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Changes in Biochemical Composition in Indian Major Carps in Relation to size

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Total biochemical composition of freshwater major carps, *Labeo rohita* -(rohu); *Catla catla* (catla); *Cirrhinus mrigala* (mrigal), in relation to size was evaluated. The water soluble nitrogen fraction constituted about 21% of the total nitrogen. Salt soluble fraction constituted 55 - 60% of the total nitrogen. Non-protein nitrogen represented 12% of the total nitrogen in all the cases except mrigal of small size (9.36%). The insoluble connective tissue contributed to 2-3 per cent of total nitrogen. The monounsaturated fatty acids (MUFA) formed 31-39% of the total fatty acids and 60-68% of the MUFA in freshwater carps was C18:1. The essential amino acids contributed to 41 to 51% of the total amino acids in freshwater major carps. Aromatic amino acid content was slightly higher compared to marine fishes while the proportion of proline was less. Variations in composition in relation to size are discussed. The autolytic activity was significantly higher in small fish in all the three species

Key words: Major carps, amino acids, fatty acids, autolysis, rohu, cutala, mrigal

The composition of the fish muscle varies according to many factors such as sex, size, stages of maturity and season. Starvation resulted in a decrease in protein and lipid contents coupled with an increase in water content (Wendakoon & Shimizu, 1991). The proximate composition of a number of marine, freshwater and brackish water fish has been reported (Gopakumar, 1997; Mukundan *et. al.*, 1986). Understanding the functional properties is of utmost importance for utilizing the fish in the preparation of value added products and the functional properties depend on the composition of the meat. Earlier reports indicate that changes in composition may occur as a result of gonadal maturity (Itoh *et al*, 1995; Montecchia *et al*, 1997). The properties of actomyosin are particularly affected during maturation. Pre-spawning fish was found to contain lower actomyosin content (Montecchia *et al.*, 1997). However, information on the changes in composition with size of the fish is relatively scanty. Hence, an attempt has been made to study the biochemical composition of freshwater major carps - rohu, catla and mrigal - in relation to size.

Materials and Methods

Fish (rohu - *Labeo rohita*, catla - *Catla catla* and mrigal - *Cirrhinus mrigala*) were collected from the culture ponds in absolutely fresh condition and brought to the laboratory partially iced. Fish of two different sizes - smaller size weighing around 500 g and a commercial size weighing above 1000 g were selected based on the gonadal maturation stage of the fish.

The fish were washed thoroughly to remove slime, dirt etc. and skin-less bone-free fillets were made. The fillets were homogenized by passing through a hand extruder and mixed thoroughly. The temperature of the fish and the mince were maintained below 5°C throughout.

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Moisture, protein, fat and ash were determined according to the methods of AOAC (1990). The muscle lipids were extracted by using 2:1 mixture of chloroform and methanol (Folch *et al.*, 1957). The quantitative derivatisation of fatty acids to fatty acid methyl esters (FAME) was carried out using boron triflouride-methanol (BF3-CH3OH) reagent (Matcalfe *et al.*, 1966). The FAMEs were analysed by a Chrompack CP 9001 gas chromatograph equipped with a flame ionisation detector.

The samples for amino acid analyses were prepared according to the method of Ishida *et al.*, (1981). The sample thus prepared was filtered using a membrane filter of 0.45µm and 20µl was injected into Shimadzu HPLC - LC 10 AS, fitted with a packed column (ISC-07/S1504-Na). The amino acid identification was done by non-switching flow method and fluorescence method after the post column derivatisation of amino acids with o-phthalaldehyde. Tryptophan was estimated as per the method of Sastry & Tummuru (1985) after alkali hydrolysis of the sample using 5% sodium hydroxide at 110°C for 24 hours.

The soluble proteins were characterized by the method of Hashimoto *et al* (1979) with some modifications. In place of phosphate buffer, bicarbonate and sodium chloride-bicarbonate buffer of appropriate ionic strengths were used. The autolytic activity of the muscle was studied as described by Green & Babbit (1990) and the Folin positive material in the supernatant was determined by the method of Herriot, (1955). The activity was expressed as µmol tyrosine released per ml test sample per minute.

