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Analysis of Fatty Acids of Some Crustaceans

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Abstract

1. The fatty acid composition of several crustaceans with various habitats was investigated by gas-liquid chromatography (GLC) on 10% DEGS.

2. The lipids obtained from a prawn, *Penaeus japonicus*, crab, *Helice tridens tridens tridens*, and shrimp, *Palaemon paucidens*, were rich in polyunsaturated fatty acids (42.0–47.9% of total acids) and deficient in the long chain monoenoic acids. A terrestrial crab, *Sesarma dehaani*, contained less polyunsaturated fatty acids (26.3% of total acids) but more saturated acids.

3. In all crustaceans examined, most of the polyunsaturated fatty acids were composed of the ω3-type of acids, especially eicosapentaenoic acid ($C_{20:5\omega3}$).

4. The shrimp, *P. paucidens*, and the crab, *S. dehaani*, comprised more monoenoic acids (38.6–40.5% of total acids) as compared with the prawn, *P. japonicus* and crab, *H. tridens tridens tridens*.

It is well known that marine animals generally contain large amounts of polyunsaturated fatty acids with a long carbon chain, whereas terrestrial animals involve relatively large amounts of saturated C_{16} and C_{18} acids. As to the crustaceans, many reports have been presented about the fatty acid composition of lipids; for example, crabs, *Pleuroncodes planipes* (Pierce *et al.*, 1969; van der Veen *et al.*, 1971), *Cancer magister* (Allen, 1971) and *Xiphosura* (*Limulus*) *polyphemus* (van der Horst *et al.*, 1973), shrimps, *Pandalus borealis* (Ackman & Eaton, 1967) and *Crangon septemspinosus* (Ackman & Hooper, 1973), lobsters, *Jasus lalandii* (de Koning & McMullan, 1966) and *Homarus americanus* (Brockerhoff *et al.*, 1968), prawn, *Penaeus japonicus* (Guary, 1973), mysids, *Neomysis interger* (Linford, 1965), euphausids, *Meganyctiphanes norvegica* (Ackman & Eaton, 1967), *Euphausia superba* (Hansen, 1969), *Thysanoessa inermis* (Ackman *et al.*, 1970), and *Euphausia* sp. (Saiki *et al.*, 1959; Jeffrey *et al.*, 1966; Pierce *et al.*, 1969; van der Veen *et al.*, 1971), and copepods (Ackman & Hooper, 1970; Morris, 1971). Data available up to the present have shown that the fatty acid composition of marine crustaceans was essentially similar to that of marine fish. Also, it has been pointed out that the fatty acid compositions varied according to several factors such as sampling seasons, dietary habitats, and physiological conditions. Recently, the investigation of lipid metabolism in crustaceans has been actively carried out. However, the information on fatty acid metabolism during molting process and on the nutritional requirements of crustaceans for lipids is still a little. Prior to investigating these subjects, the authors examined the fatty acid

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composition of several crustaceans with various types of habitats to elucidate their principal fatty acids.

This paper deals with the fatty acid composition of a prawn, *Penaeus japonicus* BATE (Japanese name, Kurumaebi), crabs, *Helice tridens tridens tridens* DE HAAN (Japanese name, Ashiharagani) and *Sesarma dehaani* H. MILNE-EDWARDS (Japanese name, Kurobenkeigani), and shrimp, *Palaemon paucidens* DE HAAN (Japanese name, Sujiebi).

Materials and Methods

Animals The prawn, *P. japonicus*, was obtained from Darumaya-Sangyo Co., Kagoshima. The crabs, *H. tridens tridens tridens* and *S. dehaani*, were collected in the marsh quite near brackish water at Ibusuki, Kagoshima. The crab, *H. tridens tridens tridens*, was inhabitting in the wet mud of relatively shallow depths, whereas *S. dehaani* was found in the hole of relatively dry mud. The shrimp, *P. paucidens*, was harvested in the Lake of Ikeda (fresh water), Kagoshima.

