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Research Article

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Investigation about the lipid composition of some freshwater fishes

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ABSTRACT
In this work we were study about the lipid composition of some fresh water fishes collected from Darbhanga locality. Fish is consumed in this Gangetic plain mainly for protein and lipids. Fishes contain unsaturated fatty acids as lipid-component. Accordingly, the study was conducted by selecting two river stretches of Darbhanga District. These included stretches of river Kamala located in village Pokharam and stretches of river Balan located in village Pohaddi. Fish collections was done with bag nets of standardized dimensions with several mesh-sizes. There is selection of *Labeo rohita* (Rohu), *Labeo calbasu* and *Catla catla* a plankton feeder, phytoplankton and detritus feeder respectively in water column. The health clues hidden in fish flesh consumption encountered through popular dailies and reviews (The Times of India, Sunday review, July.18 Aug.8 and Aug.22 and Dec.18, 2001) attracted the present attention on this particular problem. According to fat extraction by Soxhlet extraction method; fat content of *Labeo rohita* varied between 23-30% while the iodine value was in range of 90-102. The level of triglyceride could be measured between 402-410 mgm (%). The saponification value was recorded between 101-171.2 and acid value between 8.81and 9.21. Whereas, the fat contents of *Catla catla* was recorded between 24.6-26.7%, Iodine value in the range of 96-97, saponification value between 152.4-158.7 and acid value between 7.8-8.61. These values in *L. calbasu* were recorded as: fat content 25.6 - 27.1%; Iodine value 96-98; Saponification value 133.4-140.3 and acid value 6.93-7.6. The findings suggest that the total fat content in all the major carps were comparable to each other. The study also suggests that in general the fat content of fish increases during winter season probably on account of availability of quality food. However, with regard to ω6 and ω3 profile the *Labeo rohita* and *Labeo calbasu* the phytoplankton and detritus feeders respectively shows a better range of ω3 fatty acids than *Catla catla*, a zooplankton feeder which appears to be rich in ω6 fatty acids.
Keywords: Fresh Water Fishes, Fatty acids, Fat content, Iodine value, Saponification value, ω3, ω6 profile.

INTRODUCTION

The health clues hidden in fish flesh consumption encountered through popular dailies and reviews (The Times of India, Sunday review, July.18 Aug.8 and Aug.22 and Dec.18, 2001) attracted the present attention on this particular problem. Fish constitute one of the staple dietary items in many states viz. Bihar, West Bengal, Orissa, and many of the coastal states. But in Mithila the major part of the North Bihar geographically located at the latitude 260 27' 26.81 North and longitude 860 11' 20.98 East that constitutes Indian Gangatic planes, the fish is not only adored for its dietary value but has intricate relationship with the cultural heritage. The region where 'Machh-Bhat' (fish consumed with boiled rice) is taken as one of the precious meals. The region has been bestowed with an intricate riverine network thousands of fresh water pounds and a good deal of chaurs and mauns (wetlands) and to cope with needs of the social frame work. Many isolated reports highlight the rich biodiversity of fish in particular (A.R. Khan 2003). According to study made through M. Sc. desertation about 106 species have been reported (Verma, 1989). Kumar et.al. (2011) have reported about small wetlands and fishes from wetland of Bihar. One of the earlier works (Schaper claus, 1933) has classified the natural food of fishes under three groups, viz. (a) 'Main food' or the natural food which the fish prefers under favourable conditions and on which it thrives best, (b) 'Occasional food' or the natural food that is well liked and consumed as and when available, and (c) 'emergency food' which is ingested when the preferred food items are not available, and on which the fish are just able to survive. Das and Moitra (1955) reported that herbivores and carnivores always show a definite peak period in feeding while omnivores show little variation through the year. Fish is consumed in this Gangatic plain mainly for proteins and lipids. In this part of the country orthodox hindues take fish as part of their diet avoiding meat and chicken. Fatty acids are straight chain hydrocarbon having carboxylic group at one end.