Results and Discussion

Average moisture content (Table 1) noticed for fresh rohu, catla and mrigal flesh was in the range of 77 -81%. The higher moisture content of the fish flesh noticed was due to the partial icing of the fish for 24 hours prior to processing. Moisture content in the range of 76 to 77% has been reported for the same fish (Mukundan *et al*, 1986; Joseph *et al.*, 1990; Gopakumar, 1997). Larger sized fish had marginally higher moisture levels, 2.8% in rohu, 3.1% in mrigal and only 1.9% in cutla.a

Protein content varied from 16-19% in the samples analysed with the lowest value of 16.13% for rohu - big, and the highest value of 19.25% for mrigal - small. These values were in agreement with those reported earlier (Gopakumar, 1997). In rohu and mrigal, the proportion of protein was higher in the smaller fish. The difference was more than 10% in these cases. In catla, there was only marginal difference between the two groups.

The total protein is the sum of different protein fractions - water-soluble sarcoplasmic

Table 1. Proximate composition* of rohu, catla and mrigal

	Rohu		Catla		Mrigal	
	Small	Big	Small	Big	Small	Big
Weight (g) / length (cm)	560 / 39	1200 / 57	560 / 36	1350 / 45	560 / 38	1600 / 56
Weight of gonad, (g)	2.25	4.07	2.21	3.98	2.78	4.35
Moisture %	79.26	81.75	80.72	79.23	77.82	80.00
Crude protein, % (N x 6.25)	18.41	16.13	16.98	16.54	19.25	16.29
Fat, %	1.01(4.99)	2.43 (13.32)	1.12 (5.81)	2.09 (10.84	1.25 (5.64)	2.13 (10.65)
Carbohydrate, %	0.85 (4.10)	1.82 (8.78)	1.12 (5.81)	1.89 (9.80)	1.12 (5.05)	1.95 (9.75)
Ash, %	1.02 (4.92)	1.15 (5.54)	1.17 (6.09)	1.32 (6.85)	1.56 (7.03)	1.97 (9.85)
Calorie	86.13	93.67	82.48	92.53	92.73	92.13

* Values are average of triplicate analysis
Value in parenthesis denote dry weight basis

proteins including the non-protein nitrogen, the salt soluble myofibrillar protein and the stroma proteins and these individual proteins determine the texture, and other functional properties. The water-soluble nitrogen fraction (Table 2) constituted about 18 -25% of total nitrogen. The values were in the lower range in rohu - big and catla - small. In these two cases the alkali soluble denatured protein content showed an increase, suggesting the possible denaturation of water-soluble proteins during the handling process. These results are comparable to other reports in these fish (Joseph *et al.*, 1990). No correlation could be established in relation to size.

The salt soluble fraction (Table 2) reflect the actual content of structural proteins, i.e the myofibrillar proteins which are of significance as far as the production of surimi and similar products are concerned. The salt soluble fraction (SSN) constituted 55 - 60% of total nitrogen except in catla-small and mrigal-big where the contents were marginally lower. This could be due to the insolubilization of this fraction as a result of the handling condition and is explained by the increased content of alkali soluble nitrogen.

The non-protein nitrogen constituted about 13.14% (Table 2) of the total nitrogen in all the cases excepting mrigal small (9.36%). Not much difference was noticed between small and big fish in the case of rohu and catla but more than 30% increase was noticed in big mrigal compared to the small fish. Generally the non-protein nitrogen constitutes about 9 - 18 per cent in teleosts and 33 - 38 per cent in elasmobranchs (Haard *et al.*, 1994). These water-soluble fractions are of significance to food technologists because of their association with the taste of the seafood and contribution to spoilage. Joseph *et. al.*, (1990) reported similar results in the same fish during ice storage studies. The insoluble connective tissue contributed to 2-3 per cent of total nitrogen, which is well within the range of 2-5 per cent reported for bony fish (Sikorski & Borderas, 1994).

