LIPIDS AND FATTY ACIDS OF THE BIVALVE *ANADARA GRANOSA* FROM LOWER LONG SAND OF GANGETIC DELTAIC REGION OF WEST BENGAL

ABSTRACT

The lipids of flesh and combined hepatopancreas and gonad of *Anadara granosa* were fractionated into hydrocarbons, sterol esters, triglycerides, wax esters and polar lipids. Fatty acids of total lipids, sterol esters, triglycerides and wax esters were analysed by gas chromatography. Significant quantitative variations were observed in the fatty acid compositions of the various lipid components of the two samples studied.

BIVALVES are valued seafoods of maritime countries worldwide, of which mussels, scallops, oysters and clams are of greatest commercial importance (Joseph, 1982). In addition to their commercial value, current interest in the role of dietary polyunsaturated fatty acids in human health, particularly that of eicosapentaenoid acid (20 : 5 ω 3) in amelioration of certain cardiovascular diseases (Dyerberg *et al.*, 1978), also focuses attention on marine fishery products, which are excellent sources for polyunsaturates.

The Sunderbans mangrove ecosystem supports a luxuriant benthic macrofauna, of which *Anadara granosa* are found in abundance among the bivalves. The present investigation on the lipids and fatty acids

of *A. granosa* is a part of a continuing study of the lipids of animals, their ecological aspects and commercial potentiality.

Authors are thankful to Professors B. B. Biswas, Director and J. Dutta, Chairman, Department of Chemistry, Bose Institute, Calcutta for providing laboratory facilities. Thanks are due to Mr. Prabhat Kumar Gayen of S. D. Marine Biological Research Institute, Sagar Island for field assistance.

Sample collection: Samples of *A. granosa* were collected from Chuksar Island of Sunderbans. Animals were disected, flesh and combined hepatopancreas and gonad were collected and extracted for lipids according to Bligh and Dyer (1959). Tissues were homogenized with methanol-chloroform water (2 : 1 : 0.8 v/v), centrifuged and the solvent layer withdrawn. Residue was further extracted twice in the same way. The extracts were pooled and washed thrice with distilled water and dried over anhydrous sodium sulphate. Solvent was evaporated in a rotary evaporator under vacuum at room temperature. Lipids were weighed, redissolved in distilled n-hexane and stored in a deep freezer at — 18°C.

Saponification of total lipid, extraction of nonsaponifiables and methylation of fatty acids : A portion of total lipid was saponified by refluxing it with methanolic potassium hydroxide for two hours. Methanol was removed under vacuum, residue was dissolved in distilled water and nonsaponifiables were extracted three times by diethyl ether. Pooled ethereal extracts were dried over anhydrous sodium sulphate and weighed to give the nonsaponifiables. After extraction of the nonsaponifiables, the aqueous layer was acidified with 4N sulphuric acid and the free fatty acids liberated were extracted thrice with diethyl ether. Pooled ethereal extract of fatty acids was dried over anhydrous sodium sulphate and finally solvent was removed under vacuum. The saponification procedure and extraction of nonsaponifiables and fatty acids were done according to Hilditch and Williams (1964). The dried fatty acids were methylated using diazomethane gas (Schlenk and Gallerman, 1960).

Fractionation of total lipid into neutral, phospho—and glycolipids : An aliquot of total lipid was fractionated by column chromatography on silicic acid (Rouser *et al.*, 1967). The neutral, glyco — and phospholipids were eluted with chloroform, acetone and methanol respectively.

Fractionation of neutral lipids into various lipid classes : The neutral lipid was fractionated into hydrocarbon, wax ester, sterol ester, triglyceride and sterol, by preparative thin layer chromatography, using a solvent system of light petroleum ether (40°-60°C), diethyl ether and acetic acid (80 : 20 : 1 v/v/v). Components thus separated were recovered following usual procedures and finally weighed (Mangold, 1969).

