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GAS CHROMATOGRAPHIC STUDIES ON THE FATTY ACID COMPOSITION OF SOME FRESH-WATER CRUSTACEANS

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A very significant part of the enormous biomass of water organisms consists of lipids. Mankind utilizes of this quantity about 1 million metric ton a year. This rate will only increase in the coming years due to the quick progress in sea-fisheries (SWAIN 1958). As the utilization of fish fats depends on their chemical composition numerous data are already known in this field (HILDITCH 1956).

According to these analyses the fats of fishes differ characteristically from those of land animals. Experiments in aquaria showed (KELLY et al. 1958, FARKAS and HERODEK 1964) that food determines basically the composition of fats in fishes. As a source of the fat in fishes primarily plankton crustaceans can be taken into account. With fractionated destillation the fatty acid composition of altogether four crustacean species was investigated previously (LOVERN 1935). In our earlier papers (FARKAS and HERODEK 1959, 1961, 1962, 1964) we published paper chromatographic analysis on the composition of fatty acids of ten species.

The resolving power of both fractionated distillation and paper chromatography is rather restricted. The aim of this paper is to give a more detailed and correct picture on the fatty acid composition of several crustaceans with gas chromatographic method.

Material and methods

Plankton crustaceans were sampled with a No. 6 net trawled by a rowboat. Living animals, kept in water were brought to the laboratory where the species composition of the samples were determined. If a sample contained Cladocerans and Copepods the water was bubbled through in order to separate them, in this way the Cladocerans were brought to the surface while the Copepods stayed at the bottom. Only samples were determined where the species to be investigated was present in at least 99%. Therefore the analytical data are characteristic for the given species. The needed quantity of the Amphipods were collected with pincers.

From each species samples of some grams in fresh weight were obtained. The water was blotted from the animals with filter-paper then they were ground-

ed with anhydrous Na_2SO_4. The fat was extracted in N_2 atmosphere under reflux three times with petroleum ether. For 1 g sample 30 ml solvent was used. An aliquot of the extract containing 40 mg lipid was evaporated under N_2 and taken up in 1 ml hexane. Fatty acids of this lipid were transmethylated with absolute methanol containing 4% of cc HCl. To the lipids dissolved in 1 ml hexane 5 ml of this hydrochloric acid-methanol mixture was added and the samples were kept for 4 hours at 80° C in a heating block. When they cooled down the upper phase containing the fatty acid methyl esters was removed.

The analysis was carried out by a Chrom III. IKZ (Laboratorni Pristroje CSSR) gas chromatograph. The length of the column was 3 m, the inner diameter was 6 mm. 20% ethylene glycolsuccinate on 80—100 mesh Chromosorb W was used as stationary phase. The carrier gas was N_2, its flow rate 100 ml/min. The column pressure was 1.5 atmosphere, its temperature 184° C. The equipment operated with flame ionization detector. The fatty acid methyl esters, dissolved in hexane were injected with a microsyringe. For the time of injection the temperature of the sample heater was 240° C. The identification of the peaks took place by comparing the chromatogram with that of standard fatty acids and of samples of known composition. Peak areas were determined by triangulation. The correction factors for the individual fatty acids were determined by means of known fatty acid mixtures of similar composition as the samples. The percentual fatty acid composition was calculated in using these factors.

Results and discussion

In *Fig. 1* the gas chromatogram of the fatty acid methyl esters of a plankton crustacean species is demonstrated.

The fatty acid composition of different samples is summarized in *Table 1*. The accepted abbreviation techniques were used here, too, fatty acids are characterized with two numbers, the first giving the length of the carbon chain, the second after the colon (:) is the number of double bonds for 1 molecule.

From *Table 1* it can be seen that the same fatty acids are present in all crustaceans only with quantitative differences. These fatty acids are the

Table 1

The fatty acid composition of freshwater crustaceans (per cent wt.)

	14 : 0	14 : 1	14 : 2	16 : 0	16 : 1	16 : 2	18 : 0
1. *Cyclops vicinus* ULJ.	7.8	0.3	0.3	13.6	5.9	0.7	4.4
2. *Eudiaptomus gracilis* (G. O. SARS	9.7	tr	tr	17.7	7.5	0.2	3.8
3. *Daphnia cucullata* (G. O. SARS)	7.0	0.8	0.1	16.4	5.7		6.8
4. *Daphnia longispina* O. F. MÜLLER	2.5	3.0	0.8	11.7	14.6	0.8	3.7
5. *Simocephalus vetulus* V. F. M.	3.5	3.8	1.4	12.8	12.1		5.1
6. *Bosmina longirostris* f. *pellucida* STINGELIN	2.5	2.3	2.8	14.0	10.3		7.4
7. *Gammarus (Rivulogammarus) roeseli* GERVAIS	1.4	1.5	1.7	12.1	12.3		4.1