MATERIALS AND METHODS

Study of fish samples: The fish samples were collected from three sampling sites each of the water resources *viz.*, stretches of river Kamala located in village Pokharam and stretches of river Balan located in village Pohaddi. were brought to the laboratory soon after registering the physical data of the fish and other relevant information. The collection of many of the smaller sized fish were made with the help of 'bag net' which is like a butterfly net strongly built with thick mesh and long handle. The trapped fish were collected from the shore and offshore deeper area (using a boat on rent) of the water bodies under investigation. However, many of the larger samples were purchased from fish farmers readily following harvesting. The weight of the fish was taken at site, samples were rushed to the laboratory in "freeze transportation bags" for further analysis.

The identification of fish was made with the help of Jhingaran (1983), Jayram (1981), Talwar and Jhingaran (1991) and Munshi and Srivastava (1988). The data on overall catch statistics were collected from various fishermen of the locality involved in fisheries operation of the area under investigation.

Extraction of Lipid

Lipid content in the sample was extracted by Soxhlet apparatus using diethyl ether as a solvent. Fat content of the tissues when brought in contact with the volatile organic solvent is extruded. The decrease in the weight of material or increase in weight of the solvent indicates amount of the fat present in the material.

Since moisture present in the flesh gives misleading results, the sample was first oven dried. An auto calibrated electronic balance (AND Japan made HR 200 series) was used to weigh the tissue. The tissue was placed in a porous thimble (Whatman) and placed inside the extraction unit (Soxhlet apparatus). The solvent (diethyl ether) was filled in the flask. The extraction unit was properly connected to the flask and the condenser. The flask containing the solvent was gently heated with the help of a digital heating mantle set at 36^0C. The evaporating solvent goes to the condensing unit where after condensation it falls drop by drop into the thimble placed with the tissue for extraction of fat. The solvent extracts lipid from the tissue and in each cycle, lipid comes down to solvent flask. The process of extraction is repeated till the fat present in the tissue is extracted. The process is continued for 2-4 hours. After the completion of fat extraction, the sample is weighed again and the lipid percent present in the sample is calculated by the following formula:

$$\% \, Fat = \frac{Initial\ weight\ of\ the\ sample\ (g) - Final\ weight\ of\ the\ sample}{\text{Intitial weight of the sample (g)}}$$

After extraction, fat was stored in vials for further analysis.

Iodine value of Fat

The iodine number of fats was estimated on the principle that halogens add across the double bond of unsaturated fatty acids to form addition compounds. Iodine mono chloride (ICl) is allowed to react with the fat in dark. The amount of Iodine consumed is then determined by titrating the iodine released (after adding KI) with the standard thiosulphate and comparing with a blank in which the fat is omitted. The reaction mixture is kept in the dark and titration carried out as quickly as possible since halogens are oxidized in the light. The iodine number is the number of grams of iodine taken up by 100gm of fat.

Calculation-

(BT – T) x 6.35 g per 100g of fat

where,

BT = Blank test readings and,

T = Test readings.

The fat content of the fish under investigation both *L. rohita* and *C. batrachus* has been preserved in the laboratory.

Serum Triglyceride

It was analyzed in a local diagnostic clinic. Each time a fresh sample of fish blood was used for these purposes.

Analysis of Fatty Acids (Thin Layer Chromatography)

Lipids are extremely diverse group of compounds consisting tri, di and monoacylglycerols, free fatty acids, phospholipids, sterols, caretonoids and vitamins A and D. Besides, most of these groups themselves are chemically complex. For example, all triglycerides are esters of glycerol and three fatty acid molecules which may have different chain lengths, further branching unsaturation and position on the glycerol molecule thus even a lipid which consists of only triacylglycerols may exhibit enormous variation in their chemical identity.

The common fatty acids of animal and plant origin have even numbered chains of 16 to 22 carbon atoms with 0to 6 double bonds of its configuration in which methylene interrupted double bond systems predominate. However, nature provides countless exceptions and there can be innumerable other structural features. In animals much of the dietary lipid is hydrolyzed to free fatty acids before it is absorbed, cleaved and utilized for lipid synthesis.

Though SH-1, 2-Diacylglycerols are minor components of most tissues in quantitative terms but they are very important in animal tissues. They are formed as intermediates in the biosynthesis of tri-acylglycerols. The fatty acid compositions of the di-acylglycerol thus, reflect the composition of the parent phospholipids and tends to be enriched in stearic and arachidonic acids. But di-acylglycerols must contain poly unsaturated fatty acids to complete their function.