Fatty acid methylesters From four animals of each crustacean, lipids were extracted with chloroform-methanol-water (Bligh & Dyer, 1959) and converted to fatty acid methylesters by direct transesterification as follows: To 10 mg of lipids, 4 ml of HCl (5%)-methanol and 0.5 ml of dry benzene were added, and this mixture was refluxed over calcium chloride at 60°C for 2 hr. After cooling, the reaction mixture was diluted with 2 volumes of distilled water, and then fatty acid methylesters were extracted with petroleum ether 3 times, dehydrated over anhydrous sodium sulfate, and concentrated to dryness under nitrogen gas.

Gas-liquid chromatography (GLC) GLC was carried out as shown in Table 1. The identification of peaks in GLC was performed in consideration of the followings: a comparison of relative retention times (RRT) to stearic acid ($C_{18:0}$) methylester of samples with standards, a semilog plot of RRT of fatty acid methylesters vs. the number of carbon atoms (Herb *et al.*, 1962; Ackman, 1963), end carbon chain (ECC) (Ackman, 1963a) and equivalent chain length (ECL) (Hofstetter *et al.*, 1965) values, and separation factor (Ackman, 1963b). An aliquot of samples was hydrogenated with platinum oxide in *n*-hexane and the hydrogenates were analysed by GLC to confirm the identity of peaks. In addition, the peaks of chromatogram

Table 1. Conditions used in GLC.

Instrument:	Shimadzu Gas Chromatograph GC-4BP
Column:	Stainless steel, 4 mm i. d. × 3 m long
Column packing:	10% Diethylene glycol succinate polyester on 60–80 mesh Shimalite W
Column temperature:	185°C or 195°C
Carrier gas:	Nitrogen 40 ml/min.
Detector:	Flame ionization detector
Sample size:	1.0 μl (hexane solution)

were compared with those of the methylesters prepared from cod-liver oil as secondary reference standards (Ackman & Burger, 1965).

Results and Discussion

The fatty acid composition of the lipids obtained from four species of crustaceans is listed in Table 2. The fatty acid composition of prawn, *P. japonicus*, resembled that of the crab, *H. tridens tridens tridens*. The lipids from these crustaceans were rich in polyunsaturated acids and relatively low in monoenes as compared with other species examined. As to the above two crustaceans, the lipids comprised saturated acids (26–28% of total acids) such as palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$), and also monoenoic acids (25–28% of total acids) such as palmitoleic acid ($C_{16:1}$) and oleic acid ($C_{18:1}$). Palmitic and oleic acids consituted about half of the saturated and monounsaturated acid fractions, respectively. Also, these crustaceans contained high percentage of polyunsaturated acids (44.1–44.9%), in which eicosapentaenoic ($C_{20:5\omega3}$) and docosahexaenoic ($C_{22:6\omega3}$) acids as predominant acids. The large amounts of the ω3-type of polyunsaturated acids have been reported to be characteristic of lipids occurring in marine crustaceans and fish (Brockerhoff *et al.*, 1963; Ackman *et al.*, 1964, 1971; Addison *et al.*, 1972; Grunger *et al.*, 1964; Malins & Wekell, 1970; van der Horst, 1970; Guary, 1973). The above two crustaceans also contained relatively large quantities of the ω6-type of polyunsaturated acids, especially arachidonic acid ($C_{20:4\omega6}$) (5.8–9.3% of total acids).

The fatty acid composition of the fresh water shrimp, *P. paucidens*, was considerably different with those of *P. japonicus* and *H. tridens tridens tridens*, especially in the content of saturated (17.4% of total acids) and monounsaturated (40.5% of total acids) acids. The shrimp, *P. paucidens*, contained more $C_{16:1}$ and $C_{18:1}$ acids, and less $C_{18:0}$ acid. Regarding the polyunsaturated acids, this shrimp revealed higher percentage composition of $C_{20:4\omega3}$ (6.4% of total acids) and lower linoleic acid ($C_{18:2\omega6}$). Generally, fresh water crustaceans have been demonstrated to give the different fatty acid spectra with those of marine ones. For instance, O'Connor and Gilbert (1968) have pointed out that the fresh water (*Orconectes virilis*) and terrestrial (*Gecarcinus lateralis*) crustaceans contained high portion of $C_{16:1}$ and $C_{18:1}$ acids and relatively low polyunsaturated acids. Such a feature has been also observed in the fresh water crayfish, *Orconectes rusticus* (Wolfe *et al.*, 1965). In the present study, the shrimp, *P. paucidens*, was also shown to give the fatty acid spectrum typical for fresh water species.