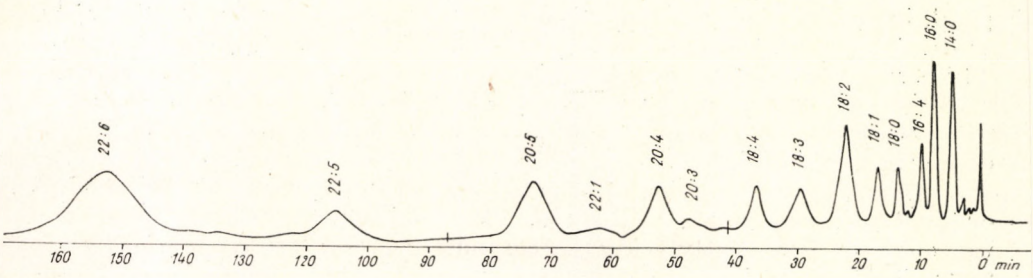

Figure 1. — 1. ábra

The gas chromatogram of the plankton crustacea an Cyclops vicinus

A Cyclops vicinus planktonrák zsírsav-metilésztereinek gázkromatogramja

same ones considered as characteristic for fats in fishes and even their quantitative relations are greatly resembling to those in fishes (GRUGER 1964, FARKAS and HERODEK 1967). The similarity of the fatty acid composition of crustaceans and fishes also at the resolving level of gas chromatography furnishes further evidence for the hypothesis that plankton crustaceans are the main source of fats for fishes. The only fatty acid occurring in much lower quantities in crustaceans than in fishes is oleic acid. It seems that fishes, like land animals, store their endogenous fatty acids mainly in the form of oleic acid.

Among the species of *Table 1* there are two Copepods *(Cyclops vicinus* and *Eudiaptomus gracilis)*, sampled in the area before our institute from Lake Balaton at 6 C° water temperature, on the 16th and on the 23rd November resp., 1966. The fatty acid composition of these two species is extraordinarily similar. This may be explained by the identic biotop, equal living habits and the relationship. On the other hand the fatty acid composition of *Daphnia cucullata*, sampled at the same time and from the same place of Lake Balaton was definitely different, deviating chiefly by the lower level of 22 : 6 fatty acids in it. In contrary to the above-mentioned two species *Daphnia cucullata* belongs to the Cladocerans. On the 29th November three further Cladocera species were sampled, *Daphnia longispina* and *Bosmina longirostris* from the

— *1. Táblázat*

Édesvízi rákok zsírsav-összetétele súlyszázalékban

18 : 1	18 : 2	18 : 3	18 : 4	20 : 2	20 : 4	22 : 1	20 : 5	22 : 4	22 : 5	22 : 6
5.2	9.9	6.6	9.2	1.5	9.7	1.1	11.9	0.2	4.6	10.1
6.1	10.6	6.9	8.8	1.7	4.2		10.5	tr	3.4	8.9
8.3	15.5	8.2	6.3	1.1	6.7		7.6		5.4	4.1
10.1	4.2	11.3	15.8	0.7	1.3	0.6	17.4			1.5
11.8	6.0	6.8	7.8	2.0	6.6		18.9		0.4	1.0
19.3	4.1	5.7	2.8	1.7	5.4		21.7			
24.5	16.0	6.7	1.9		7.3		10.5			

pond Belső-tó in Tihany and *Simocephalus vetulus* from Lake Velencei-tó. Water temperature of both lakes was 3 °C. In the fatty acid composition of all the four Cladocerans there are definite differences, however, in all of them there is much more less 22 : 6 than in Copepods. To decide the generality of this phenomenon will be only possible in the knowledge of analytical records of much more species.

One Amphipod species, *Gammarus roeseli* was sampled from the detritus of the Aszófői-Séd brook. In this crustacean similarly to *Bosmina longirostris* we could not detect fatty acid of 22 carbon atoms and it also contained great amounts of oleic acid. The crustaceans analyzed show a certain regularity insofar that the less 22 : 6 is present the higher is the level of 18 : 1.

