Studies on Lipid Profiles and Fatty Acid Composition of Roe from *Rohu* (*Labeo rohita*) and *Murrel* (*Channa striatus*)

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Abstract: The lipid profiles and fatty acid composition of roes from two common fresh water fish viz., *rohu* (*Labeo rohita*) and *murrel* (*Channa striatus*) have been studied. The dehydrated fish egg was ground to fine powder and extracted with chloroform/methanol (2:1, v/v) to recover the total lipid in 20.2 and 22.7% on dry weight basis. The fat free meal was found to contain 70 and 58% protein in *rohu* and *murrel*, respectively. The total lipids were separated into neutral lipids, glycolipids and phospholipids using silica gel column chromatography and found to be 43.8, 72.9% neutral lipids; 12.7, 9.4% glycolipids and 43.5, 17.7% phospholipids in *rohu* and *murrel* roes, respectively. The fatty acid compositions of all lipid classes were analysed by GC and GC-MS. Among the saturated fatty acids, hexadecanoic acid (16:0) was found to be 30.2 and 30.4% respectively in *rohu* and *murrel* total lipid. n-3 Fatty acids namely eicosapentaenoic acid (20:5, EPA) was observed to an extent of 1.5 and 0.6% and docosahexaenoic acid (22:6, DHA) to an extent of 11.8 and 6.1% respectively in *rohu* and *murrel* total lipids. Stearic acid (18:0) was also seen at 12.2 and 6% respectively in *rohu* and *murrel* lipids. The fatty acid composition was found to be almost similar in all the lipid classes. The study indicated that *rohu* and *murrel* fish egg lipids are good sources for polyunsaturated n-3 fatty acids.

Key words: *Labeo rohita*, *Channa striatus*, fish roes, fatty acids, neutral lipids, glycolipids, phospholipids

1 INTRODUCTION

Fish roe is a very popular protein source throughout the world with premium prices for selected roe products. Salted roes, canned roe and roe sausages are the common products available in the markets. In India, the fish roe obtained during dressing of fish in the fresh fish markets is either discarded or sold at very low price, as few people prefer to consume the material. The fresh roes are highly perishable commodities due to their high moisture, protein and lipid contents. Microbial spoilage of roes is well documented in the literature¹, and some reports are available on pasteurization, blanching in boiling water and freezing to arrest the spoilage to some extent. Lactic acid bacteria, *enterobacteriaceae* and *Vibrio* species were responsible for bad odours and spoilage in lightly salted *lump* fish roe stored at 5°C². CaCl₂, MgCl₂ and KCl at specific concentrations are reported in brining of cod products without affecting the sensory or microbiological quality. In some

studies, the egg is homogenized and dried to obtain a free flowing powder containing as high as 65% protein and 25-30% of fat content³.

Fish oil is rich in n-3 fatty acids, and are found in many marine and cold water fish species^{4,7}. Some of the literature reports on roe lipid composition like study on immature *pollock* roe were found to contain 21.2% palmitic acid (16:0), 21.1% docosahexaenoic acid (DHA), 19% eicosapentaenoic acid (EPA) and 11.6% oleic acid as the main fatty acids⁸. Mukhopadhyay *et al.*⁹ studied the lipid profiles of some Indian fresh water fish and reported higher contents of phospholipids (37%) along with EPA and DHA in all the lipid classes. Shirai, *et al.*¹⁰ reported the total lipid (TL) and different lipid classes namely the sterol esters (SE), triacylglycerols (TG), phospholipids (PL), and the individual phospholipids such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in fish roe products viz. Ikura, Tarako, Tobiko and Kazunoko. The researchers found that

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Accepted March 28, 2010 (received for review January 12, 2010)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/>

the major fatty acids in these products were 16:0, 18:1, 20:5, and 22:6 and concluded that fish roe products may be categorized under health foods.

Joe *et al.*¹¹ studied the lipid composition in different components of a mullet type fish (*Liza carinata*) such as roe, muscle and viscera. Roe lipids had high content of wax esters (63%) and low triglycerides (9.9%) in contrast to muscle which contained excess of triglycerides (66%) and traces of wax esters. Roe wax esters were significantly rich in monounsaturated fatty acids such as 16:1, 17:1 and 18:1 and also n-3 fatty acids.