TABLE 1. *Compositions (% w/w) of various lipids of flesh and combined hepatopancreas and gonad of* Anadara granosa

Lipid components	Flesh	Hepatopancreas and gonad
Total lipid*	0.65	1.86
Neutral lipid**	78.40	88.40
Hydrocarbon***	18.70	23.20
Wax ester***	20.10	14.70
Sterol ester***	21.00	34.80
Triglyceride***	30.90	19.30
Sterol***	9.30	8.00
Phospholipid**	16.50	11.00
Glycolipid**	5.10	0.60
Nonsaponifiable**	35.00	21.10

* Percent w/w of fresh tissue
** Percent w/w of total lipid
*** Percent w/w of neutral lipid

TABLE 2. *Fatty acid compositions (% w/w) of various lipids of* Anadara granosa

Component acids [a, b]	Total lipid		Sterol ester		Wax ester		Triglyceride	
	F [c]	HPG [d]	F	HPG	F	HPG	F	HPG
14 : 0	2.5	4.2	8.7	4.0	12.5	4.6	3.8	9.5
14 : 1	0.2	2.0	6.3	6.4	6.0	5.5	4.1	0.3
15 : 0	3.9	0.2	5.5	5.7	6.6	8.8	0.5	4.0
16 : 0	9.0	15.3	15.6	16.3	11.0	13.9	11.9	14.4
16 : 1	1.3	2.0	3.1	7.1	7.2	7.4	13.4	3.1
17 : 0	6.0	3.2	2.5	4.2	1.2	7.8	3.4	5.7
17 : 1	1.2	1.1	1.0	1.0	5.6	2.5	3.0	2.8
18 : 0	8.0	7.7	6.5	8.7	5.0	7.4	2.7	4.0
18 : 1	3.7	8.0	6.2	6.3	0.1	5.5	7.0	5.4
18 : 2ω6	6.1	4.3	0.5	2.7	0.6	2.0	1.8	6.8
20 : 0	0.1	1.8	0.5	6.4	—	2.3	0.1	6.6
18 : 3ω3	3.4	0.7	1.7	3.5	1.7	3.1	10.1	3.6
20 : 1								
18 : 4ω3	0.4	4.9	0.1	1.0	4.3	0.2	1.2	3.3
20 : 2ω6	10.0	1.3	3.4	0.9	1.2	0.3	2.1	1.2
20 : 3ω9	1.0	1.3	3.4	1.0	0.2	1.5	2.4	1.4
22 : 0	0.1	1.1	2.5	1.5	2.5	1.0	1.4	1.1
20 : 4ω6	0.7	3.9	1.7	4.1	0.2	1.8	5.8	1.1
22 : 1								
22 : 2	3.7	8.3	1.0	2.1	0.2	0.3	3.0	1.3
20 : 5ω3	15.1	12.0	10.2	9.4	9.7	10.2	9.5	10.4
22 : 4ω6	7.6	2.6	—	0.2	0.2	0.5	0.3	2.1
22 : 4ω3	0.6	2.5	—	1.0	0.6	1.1	0.1	1.2
21 : 5ω3								
22 : 5ω6	0.1	1.1	3.1	0.1	3.6	0.6	1.1	0.3
22 : 5ω3	0.1	0.5	1.2	1.4	4.6	1.0	0.1	—
22 : 6ω3	15.2	10.0	15.3	11.0	15.2	10.7	11.2	10.4

[a] First and second figures represents carbon number : number of double bonds

[b] ω values represent the carbon number of the methyl end chain from the center of the double bond farthest from carboxyl group

[c] Flesh

[d] Hepatopancreas and gonad

Gas liquid chromatography (GLC) of the fatty acid methyl esters (FAME): The instrument used was a Pye Unicam model 104 gas chromatograph equipped with dual glass column (1.8 m x 3 mm) and dual flame ionisation detector. Fatty acid methyl esters were analysed on 10% DEGS liquid phase supported on 80-100 mesh Diatomite C. Peaks were identified by using relative retention times and equivalent chain length values (Misra *et al.*, 1983 a). Cod liver oil FAME was also used as secondary standard for the identification of the peaks (Ackman and Burgher, 1965).

Analysis of the fatty acids of wax esters and sterol esters: Wax esters were lipolysed on TLC plates using lipid free porcine pancreatic lipase (Misra *et al.*, 1984 a) and fatty acids thus extracted was analysed by GLC (Misra *et al.*, 1984 b) Sterol esters were also lipolysed on TLC plate (Misra *et al.*, 1984 b) and fatty acids were analysed.