Previously we demonstrated that the fatty acid composition of freshwater crustaceans changes according to a regular yearly cycle (FARKAS and HERODEK 1964). By altering the composition of their fat the crustaceans can assure that the melting point of the fat should be some degrees lower than the water temperature, that means that the fats can stay all the year round in the suitable liquid state. In this sense data of *Table 1* do not represent a constant, unalterable property. A comparison among the different species is possible only in that case if it takes place among samples collected at the same time and at similar water temperatures. In our present investigations all species were sampled in the second half of November. Samples collected during summer presumedly will prove to be more saturated. According to our earlier results (FARKAS and HERODEK 1962) parallel with the decrease of water temperature the iodine value of the fat increases, demonstrating the rising unsaturation. Furthermore we found paper chromatographically that the quantity of fatty acids of 20 and 22 carbon atoms increases too. We supposed that these can be penta- and hexaenoic fatty acids and in their quality may contribute to the decrease of the fat's melting point to a great extent. Paper chromatographically this hypothesis could not be stated unequivocally, but our recent gas chromatographic data justify this hypothesis. The 20 : 4 and 22 : 5 fatty acids originate from linoleic acid, the 20 : 5 and 22 : 6 fatty acids from the linolenoic fatty acid by chain elongation and by dehydrogenation in divinyle-methan rhythm (MEAD 1960). The data of *Table 1* show that in crustaceans more fatty acid originates in the linolenoic than in the linoleic acid.

Summary

The fatty acid compositions of the following crustaceans were determined: *Cyclops vicinus* ULJ., *Eudiaptomus gracilis* (G. O. SARS), *Daphnia cucullata* G. O. SARS, *Daphnia longispina* O. F. MÜLLER, *Simocephalus vetulus* V. F. M., *Bosmina longirostris* f. *pellucida* STINGELIN, *Gammarus (Rivulogammarus) roeseli* GERVAIS.

From the different species the same fatty acids could be demonstrated. The fatty acid composition of crustaceans is greatly similar to those published for fishes suggesting that the bulk of the fats in fishes originates in the crustacean plankton.

There was more C_{22} polyunsaturated fatty acid in the investigated Copepods than in the Cladocerans. The changes in the quantity of oleic acid in the different samples are showing an inverse tendency to the quantity of C_{22} polyunsaturated fatty acids.

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NÉHÁNY ÉDESVÍZI RÁK ZSÍRSAV-ÖSSZETÉTELÉNEK GÁZKROMATOGRÁFIÁS ELEMZÉSE

Herodek Sándor és *Farkas Tibor*

Összefoglalás

A következő rákok zsírsav-összetételét határoztuk meg: *Cyclops vicinus* ULJ., *Eudiaptomus gracilis* (G. O. SARS), *Daphnia cucullata* G. O. SARS, *Daphnia longispina* O. F. MÜLLER, *Simocephalus vetulus* V. F. M., *Bosmina longirostris* f. *pellucida* STINGELIN, *Gammarus (Rivulogammarus) roeseli* GERVAIS.

A különböző fajokból ugyanazokat a zsírsavakat lehetett kimutatni. A rákok zsírsavösszetétele nagyban hasonlít a halakról közölt adatokhoz, ami arra utal, hogy a halak zsírsavainak zöme a crustacea planktonból származik.

A vizsgált Copepodákban több C_{22} többszörösen telítetlen zsírsav volt, mint a Cladocerákban. Az egyes minták között az olajsav mennyisége a C_{22} többszörösen telítetlen zsírsavak mennyiségével ellentétes irányban változik.

Köszönetnyilvánítás

Köszönetünket fejezzük ki dr. PONYI JENŐNEK a rákok meghatározásáért, és a rákokra vonatkozó hasznos felvilágosításaiért.

АНАЛИЗ СОСТАВА ЖИРНЫХ КИСЛОТ НЕКОТОРЫХ ПРЕСНОВОДНЫХ РАКОВ ПОСРЕДСТВОМ ГАЗОВОЙ ХРОМАТОГРАФИИ

Шандор Херодек и *Тибор Фаркаш*

Состав жирных кислот определялся у следующих видов: *Cyclops vicinus* Uli. *Eudiaptomus gracilis* (G.O. Sars), *Daphnia cucullata* G.O. Sars, *Daphnia longispina* O.F. Müller *Simocephalus vetulus* V.F.M., *Bosmina longirostris* f. *pellucida* Stingelin, *Gammarus (rivulogammarus) roeseli* Gervais.

У разных видов обнаруживается один и тот же состав жирных кислот. Состав жирных кислот раков совпадает с составом жирных кислот рыб, что указывает на то, что бóльшая часть жирных кислот рыб происходит из планктонных ракообразных.

В копеподах было найдено больше C_{22} ненасыщенных жирных кислот чем в кладоцерах. В отдельных пробах содержание олеиновой кислоты изменялось обратно пропорциоально количеству многократно-ненасыщенных жирных кислот.