PHOSPHOLIPIDS OF FIVE INDIAN FOOD FISHES

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The phospholipids of five Indian food fishes, viz; sardine, pomfret, mackerel, anchovies and thrissocles were fractionated quantitatively using column and thin layer chromatographic techniques and the results reported in this communication.

INTRODUCTION

Oil sardine *(Sardinella longiceps)*, pomfret *(Pampus argenteus)*, mackerel *(Rastrelliger kanagurta)*, *Anchoviella* and *Thrissocles mystax* constitute five major food fishes of India. The body oil of oil sardine finds large scale application in leather and paint technology and in the maintenance of fishing crafts. Phospholipids composition of these fishes have not yet been studied in detail. Component fatty acids of the phospholipids of mackerel, pomfret and sardine have been reported earlier (Gopakumar and Nair, 1967 a, b). The present paper reports the summary of the study on phospholipid composition of the fishes mentioned above.

MATERIALS AND METHODS

The lipids were extracted from the muscle by the method of Bligh and Dyer (1959). The chloroform extract was washed thrice by the procedure of Folch *et al* (1957), evaporated in a current of carbondioxide and the residual lipids dried *in vacuo* and weighed.

Column chromatography: A column was prepared of 100 g activated silicic acid (silica gel, 100 mesh, for chromatography according to Ramsey and Patterson, Fluka, A. G. Switzerland). The sample of lipid was applied in chloroform on the column and neutral lipids were eluted out with chloroform. The phospholipids were eluted with increasing concentration of methanol in chloroform and finally with pure methanol. 10 ml fractions were collected at a rate of 1.5 ml per minute. 120 fractions were collected. The phosphorus content in each fraction was determined by King's method (1932) Depending on the phosphorus content 7 fractions were made, each of which was further analysed by quantitative thin layer chromatography.

The same method was applied for lipids of mackerel, sardine and pomfret. But in case of anchovies and thrissocles the phospholipid separated by column chromatography was further separated into different components by quantitative two-dimensional thin layer chromatography on silica gel G.

Thin layer chromatography: Activated silica gel G (according to Stahl E. Merk) was applied on 20 cm x 20 cm plates to a thickness of 1000μ. The lipid sample was applied on the gel and developed by the procedure of Rouser *et al* (1965). Individual phospholipids were identified by comparison of Rf values with those of reference standards, viz; phosphatidyl choline (PC), lysophosphatidyl choline (LPC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), phosphatidyl inositol (PI) and sphingomyelin (SP), Kochlight laboratories, Colnbrook, Bucks, England. The plates were sprayed with 1) ninhydrin for amino phosphatides, 2) Dragend roof's reagent (Bregoff-Delwiche, Stahl, E) for choline containing lipids and 3) iodine as a general spraying reagent. The spots developed with iodine were scraped off and iodine was allowed to sublime. The phospholipid contents in them were determined by estimation of phosphorus contents. The results given in Table I show that phosphatidyl choline and phosphatidyl ethanolamine are the two principal components of phospholipids of all fishes investigated. Total phospholipid content expressed on the wet weight of the tissue showed the minimum value, namely 0.32% for anchovies. It has been observed by Ackman *et al*, (1969) that in most marine species of fish PC will be about 60% of the muscle phospholipid and PE 20%. The values observed here are well in agreement with those of most species occurring in cold waters (Dekoning, 1967) Phospholipids of pomfret were outstanding, for they contained a high % of cardiolipins and the lowest amount of PC compared to other species investigated. A note-worthy feature of the phospholipids of sardine, mackerel and pomfret is that they contain a high % of lysophosphatidyl ethanolamine to the extent of 3.9,3.5 and 4.2 respectively. These fishes analysed were very fresh and uniced. It is very likely that postmortem lipid hydrolysis would have taken place in these fishes before analysis. It has been shown by Olley *et al* (1969) that in case of haddock, PC and PE containing C_{16}, C_{18} and $C_{20:1}$ acids are predominantly hydrolysed. The higher amount of PE and PC which are prone to hydrolysis, may contribute to larger quantities of free fatty acids (F. F. A) formed in the fish during frozen storage. Davidkova and Khan (1967) showed that about 70% of the total increase in F.F.A content of the muscle was probably due

TABLE I

	Sardine	Pomfret	Mackerel	Anchoviella	Thrissocles
Total lipids (g/100 g wet tissue)	6.0	4.5	6.0	0.7	5.4
Phospholipids (g/100 g wet tissue)	0.8	0.9	0.8	0.3	0.9
Phosphatidyl serine: % of total phospholipids	3.9	3.3	1.2	4.0	5.0
Phosphatidyl ethanolamine: ,,	15.5	22.7	22.1	19.0	21.2
Phosphatidyl inositol: ,,	4.0	2.2	6.5	5.0	3.2
Phosphatidyl choline: ,,	61.8	51.6	60.0	63.0	57.4
Lysophosphatidyl choline: ,,	2.0	3.5	0.5	0.4	2.4
Sphingomyelin: ,,	4.6	5.7	2.0	1.2	3.8
Phosphatidic acids: ,, (cardiolipins)	4.1	10.0	4.2	6.0	2.8

(Plasmalogens were included in the diester fractions)

to phospholipase B activity, and the remaining 30% to the breakdown of triglycerides. Hence it is likely that their lyso derivatives would have been subjected to further hydrolysis if they were kept in chilled storage.

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