Fat content of rohu, catla and mrigal were in the range 1.0- 2.5%. A clear distinction in fat content between small and big fish was seen after calculating on dry weight basis. About three-fold increase was noticed in big fish in the case of rohu while in catla and mrigal the increase was about two fold.

Ash content of the fish showed only a marginal variation between species (Table 1). Variations in ash content with size of the fish also did not show any clear trend. The large sized mrigal had higher ash content, but in the case of the other two species, the differences were insignificant.

Though the carbohydrate content is almost negligible in fish, the content in freshwater fish was slightly higher. The carbohydrate content almost doubled in all the three fish with size (Table 1). The calorific value of the fish increased with size and may be related to the increase in fat content with size.

Table 2. Different nitrogenous fractions* in rohu, catla and mrigal

	Rohu		Catla		Mrigal	
	Small	Big	Small	Big	Small	Big
Water soluble nitrogen	21.69	18.61	19.81	25.77	21.42	21.16
Salt soluble nitrogen	58.31	55.43	50.94	54.64	54.36	51.54
Non protein nitrogen	12.88	12.79	13.84	12.72	9.36	14.33
Alkali soluble nitrogen	1.69	10.08	10.89	6.56	8.35	8.92
Stroma nitrogen	2.37	3.49	2.63	2.09	2.82	2.68

* Per cent of total nitrogen
Values are average of triplicate analysis

Table 3. Fatty acid composition* of fish meat from rohu, catla and mrigal (area %)

Fatty acid	Rohu		Catla		Mrigal	
	Small	Big	Small	Big	Small	Big
C12	0.52	0.26	0.00	0.25	0.83	0.26
C13	0.15	0.00	0.40	0.26	0.26	0.00
C14	5.12	2.86	2.20	3.05	4.39	2.86
C14:1	0.59	1.45	0.77	1.91	0.97	2.86
C15	2.75	1.34	1.63	1.21	2.39	1.34
C16	21.58	25.08	23.10	25.65	19.43	27.28
C16:1	9.64	8.36	8.95	9.12	9.24	8.28
C17	1.84	3.66	2.25	4.05	2.10	0.96
C18	3.65	0.44	0.21	0:31	4.21	0.44
C18:1	22.35	24.88	26.36	26.17	19.54	25.56
C18:2	6.25	5.77	5.00	6.12	7.34	4.99
C18:3	2.56	2.45	3.10	2.25	2.35	1.90
C18:4	1.95	1.79	2.04	1.95	1.42	1.36
C19	0.00	0.30	0.48	0.52	0.00	0.30
C20:1	0.18	0.01	0.25	0.18	0.23	0.00
C20:2	0.84	0.79	0.80	0.94	0.75	0.89
C20:4	5.28	4.32	4.88	4.57	6.23	4.58
C20:5	2.64	1.41	2.80	2.75	2.56	1.50
C22:1	0.62	0.49	0.92	0.88	0.51	0.56
C22:6	8.12	7.96	6.00	6.12	7.46	7.86
C24:1	1.51	1.20	1.95	1.45	1.48	1.20
Total SFA	35.61	33.94	30.27	35.3	33.61	33.44
Total MUFA	34.89	36.39	39.20	39.71	31.97	38.46
Total PUFA	27.64	24.49	24.62	24.70	28.11	23.08

* Values are average of triplicate analysis

The fatty acid composition was slightly different from that of marine fish (Table 3). Generally, saturated fatty acids (SFA) form 15 - 35% of total fatty acids in marine fish (Ackman, 1989). SFA constituted 30 - 36 % in the three fish with the highest proportion in rohu-small and the lowest in catla-small. This included about 2-5% of odd numbered SFA also. Among the SFA, palmitic acid (16:0) content was 20-25% of the total fatty acids and is comparable to that in marine fish (Nair, 1998). Palmitic acid accounts for upto 60% of SFA in marine fish (Ackman & Eaten, 1966) and in the freshwater major carps analysed in the study, 16:0 constituted 60-80% of the total SFA, the exception being mrigal-small where it was just 58%.