In *P. japonicus*, *H. tridens tridens tridens* and *P. paucidens*, the major polyunsaturated acid was $C_{20:5\omega3}$ rather than $C_{22:6\omega3}$, contrary to marine vertebrates which contained $C_{22:6\omega3}$ as a major polyunsaturated acid instead of $C_{20:5\omega3}$. This observation has been reported about many crustaceans: the lobster, *J. lalandii* (de Koning & McMullan, 1966), crabs, *Callinectes sapidus* (Whitney, 1969) and *C. magister* (Allen, 1971), crayfish, *O. rusticus* (Wolfe *et al.*, 1965), copepods *Temora longicornis* (Ackman & Hooper, 1970)

and *Chionoecetes opilio* (Addison *et al.*, 1972), and euphausiids (Yamada, 1964; Mauchlin & Fisher, 1969; Pierce *et al.*, 1969; Hansen & Meiklen, 1970).

Table 2. Fatty acid composition of the crustaceans, *P. japonicus*, *H. tridens*, *S. dehaani*, and *P. paucidens*.

Fatty acid	RRT*	ECL**	% Composition			
			P. japonicus	*H. tridens*	*S. dehaani*	*P. paucidens*
14: 0	0.32		0.7	1.9	7.9	2.6
14: 1	0.37	14.38	—	1.2	3.0	—
15: 0	0.42		0.3	0.8	2.8	0.5
15: 1	0.48	15.38	0.6	0.5	0.8	0.4
16: 0	0.56		15.6	14.8	15.8	13.0
16: 1	0.63	16.37	3.6	9.1	15.8	14.0
17: 0	0.73		1.3	3.7	4.0	—
16: 2	0.85	17.38	1.3	2.4	2.9	0.9
18: 0	1.00		9.2	6.8	4.2	4.0
18: 1	1.13	18.38	19.5	14.3	18.6	24.5
18: 2ω6	1.39	19.04	6.6	4.5	8.0	1.7
18: 3ω6	1.65	19.65	—	0.7	0.5	0.9
18: 3ω3	1.83	20.05	0.5	1.0	6.5	—
20: 1	2.05	20.30	0.9	2.7	0.5	1.7
20: 2ω6	2.52	21.05	0.5	0.6	0.5	0.8
20: 3ω6	2.92	21.53	—	0.1	0.1	—
20: 4ω6	3.28	22.08	9.3	5.8	3.9	5.2
20: 4ω3	3.76	22.40	0.6	0.6	0.4	6.4
20: 5ω3	4.28	23.03	14.4	18.9	6.3	16.8
?	4.83	23.30	—	—	0.3	—
22: 4ω6	6.32	24.12	0.3	0.6	0.2	0.5
?	6.40	24.28	0.5	—	—	—
22: 5ω6	7.08	24.62	1.5	0.6	0.3	0.5
22: 5ω3	8.06	25.07	0.8	1.0	0.2	0.5
22: 6ω3	9.04	25.45	11.7	7.1	2.2	7.3
24: 4	12.90	26.65	0.4	0.3	0.1	1.5
Total						
Saturated acids			26.9	28.0	35.1	17.4
Monoenoic acids			25.1	27.8	38.6	40.5
Polyenoic acids			47.9	44.1	26.3	42.0

The shorthand designation is as follows: The number preceding the colon gives the number of carbons in the chain; the number of double bonds is shown by the figure following the colon; the ω-notation indicates the position of double bond from the terminal methyl group.

* Relative retention time to stearic acid methylester ($C_{18:0}$).

** Equivalent chain length.

In the case of the crab, *S. dehaani*, the lipids were rich in monounsaturated acids (38.6% of total acids) as well as the shrimp. *P. paucidens*. Also, the presence of large amounts of $C_{16:1}$ was typical for terrestrial species. However, this crab differed from the shrimp, *P. paucidens*, in the respect that the percentage composition of polyunsaturated acids such as $C_{20:5\omega3}$ and $C_{22:6\omega3}$ was low. In addition, it was notable that this crab contained substancial amounts of myristic ($C_{14:0}$) and linolenic ($C_{18:3\omega3}$) acids which were present in minute quantities in other crustaceans examined. The unique fatty acid spectrum of *S. dehaani* may be partly attributed to the special habitat on diets in this crustacean, but this point warrants further detailed study.