Moriya *et al.*¹² found that phospholipids were the major class of fatty acids in herring roe with palmitic acid (25.8%), oleic acid (13.2%), docosahexaenoic acid (DHA) (21.6%) and eicosapentaenoic acid (EPA) (14.4%). Cengarle *et al.*¹³ found that roes of several marine species were found to be rich in n-3 PUFA with the optimum ratio between saturated and monounsaturated fatty acids. In their study, they noticed that EPA and DHA together constituted more than 35% in the roe lipids. Mendez *et al.*¹⁴ reported a lipid content of 6.6% (dry basis) in southwest Atlantic Hake (*Merluccius hubbsi*) roe, of which 27.6% were waxes, 42% triacylglycerols, 14% phospholipids and 5.7% cholesterol. The PUFA were 45% of the total lipid, which makes this marine lipid an attractive substitute for PUFA enrichment in normal diets. The lipid classes and fatty acid composition of eggs from the Atlantic halibut (*Hippoglossus hippoglossus*) were studied by Peterson *et al.*¹⁵ and they reported neutral lipids as major class which constituted 30% of the total lipid.

The present study aims at investigating the lipid profile of two fresh water fish roes namely rohu (*Labeo rohita*) and murrel fish (*Channa striatus*) to find out the composition of lipid classes and their fatty acid compositions.

2 EXPERIMENTAL

2.1 Sample collection and materials

Inland fish roes from rohu and murrel fish were collected from local fresh fish market. They were kept under refrigeration (5-8°C) for not more than 24 h before processing. Chloroform, acetone, methanol, ethyl acetate, sulfuric acid, acetic acid, hydrochloric acid, resorcinol, α -naphthol, ammonium molybdate, perchloric acid, bismuth nitrate and ninhydrin used in this study were of analytical grade and were procured from Sd Fine Chemicals, Mumbai, India. The reagents were prepared as per standard procedures for the development of TLC plates¹⁶⁻¹⁸. Glass coated TLC plates (20 × 20 cm) were procured from Sigma-Aldrich, USA.

The roes were cleaned to remove fins, scales and other blood vessels, and immediately ground in a blender into a homogenous mass. The homogenized material was anal-

ysed for moisture, protein and fat content as per standard AOAC procedures¹⁹.

2.2 Dehydration of roe

The homogenized mass (500 g) was spread in SS trays up to 2 cm in height and dried in a cabinet tray dryer (Chemida, Mumbai, India) at 55°C for 10 h. The dried flakes were ground in mixer grinder (Sumeet Food Processor, Mumbai, India) to a fine powder to pass through 30 BS mesh (500 μ). The moisture content of the powder was analysed by determining the loss on drying in an air oven at 100 ± 3 °C for 8 h.

2.3 Extraction of lipid

The dry powder (100 g) was extracted at room temperature using the method of Folch *et al.*²⁰ to recover the total lipid (20 g, rohu and 22.7 g, murrel) using a solid-solvent ratio of 1:4 in 3 successive extractions. The pooled solvent extracts were distilled in a rotary vacuum evaporator at ≤ 50°C to obtain the lipid. The lipid was stored in a refrigerator at 4-6°C for further analysis.

2.4 Lipid class analysis and fatty acid composition

The total lipid was analysed for free fatty acid content (FFA), peroxide value (PV), and phosphorous content as per standard AOCS methods²¹. Fatty acid methyl esters (FAMES) of the lipid were prepared by transesterification using 2% sulphuric acid in methanol. The FAMES were extracted into ethyl acetate and thoroughly washed with water to make them free of acid and dried over anhydrous sodium sulphate. The dried esters were analysed by GC and GC-MS.

2.5 Column chromatographic separation of lipids

The total lipids (TL) of rohu and murrel (1 g) were separated into different lipid classes by silicic acid column chromatography. The column was successively eluted using chloroform, acetone and methanol to separate neutral lipids, glycolipids and phospholipids, respectively²². The lipid fractions were qualitatively analyzed by TLC for identifying triglycerides, glycolipids and phospholipid components. Different combinations of solvent systems such as hexane : ethyl acetate (9:1, v/v) for neutral lipids, chloroform : methanol : acetic acid : water (170:24:25:4, v/v/v/v) for glycolipids and chloroform : methanol : water (65:25:4 v/v/v) for phospholipids were used for developing the TLC. The eluted spots were identified by spraying with spray reagents namely α -naphthol for glycolipids¹⁶, ammonium molybdate-perchloric acid for phospholipids¹⁶, Dragendorff reagent¹⁸ for phosphatidylcholine (PC) and lysophosphatidylcholine, and ninhydrin^{16, 18} for phosphatidylethanolamine (PE).

2.6 Fatty acid analysis

The individual lipid fractions were converted into fatty acid methyl esters by refluxing with 2% methanolic sulphuric acid for 6 h. The esters were extracted using ethyl acetate, washed with water and dried over anhydrous sodium sulphate and determined for their fatty acid profiles.