Nearly all the commercially important fats and oils of animals and plant origin contain almost exclusively of the simple lipid class diglycerols. Most of these are depots fats with main function to be a store of energy.

Lipids are usually defined as these components that are soluble in organic solvents (such as ether, hexane or chloroform) but are insoluble in water. The lipid fraction of fatty food contains a complex mixture of different type of molecules (refer 1st para). Even so triglycerols are major component of most foods, typically making up more 95-99% of the total lipid present.

The Triglycerols are esters of three fatty acids and a glycerol molecule. The fatty acids normally found in foods very in chain length, degree of unsaturation and position of glycerol molecules. Consequently, the triacylglycerol fraction itself consists of a complex mixture of different types of molecules. Each type of fat has a different profile of lipids present which determines the precise nature of its nutritional and physicochemical properties. The lipids present in teleost fish species may be divided into two major groups, the phospholipids and triglycerides. Fish oils, in particular are characterized by a relatively high proportion of unsaturated acids present as triglycerides (Brocklesby 1941, Cruger et al. 1964)

Sample preparation

It was the foremost task to ascertain that the sample chosen for analysis represents the lipid present in the original from. During present work solvent extraction method was followed that has already been explained earlier in the same chapter. Oxidation of unsaturated lipids was provided by using butylated hydroxyl toluene as antioxidant.

Separation by chromatography

Chromatography is one of the most powerful analytical tools for separating and analyzing the properties of lipids. However thin layer chromatography (TLC) is used mainly to separate and determine the concentration of different types of lipids groups in foods i.e., triglycerides diglycerides monoacylglcerides, cholesterol cholesterol oxides and phospholipids. Following lipid extraction two-dimensional TLC procedure was used as prescribed for complete lipid. In first set of experiment was silica gel G precoated glass of experiment Silica gel precoated glass plates (OB 107085) were used. The chromatoplates activated at for 2hrs. in hot air oven were spotted with 50ml of the extracted lipid (50mg butylated hydroxyl toluene, BHT as an antioxidant) 2cm. Above the base line in first mobility phase triglyceride standard (MDT –12 SIGMA) was used as standard. The plate was allowed for first development mobile phase containing chloroform: methanol: water (60:30:5 v/v) was used and chromatoplates were allowed to full length of the chromatoplates in less polar mobile containing hexane: diethyl ether: acetic acid (80:20:1.5 v/v)

The process allows the movement of triglycerides approximately near the top of the plate while the complex lipids like phospholipids and glycolipids remain at the base of if they were single lipid class. The component was detected by spraying the plate with ethanol phosphomolybdic acid reagent commercially available as a 20% w/v solution followed by heating it in an oven. All the lipid appears as dark spot on a light-yellow background.

Identification of essential fatty acids

In order to separate free fatty acids from triglyceride fraction as well as complex lipid fraction (phospholipids/gylcolipids that remain at the base of the chromatoplate) the spots were attentively demarketed using a pointed needle and scrapped off in vials. The vials were added with 5ml of diethyl ether along with 5gm of antioxidant butylated hydroxy toluene (BHT) and centrifuged. The aliquot free of silica gel G particles was subjected to two different mobile phase each for triglycerides and complex lipid fraction. Two fresh chromatoplates (activated) were spotted with 10 ml of triglycerides of complex lipid following the same techniques as applied above. For triglycerides the mobile phase containing acetonitrile: acetone: water was used while for the complex lipid group hexane: diethyl ether: acetic acid (70:30:1.5 v/v/v) was used for comparison. The plates were allowed to develop full length with approximate elution time taken calculated to 2 – 1.5 hr. The plates were sprayed with ethanol phosphomolybdic acid reagent as earlier and dark spots were identified visually in comparison to control. The result has been presented in (+) to (++++) as assessed in comparison to control.