References

ACKMAN, R. G. (1963a): Structural correlation of unsaturated fatty acid esters through graphical comparison of gas-liquid chromatographic retention times on a polyester substrate. *J. Am. Oil Chem. Soc.*, **40**, 558–564.

ACKMAN, R. G. (1963b): An analysis of separation factors applicable in the gas-liquid chromatography of unsaturated fatty acid methyl esters on a polyester substrate. *J. Am. Oil Chem. Soc.*, **40**, 564–567.

ACKMAN, R. G., JANGAARD, P. M., HOYLE, R. J., and BROCKERHOFF, H. (1964): Origin of marine fatty acids. I. Analyses of the fatty acids produced by the diatom *Skeletonema costatum*. *J. Fish. Res. Bd. Can.*, **21**, 747–756.

ACKMAN, R. G. and BURGER, R. D. (1965): Cod liver oil fatty acids as secondary reference standards in the GLC of polyunsaturated fatty acids of animal origin: analysis of a dermal oil of the Atlantic leatherback turtle. *J. Am. Oil Chem. Soc.*, **42**, 38–42.

ACKMAN, R. G. and EATON, C. A. (1967): Fatty acid composition of the decapod shrimp, *Pandalus borealis*, in relation to that of the euphausid, *Meganyctiphanes norvegica*. *J. Fish. Res. Bd. Can.*, **24**, 467–470.

ACKMAN, R. G. and HOOPER, S. N. (1970): Analysis of fatty acids from Newfoundland copepods and sea water with remarks on the occurrence of arachidonic acid. *Lipids*, **5**, 417–421.

ACKMAN, R. G., EATON, C. A., SIPOS, J. C., HOOPER, S. N., and CASTELL, J. D. (1970): Lipids and fatty acids of two species of North Atlantic krill (*Meganytiphanes norvegica* and *Thysanoëssa inermis*) and their role in the aquatic food web. *J. Fish. Res. Bd. Can.*, **27**, 513–533.

ACKMAN, R. G., EATON, C. A., and KE, P. J. (1971): The distribution of saturated and isoprenoid fatty acids in the lipids of three species of molluscs, *Littorina littorea*, *Crassostrea virginica* and *Venus mercenaria*. *Comp. Biochem. Physiol.*, **39B**, 579–587.

ACKMAN, R. G. and HOOPER, S. N. (1973): Non-methylene interrupted fatty acids in lipids of shallow marine invertebrates: a comparison of two molluscs (*Littorina littorea* and *Lunatia triseriata*) with the sand shrimp (*Crangon septemspinosus*). *Comp. Biochem. Physiol.*, **46B**, 153–165.

ADDISON, R. F., ACKMAN, R. G., and HINGLEY, J. (1972): Lipid composition of the queen crab (*Chionoecetes opilio*). *J. Fish. Res. Bd. Can.*, **29**, 407–411.

ALLEN, W. V. (1971): Amino acid and fatty acid composition of tissues of the Dungeness crab (*Cancer magister*). *J. Fish. Res. Bd. Can.*, **28**, 1191–1195.

BLIGH, E. G. and DYER, W. J. (1959): Rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**, 911–917.

BROCKERHOFF, H., ACKMAN, R. G., and HOYLE, R. J. (1963): Specific distribution of fatty acids in marine lipids. *Archs. Biochem. Biophys.*, **100**, 9–12.

BROCKERHOFF, H., HOYLE, R. J., HWANG, P. C., and LITCHFIELD, C. (1968): Positional distribu-

tion of fatty acids in depot triglycerides of aquatic animals. *Lipids*, **3**, 24–29.

DE KONING, A. J. and MCMULLAN, K. B. (1966): Phospholipids of marine origin: the rock lobster (*Jasus lalandii*). *J. Sci. Food Agr.*, **17**, 117–120.

GRUNGER, E. H. JR., NELSON, R. W., and STANSBY, M. E. (1964): Fatty acid composition of oils from 21 species of marine fish, fresh water fish and shellfish. *J. Am. Oil Chem. Soc.*, **41**, 662–667.