Analysis of the fatty acids of triglycerides: The triglycerides were saponified and fatty acids were analysed following the methodology stated under the saponification of the total lipid.

Results and discussion

Percentages of total lipids and various other lipids of flesh and combined hepatopancreas and gonad of *Anadara granosa* have been presented in Table 1. Significant differences were observed in the compositions of total lipids, glycolipids, sterol esters and nonsaponifiables. The nonsaponifiable content was comparable to those of the bivalve of *Macoma* sp. (Misra *et al.*, 1985) and the gastropod mollusc, *Cerethidea cingulata* (Dutta *et al.*, 1986) of this ecosystem.

The fatty acid compositions of total lipids, sterol esters, wax esters and triglycerides have been presented in Table 2. It is revealed from Table 2, that among the saturated fatty acids, 14 : 0, 16 : 0 and 18 : 0 were the major components. Among the unsaturated fatty acids, 20 : 5 ω 3 and 22 : 6 ω 3 are the major components in all the samples. It is revealed from the present study that the levels of the two biologically active fatty acids, *viz.* eicosapentaenoic acid (20 : 5 ω 3) and docosahexaenoic acid (22 : 6 ω 3) were comparable to those of the bivalve of *Macoma* sp.(Misra S *et al.*, 1985). It is also interesting to note that the occurrence of ω 3 series of polyunsaturated acids were always much higher in all of the detritivorous animals of this area studied so far. This observation indicates that the biosynthesis of ω 3 acids were much higher than the other polyunsaturates and probably due to the presence of higher levels of the precursor linolenic acid (18 : 3ω3) in the detritus, which should be confirmed to establish an important food chain.

Department of Chemistry,
Bose Institute,
93/1 A.P.C. Road,
Calcutta-700 009.

SUBHASISH CHATTOPADHYAY
PRASANTA K. PAL
AMITABHA GHOSH
SUNITI MISRA*
TARUN K. DAM*
AMALESH CHOUDHURY*

REFERENCES

ACKMAN, R. G. AND R. D. BURGHER 1965. *J. Am. Oil Chem. Soc.*, **42** : 38-42.

BLIGH, E. G. AND W. J. DYER 1959. *Can. J. Biochem. Physiol.*, **37** : 911-917.

DUTTA, A. K., P. K. PAL, A. GHOSH, S. MISRA, S. NANDI AND A. CHOUDHURY 1986. *J. Am. Oil Chem. Soc.*, **63** : 223-225.

DYERBERG, J., H. O. BANG, E. STOFFERSON, S. MONCADA AND V. R. VANE 1978. *Lancet*, **2** : 117-119.

* Present address : Department of Marine Science, University of Calcutta, 35 B. C. Road, Calcutta 700 019.

HILDITCH, T. P. AND P. N. WILLIAMS 1964. *The Chemical Constitution of Natural Fats.* Chapman and Hall, London, 4th edn., pp. 676-711.

MANGOLD H. K. 1969. Thin layer chromatography of lipids. *In:* E. Stahl (Ed.) *Thin Layer Chromatography.* Springer-Verlag, New York, N.Y. pp. 374-379.

MISRA, S., A. K. DUTTA, T. DHAR, A. GHOSH, A. CHOUDHURY AND J. DUTTA 1983 a. *J. Sci. Food Agric.*, **34** : 1413-1418.

———, A. CHOUDHURY, A. K. DUTTA, A. GHOSH AND J. DUTTA 1983 b. *J. Chromatogr.*, **280** : 313-320.

———, ———, ———, J. DUTTA AND A GHOSH 1984 a. *Lipids*, **19** : 302-303.

———, ———, ——— AND A. GHOSH 1984 b. *Phytochemistry*, **23** : 2823-2827.

———, M. K. GHOSH, A. CHOUDHURY, A. K. DUTTA, P. K. PAL AND A. GHOSH 1985. *J. Sci. Food Agric.*, **36** : 1193-1196.

ROUSER, G., G. KRITCHEVSKI AND A. YAMAMOTO 1967. *In*: G. V. Merinetti (Ed.) *Lipid Chromatographic Analysis.* Marcel Dekker, New York, N.Y. Vol. 1. pp. 118-119.

SCHLENK, H. AND J. L. GALLERMAN 1960. *Anal. Chem.*, **32** : 1412-1414.