The GC-FID analyses were performed with an Agilent 6850 series gas chromatograph equipped with an FID detector using a DB-225 capillary column (30 m × 0.25 mm i.d.). The column temperature was initially maintained at 160°C for 2 min, increased to 220°C at 5°C/min and maintained for 10 min at 220°C. The carrier gas was nitrogen at a flow rate of 1.5 mL/min. The injector and detector temperatures were maintained at 230 and 250°C respectively, with a split ratio of 50:1.

The GC-MS analyses were performed using an Agilent (Palo Alto, USA) 6890N gas chromatograph equipped with an HP-5 MS capillary column (30 m × 0.25 mm i.d.) connected to an Agilent 5973 mass spectrometer operating in the EI mode (70 eV; m/z 50-550; source temperature 230°C and a quadrupole temperature 150°C). The column temperature was initially maintained at 200°C for 2 min, increased to 300°C at 4°C/min, and maintained for 20 min at 300°C. The carrier gas was helium at a flow rate of 1.0 mL/min. The inlet temperature was maintained at 300°C and split ratio of 50:1. Structural assignments were based on interpretation of mass spectrometric fragmentation and confirmed by comparison of retention times as well as fragmentation pattern of authentic compounds and the spectral data obtained from the Wiley and NIST libraries.

3 RESULTS AND DISCUSSION

Fish roe from *rohu* and *murrel* were analysed for moisture, fat, protein, dry matter yield and physico-chemical characteristics of fat (Table 1). The moisture in the samples was present up to an extent of 48 and 67% in the fresh roes and it was 6.75 and 7.2% in the dehydrated rohu and murrel roe powders, respectively. The lipid contents were about 20, 22% and protein to an extent of 70, 58% in *rohu* and *murrel* fish roe respectively, on dry weight basis. The free fatty acid content was relatively higher in *rohu* lipids, which may probably be due to an active inherent lipase.

The total lipids were separated into neutral, glyco- and phospholipids using silica gel column chromatography. The composition of lipid classes is presented in Table 2. Phospholipids were considerably higher in *rohu* whereas the neutral lipids were more in *murrel* egg lipids. The TLC of phospholipid fractions clearly indicated the presence of PC by the characteristic orange spots developed by spraying with dragendorff reagent and PE by pink spots developed on spraying with ninhydrin. Lyso PC was also present to a

Table 1 Physico-chemical Analysis of Fresh Fish Egg

Property	<i>Rohu</i>	<i>Murrel</i>
Moisture, %	66.9±0.35	48.5±0.40
Dry matter yield, %	25±1.73	35±1.40
Total ash, % *	3.8±0.14	5.12±0.20
Total lipid content, % *	20.2±0.50	22.7±0.56
Total crude protein, % * % N X 6.25	70.2±0.95	58.8±1.30
FFA in total lipid, % oleic acid	7.1±0.26	2.1±0.10
Peroxide value in total lipid, meq. O ₂ /1000 g	4.3±0.17	3.1±0.20

* Values are on dry matter basis

Values are mean of triplicate analyses

Table 2 Composition of *Rohu* and *Murrel* Egg Lipid Classes

Lipid Class	Composition (wt %)	
	<i>Rohu</i>	<i>Murrel</i>
Neutral lipids	43.8±0.61	72.9±1.35
Glycolipids	12.7±0.36	9.4±0.14
Phospholipids	43.5±0.87	17.7±0.90

Values are mean of triplicate analyses

minor extent as seen from the TLC. The fatty acid composition of total lipids, triglycerides, glycolipids and phospholipids was based on GC, GC-MS data as shown in Table 3.

The fatty acid profile of total lipid showed the saturated fatty acids were to an extent of 48 and 38% with major fatty acid being palmitic acid to an extent of 30% in both *rohu* and *murrel* eggs, respectively. However, the saturated stearic (12, 6%) and monounsaturated octadecenoic acid (21.6, 30.4%) were also present in considerable quantities. The octadecadienoic acid and octadecatrenoic acids were present to an extent of 4.8, 3.1% and 8.5 and 9.7% in *rohu* and *murrel* lipids, respectively. The n-3 fatty acids were found to be 23.6 and 4.1% in the *rohu* and *murrel* egg lipids, respectively. EPA (20:5), DHA (22:6) were in the range of 1.5, 11.8% and 0.6, 6.1% in *rohu* and *murrel* lipids respectively.