GUARY, J. C. (1973): Contribution a l'étude du métabolisme des lipides chez le Crustacé Décapode *Penaeus japonicus* BATE. Thése de Doctorat de spécialité, Université d'Aix-Marseille.

HANSEN, R. P. (1969): The occurrence of phytanic acid in Antarctic krill (*Euphausia superba*). *Aust. J. Sci.*, **32**, 160.

HANSEN, R. P. and MEIKLEN, S. M. (1970): Isoprenoid fatty acids in Antarctic krill (*Euphausia superba*). *J. Sci. Food Agr.*, **21**, 204–206.

HERB, S. F., MAGIDMAN, P., LUDDY, F. E., and RIEMENSCHNEIDER, R. W. (1962): Fatty acids of cows' milk. B. Composition by gas-liquid chromatography aided by other methods of fractionation. *J. Am. Oil Chem. Soc.*, **39**, 142–146.

HOFSTETTER, H. H., SEN, N., and HOLMAN, R. T. (1965): Characterization of unsaturated fatty acids by gas-liquid chromatography. *J. Am. Oil Chem. Soc.*, **42**, 537–540.

JEFFREY, L. M., BOTTINO, N. R., and REISER, R. (1966): The distribution of fatty acid classes in lipids of Antarctic euphausids. *Antarctic J. U. S.*, **1**, 209.

LINFORD, E. (1965): Biochemical studies on marine zooplankton II. Variations in the lipid content of some Mysidaea. *J. Conseil Perm. Intern. Exploration Mer.*, **30**, 16–27.

MALINS, D. C and WEKELL, J. C. (1970): The lipid biochemistry of marine organisms. *Prog. Chem. Fats*, **10**, 339–363.

MAUCHLINE, J. and FISHER, L. R. (1969): The biology of euphausiids. In '*Advances in Marine Biology* (Edited by Russell, F. S. and Younge, M.)', Vol. 7, pp. 200–214. Academic Press, London.

MORRIS, R. J. (1971): Comparison of the composition of oceanic copepods from different depths. *Comp. Biochem. Physiol.*, **40B**, 275–281.

PIERCE, R. W., van der VEEN, J. and OLCOTT, H. S. (1969): Proximate and lipids analyses of krill (*Euphausia* species) and red crab (*Pleuronocodes planipes*). *Agric. Food Chem.*, **17**, 367–369.

O'CONNOR, J. D. and GILBERT, L. I. (1968): Aspects of lipid metabolism in crustaceans. *Am. Zoologist*, **8**, 529–539.

SAJKI, M., FANG, S., and MORI, T. (1959): Studies on the lipid of plankton. II. Fatty acid composition of lipids from Euphausiacea collected in the Antarctic and Northern Pacific oceans. *Bull. Jap. Soc. Sci. Fish.*, **24**, 837–839.

VAN DER HORST, D. J. (1970): Investigation of the synthesis and distribution of fatty acids in the lipids of the snail *Cepaea nemoralis* (L.). *Netherlands J. Zool.*, **20**, 433–444.

VAN DER HORST, D. J., OUDEJANS, R. C. H. M., PLUG, A. G., and van der SLUIS, I. (1973): Fatty acids of the female horseshoe crab *Xiphosura* (*Limulus*) *polyphemus*. *Marine Biol.*, **20**, 291–296.

VAN DER VEEN, J., MEDWADOWSKI, B., and OLCOTT, H. S. (1971): The lipids of krill (*Euphausia* species) and red crab (*Pleuroncodes planipes*). *Lipids*, **6**, 481–485.

WHITNEY. J. O. (1969): Sterols, fatty acids and sterol content in eggs and hepatopancreas of the blue crab, *Callinectes sapidus* (RATHBUN). *Acta Embryol. Exp.*, **1**, 111–121.

WOLFE, D. A., RAO, P. V., and CORNWELL, D. G. (1965): Studies on the fatty acid composition of crayfish lipids. *J. Am. Oil Chem. Soc.*, **42**, 633–637.

YAMADA, M. (1964): The lipids of plankton. *Bull. Jap. Soc. Sci. Fish.*, **30**, 673–681.