatty acid composition in the coldest period and on the whole they change symmetrically upwards and downwards. It was

fatty acid composition

18 : 2	18 : 3 ω 3	18 : 4	20 : 1	20 : 2	20 : 3 ω 6	20 : 3 ω 3	20 : 4 ω 6	20 : 4 ω 3	20 : 5 ω 3	22 : 4 ω 6	22 : 4 ω 3	22 : 5 ω 6	22 : 5 ω 3	22 : 6 ω 3
4.6	5.1	16.1	—	2.4	—	—	6.1	—	10.6	—	—	—	—	4.8
1.4	3.5	9.7	—	2.3	—	—	4.9	0.8	12.8	0.7	2.2	1.8	1.7	17.4
1.9	5.7	11.7	—	4.1	—	0.7	3.7	1.0	13.2	0.4	1.7	1.1	0.6	18.7
6.0	2.6	6.2	—	9.9	1.2	0.3	2.7	2.4	13.9	1.4	1.7	—	1.1	25.2
4.5	5.1	9.0	4.2	4.6	05.	1.7	2.0	2.2	12.3	4.8	3.0	2.9	1.7	24.4
4.6	4.6	4.2	—	0.9	—	—	4.4	0.6	10.1	0.3	2.9	2.5	—	19.8
5.3	1.1	11.4	—	3.6	—	0.7	5.4	1.1	10.4	1.0	1.7	—	1.1	8.9

found in cases of C_{14}, C_{16} and C_{18} acids equally a decrease of saturated and monoenoic acids by the cooling down of the water. No changes paralleling that of the temperature were found in the di-, tri-, and tetraenoic acids. The 20 : 5 showed a definite increase from the summer value 10.5 to the winter 13.9. The most essential change was revealed in the 22 : 6 content, which by the cooling down of the water increased from 4.8 to 25.2 per cent.

Discussion

The significance of changes in the fatty acid composition for the survival of plankton crustaceans was indicated already earlier (FARKAS and HERODEK, 1964). The more unsaturated is the fat, the lower is its melting point. Melting point determinations of the fat of plankton samples collected at different times showed it to be always with 1—2° C lower, than the lake temperature. This means, that these animals are capable to regulate the composition of their fat in such a way, that it remains in a just liquid state the whole year through. In this process — as revealed by the present gas-liquid chromatographic results — to a lesser extent 20 : 5 and principally 22 : 6 acids are involved. The total of these two acids is high enough in winter, that even every glycerolipid molecule could get to one of them. The incorporation of fatty acids with 5 or 6 double bonds can alter basically the physical properties of the lipid molecules and the state of their mixture. The inter and intra-molecular distribution of these fatty acids seems to be worthy of further examination.

The elucidation of the mechanism regulating 22 : 6 level in the fat according to the changing requirements in these some millimeter long animals seems a difficult task. It is possible, that the synthesis of fatty acids or their incorporation into the depot fat is influenced by the temperature or by the physical state of fat. In green algae 22 : 6 was not yet detected (KLENK, 1963) and until it is not demonstrated from the food it can be supposed, that crustaceans produce it themselves. In 22 : 6 acid, isolated from fishes, the positions of double bonds are identical with those of decosahexaenoic acid of mammals. Fish oil originates by far greater part in plankton crustaceans. It is therefore most likely that 22 : 6 acid of plankton crustaceans is of divinyl-methane

structure too. If so, its synthesis may proceed in the same way that was demonstrated in mammals (KLENK, 1960; MEAD, 1960) i.e.:

$$18 : 3 \rightarrow 18 : 4 \rightarrow 20 : 4 \rightarrow 20 : 5 \rightarrow 22 : 5 \rightarrow 22 : 6.$$

Crustaceans can take up plenty of linolenoic acid from algae. Whether these animals can synthetize this acid or not is not yet known. The two acids involved in the temperature adaptation are closely related, as 22 : 6 is formed from 20 : 5.

Fatty acid composition of plankton crustaceans can correspond to the temperature so well only because the temperature changes are rather slow owing to the great heat capacity of the water. Poikilothermic land animals are subject to much higher temperature changes during short periods, sometimes exceeding 10 °C a day, and of course, they are not expected to change the composition of their fat so rapidly that its melting point should be always with 1—2 °C lower than the external temperature.

Summary

For the winter months the amount of saturated and monoenoic acid content of the fat decreased.

In di-, three- and tetraenoic acids no changes paralleling that of the temperature were observed. The 22 : 5 content somewhat increased for winter.

The greatest change was found in 22 : 6, the most unsaturated fatty acid. In summer it amounts to 5 per cent, while in winter to 25 per cent of the total fatty acids. It seems that the variations in the quantity of 22 : 6 ensures the constant optimal physical state of fat in crustaceans in spite of seasonal changes of the environmental temperature.

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A COPEPODA (CRUSTACEA) PLANKTON ZSÍRJÁBAN MUTATKOZÓ ÉVSZAKOS VÁLTOZÁSOK GÁZKROMATOGRÁFIÁS VIZSGÁLATA

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Összefoglalás

A téli hónapokra lecsökkent a zsírban a telített és egyszer telítetlen zsírsavak mennyisége.

A kétszer, háromszor és négyszer telítetlen zsírsavak esetében nem észleltünk a hőmérséklet alakulásával összefüggő változást. A 22 : 5 mennyisége télire kissé megemelkedett. A legnagyobb változást a 22 : 6, a legtelítetlenebb zsírsav mutatta. Ez nyáron az összes zsírsavak 5, télen 25%-át tette ki. Úgy látszik, hogy a 22 : 6 mennyiségének változása biztosítja, hogy a hőmérséklet évi változása ellenére a rákokban a zsír mindig optimális halmazállapotú maradjon.

ИССЛЕДОВАНИЕ СЕЗОННЫХ ИЗМЕНЕНИЙ ЖИРОВ РАКООБРАЗНОГО ПЛАНКТОНА ПРИ ПОМОЩИ ГАЗОВОЙ ХРОМАТОГРАФИИ

Ш. Херодек

Зимой в составе жиров происходит снижение количества насыщенных и однократно ненасыщенных жирных кислот.

В составе двух—, трех—, и четырехкратно ненасыщенных жирных кислот не были найдены изменения, зависящие от температуры: соотношение 22:5 зимой несколько повысилось. Самое значительное изменение было обнаружено в случае наиболее ненасыщенной жирной кислоты 22:6. Эта жирная кислота составляла летом 5 процентов от всего количества жирной кислоты — а зимой 25%. Вероятно, изменение соотношения 22:6 обеспечивает в раках, несмотря на годичные колебания температуры, постоянное оптимальное состояние жира.