Determination of Acid value of fat

10.0g of fat was suspended in about 50 ml of solvent prepared by mixing equal volume of 95% (v/v) alcohol and ether neutralized to phenolphthalein and mixed thoroughly. This was titrated with 0.1 mol/litre KOH until the faint pink color persists for 20-30 seconds. The number of milli liters of standard alkali required was registered and the acid value of fat was calculated (0.1 mol/litre KOH contains 5.6 g/litre or 5.6 mg/ml).

Determination of Saponification value of fat

1g of fat was dissolved in 3 ml of solvent by mixing (equal volume of 95% v/v alcohol and ether neutralized to phenolphthalein). The content was transferred to a 250 ml conical flask by rinsing the beaker three times with a further millimeter of solvent. To this 25 ml of 0.5 mol/litre alcoholic KOH was added and attached to reflux condenser. Another condenser was set-up as blank with everything present except the fat. Both the flask was heated on boiling water for 30 minutes and then left to cool at room temperature. Cooled substrate was titrated with 0.5 ml/litre HCl and phenolphthalein indicator.

RESULTS AND DISCUSSION

The selection of the fish species was made to characterize the impact of feeding habits on the lipid profile of fish under influence of the prevalent climatic factors, as mentioned in the work plan. Table-1 illustrates feeding habit of some selected fish species known for their high economic value. The detail presented in the table has been compiled after Jhingaran (1982) and does not constitute part of the present work. The following table-1 gives an account of the fish selected for their fat analysis. This includes *Labeo rohita* (Rohu), a plankton feeder in water column, *Catla catla*, which larvae, rotifers and insect available on water surface and *Labeo calbasu* (Bhakur), a detritus feeder in the bottom of the water bodies. These three species are belonging to major carp group and hold high economic value. Details of the biochemical profiles of *Labeo rohita*

have been presented in tables (2). Table-2-4 includes biochemical profile with reference to the present parameters like Iodine value, acid value and saponification value, Triglyceride content added with length, wet weight, dry weight and percent fat content of the fish sampled during summer monsoon and winter season from rivers. Similar details of *Catla catla*, sampled from Balan river have been presented in Table-4. Further analysis of the lipid profile with special reference to omega-6 (ω_6) and omega-3 (ω_3) fatty acids had many constrains and limitations. However, the data obtained through thin layer chromatography analysis of the triglyceride component from all the species have been presented in table-5

Table 1. Details of the fish species selected for biochemical analysis (fat).

Feeding	Habitat	Economic group	Species/ common name
Panktron feeder Column feeder	Predominantly in rivers and ponds	Major carp	*Labeo rohita* (Rohu)
Surface feeder larvae, rotifers & insects	Predominantly in rivers and ponds, also recorded from wetlands	Major carp	*Catla catla* (Catla)
Detritus feeder	”	”	*Labeo calbaus* (Bhakur)
Carnivorous and predaceous	Rivers and wetlands	Predatory fish	*Wallago attus* (Boari)
Bottom feeder predaceous	Predominantly in wetlands & ponds	Live fish	*Clarius batrachus* (Mangur)

Table 2. Biochemical profile of sampled *Labeo rohita* during summer, monsoon and winter months

Month	Length	Wet weight	Dry weight	Fat content	Iodine Value	Triglyceride mgm (%)	Saponification value	Acid value
Summer	42,7±0.356	1016±0.307	90.00±0.307	21.2±0.324	93	407	160	8.7
Monsoon	40.9±1.163	996±0.614	89.5±0.614	20.8±0.112	92	404	164.4	9.12
Winter	44.7±0.169	1027±1.09	91.12±1.09	23.2±0.132	94	408	165.9	8.9

Table 3. Biochemical profile of sampled *Labeo calbasu* during summer, monsoon and winter months

Month	Length	Wet weight	Dry weight	Fat content	Iodine Value	Triglyceride mgm (%)	Saponification value	Acid value
Summer	38.7±0.269	983 ±0.289	83.10±0.042	24.6±0.327	96±0.372	398	152.4	7.8
Monsoon	41.3±1.102	1035±0.617	86.7±0.217	25.2±0.022	97±0.362	411	155.0	8.61
Winter	43.0±0.172	1062±1.14	86.9±0.326	26.7±0.098	97±0.317	409	158.7	8.03

Table 4. Biochemical profile of *sampled Catla catla* during summer monsoon and winter months