The fatty acid composition of individual components (neutral, glyco- and phospholipids) showed considerable quantities of palmitic acid (28, 30.6 and 28% in *rohu*; 29.6, 29.6 and 31.1% in *murrel*). Stearic acid was present to an extent of 6.9, 11.8, 12.9% and 6.4, 10, 10.8% and octadecenoic acid of about 21.2, 19.9, 21.1% and 32.2, 24.8, 24.9%; while octadecatrenoic acid was found to an extent of 5.5, 1.9, 3% and 7.2, 6.6, 6.8% in *rohu* and *murrel*, respectively. Phospholipid fractions of *rohu* and *murrel* contained EPA and DHA in 1.1, 14.1% and 1.0, 6.8%, respectively.

Table 3 Fatty Acid Composition (Peak area %) of Individual Lipid Fractions of *Rohu* and *Murrel* Fish Eggs

Fatty acid	<i>Rohu</i>				<i>Murrel</i>			
	Total lipid	Neutral lipids	Glycolipids	Phospholipids	Total lipid	Neutral lipids	Glycolipids	Phospholipids
14:0	2.1±0.05	5.5±0.10	5.2±0.20	2.4±0.17	1.2±0.17	3.8±0.53	2.0±0.10	1.3±0.17
16:0	30.2±0.30	28.0±0.46	30.6±1.04	28.0±1.25	30.4±0.36	29.6±0.79	29.6±0.56	31.1±0.17
17:0	3.2±0.13	2.3±0.23	3.6±0.04	2.2±0.09	—	—	—	—
18:0	12.2±0.10	6.9±0.28	11.8±0.09	12.9±0.08	6.0±0.28	6.4±0.07	10.0±0.24	10.8±0.11
Total saturates	47.7	42.7	51.2	45.5	37.6	39.8	41.6	43.2
16:1	5.3±0.09	5.3±0.09	4.4±0.12	4.4±0.05	3.9±0.03	4.1±0.09	3.0±0.13	2.0±0.11
18:1	21.6±0.56	21.2±0.07	19.9±0.22	21.1±0.22	30.4±0.17	32.2±0.30	24.8±0.26	24.9±0.26
20:1	0.6±0.02	0.8±0.03	1.0±0.13	0.3±0.04	—	—	—	—
Total mono-unsaturates	27.5	27.3	25.3	25.8	34.3	36.3	27.8	26.9
18:2	4.8±0.10	4.6±0.11	6.6±0.36	5.1±0.23	8.5±0.16	8.0±0.20	10.9±0.09	8.9±0.20
20:2	1.1±0.12	1.5±0.15	2.3±0.18	2.9±0.22	1.0±0.17	0.8±0.07	1.0±0.19	2.2±0.10
18:3	3.1±0.09	5.5±0.10	1.9±0.19	3.0±0.13	9.7±0.16	7.2±0.07	6.6±0.09	6.8±0.09
20:3	1.9±0.09	4.1±0.09	2.2±0.04	2.0±0.12	2.2±0.07	1.8±0.17	5.0±0.16	4.2±0.04
20:4	0.6±0.10	0.8±0.05	0.2±0.03	0.5±0.11	—	—	—	—
20:5	1.5±0.10	0.8±0.08	0.6±0.04	1.1±0.09	0.6±0.04	0.8±0.04	0.5±0.01	1.0±0.07
22:6	11.8±0.10	12.7±0.19	9.7±0.26	14.1±0.19	6.1±0.19	5.3±0.22	6.6±0.25	6.8±0.23
Total poly-unsaturates	24.8	30.0	23.5	28.7	27.5	23.9	30.6	27.9

Values are mean of triplicate analyses ± SD

The presence of higher amounts of n-3 fatty acids with considerable quantities of EPA and DHA were found to have lower incidence of coronary heart disease among the population who consume large amount of fish²³). DHA is also essential for the development of brain and retina in fish and any deficiency may cause abnormalities²⁴). The presence of higher amounts of DHA in fish roe lipid is known to help in healthy development of fish larvae.

4 CONCLUSION

The results of the present study showed that the *rohu* and *murrel* fish egg lipids are good sources of essential polyunsaturated fatty acids. These lipids could be successfully used as supplements in many food products. The phospholipid fractions of these fish roe lipids were particularly rich in phosphatidylcholine, which is essential for brain development and in memory enhancement. Hence, the formulations of the fish egg lipids in pharmaceutical applications could be explored further.

ACKNOWLEDGEMENT

The authors thank Dr. V. Prakash, Director, Central Food Technological Research Institute for his keen interest and the Department of Biotechnology, Government of India, for funding the project.

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