Month	Length	Wet weight	Dry weight	Fat content	Iodine Value	Triglyceride mgm (%)	Saponification value	Acid value
Summer	39.3±0.412	992±0.339	84,1±0.062	25.6±0.227	98±0.124	391	140	7.6
Monsoon	42.0±1.02	1012±0.642	86.2±0.032	27.2±0.319	96±0.216	401	133	7.0
Winter	41.8±0.679	1007±0.598	85.6±0.59	27.1±0.376	96±0.248	410	135.0	6.93

Table 5. Omega-6 and Omega-3 fatty acid (EFAs) profile of the fishes

Fish	Season	Omega-6 (ω_6)	Omega-3 (ω_3)
Labeo rohita	Summer	+	+++
	Monsoon	–	++
	Winter	++	+++
Catla catla	Summer	++	++
	Monsoon	+	+
	Winter	++	+++
Labeo calbasu	Summer	+	++
	Monsoon	–	+
	Winter	+	+++

(+) – Present
(++) – Less than the standard
(+++) – Comparable to the standard
(–) – Could not be traced

Like biochemical profile, the growth pattern of the fish in general, is highly variable depending upon their physiological status, sex, environment, season and availability of preferred food. Similarly, making up of the biochemical profile of the fish is closely related to fish intake, behavioral pattern and spousing (Halver, 2002). In particular, the lipid fraction is the component showing the greatest variation (Love, 1970). Seasonal variation in fatty acid composition of the fish species (Table 5) as observed through total lipid content and iodine values have been reported in many cases. The iodine value of fat gives a measure of average degree of saturation of lipid: The higher the iodine value, the greater the number of C=C double bonds. By definition, the iodine value is expressed as the gram of iodine observed per 100g lipid. The acid value is the numbers of milligrams of KOH required to neutralize the fatty acids present in 1g of fat and the Saponification value is the number of milligrams of KOH required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of fat. The Saponification value gives all indication of the nature of the fatty acid in the fat since the longer the

carbon chain the less acid is liberated per gram of fat hydrolyzed. This clearly indicates that the quality of fat is more unsaturated in *Labeo rohita* which is basically a plankton feeder and less in *Catla catla* which feeds on small animals. The *L. calbasu*, being a detritus feeder comes almost in the same range as shown by *L. rohita* on probably account of having multiple choice for food. Similar observations have been put forwarded by Gulzar and Zuber (2000) who determined omega-3 fatty acid composition in some freshwater fish like *Mastacembus armatus, Mystus singhala and Labeo calbasu*.

Further, table - 23 which depicts the status of omega – 6 and omega – 3 fatty acids in present fish also indicate value of omega – 3 in '+++' category both during summer and winter season is *Labeo rohita* as well as *Labeo calbasu*. The level of omega - 3 is *Catla catla* shows '++' category in both summer and winter season.

CONCLUSION

All the selected fish species were sampled separately in summer, monsoon and winter season brought freshly to the laboratory for fat extraction by Soxhlet extraction method. The fat extracted in batches were pooled and stored for further analysis. The fat contents of *Catla catla* was recorded between 24.6-26.7%, Iodine value in the range of 96-97, saponification value between 152.4-158.7 and acid value between 7.8-8.61 These values in *L. calbasu* was recorded as: fat content 25.6 - 27.1%; Iodine value 96-98; Saponification value 133.4-140.3 and acid value 6.93-7.6. The findings suggest that the total fat content in all the major carps were comparable to each other. The study also suggests that in general the fat content of fish increases during winter season probably on account of availability of quality food. However, with regard to ω_6 and ω_3 profile the *Labeo rohita and Labeo calbasu* the phytoplankton and detritus feeders respectively shows a better range of ω_3 fatty acids than *Catla catla*, a zooplankton feeder which appears to be rich in ω_6 fatty acids. Thus, the investigation suggests that while the carnivorous fish are rich in ω_6 fatty acids the phytoplankton and omnivorous fish shows a good profile of ω_3 fatty acids during summer and winter season. It is altered during monsoon season due to fish physiological activities involving maturation and subsequent reproduction as the level of fat deplete drastically.

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