The monounsaturated fatty acids (MUFA) formed 31-39% of the total fatty acids in the three major carps and its content was higher than that reported for marine fish (Nair & Gopakumar, 1978). 60-68% of the MUFA in freshwater carps was C18: 1 (oleic acid). Fish from Indian waters were reported to contain about 13% of C18: 1 (Nair, 1998), against the more than 20% seen in freshwater major carps studied.

The polyunsaturated fatty acid (PUFA) accounted for 23 - 28% of the total fatty acids among the different samples, with the maximum content in mrigal-small and minimum in mrigal-big. 68-71% of the PUFA was constituted by C18: 2 (n-6), 20: 4 (n-6) and C22: 6(n-3) with C22: 6 predominating in all the cases. The C22: 6 content, however, was comparatively less than that of marine fish. The PUFA content in the case of marine fish ranged from 28 to 57% with C20:5 and C22: 6 predominating and constituting about 50% in most cases (Nair & Gopakumar, 1978). The other principal fatty acid from marine source C20: 5 was present in comparatively lower proportions in freshwater fish. The C20: 4 (n-6) content was marginally higher but the C22: 6 was very much lower than that of marine fish.

In the case of rohu, the total SFA and PUFA content decreased with increase in size while the MUFA content increased marginally. Almost similar trend was noticed in the case of mrigal but the increase in MUFA was about 20%. In catla, the proportion of saturated fatty acids was higher in the larger fish. However, the available data do not indicate any definite relationship between fatty acid composition and the size of the fish.

Glutamic acid was the major amino acid accounting for 15 - 20% of total amino acids and this result is in accordance with the earlier report for rohu and mrigal (Mukundan *et.al.*, 1986). The glutamic acid content was marginally higher than that in marine fish (Gopakumar, 1997) and maximum content was in rohu - small and minimum in catla - big (Table 4). Aspartic acid (10-12%) occupied the second position. Marginal

Table 4. Amino acid composition of fish meat* from rohu, catla and mrigal (g/100g protein)

Amino acid	Rohu		Catla		Mrigal	
	Small	Big	Small	Big	Small	Big
Aspartic acid	10.99	11.29	10.26	11.21	11.59	12.25
Threonine	3.95	4.55	4.37	4.18	4.25	4.56
Serine	4.00	4.24	4.24	4.03	4.05	4.26
Glutamic acid	20.06	18.98	18.21	15.63	17.88	18.02
Proline	2.73	3.03	3.48	3.91	3.47	3.52
Glycine	4.99	4.95	3.31	3.19	5.00	5.02
Alanine	7.80	7.07	8.79	6.76	7.07	7.25
Cysteine	0.76	0.00	2.01	2.13	0.84	0.52
Valine	4.05	5.07	5.21	4.07	4.80	4.92
Methionine	1.79	2.18	2.25	1.86	1.91	2.14
Isoleucine	3.94	4.56	4.91	4.33	3.68	2.58
Leucine	8.58	8.89	9.33	8.45	7.09	7.98
Tyrosine	3.56	3.48	3.82	3.27	3.51	3.66
Phenylalanine	3.41	4.49	4.54	3.92	4.20	4.32
Histidine	3.78	5.09	3.95	3.83	4.97	5.28
Lysine	3.01	5.40	7.40	8.20	5.34	3.21
Tryptophan	1.12	1.49	1.27	1.18	1.39	1.45
Arginine	5.58	6.24	7.89	12.07	5.58	5.78

* Values are average of triplicate analysis

decrease in glutamic acid and a similar increase in aspartic acid were noticed with growth.

The essential amino acids contributed to 41 to 51% of the total amino acids. Catla showed a marginally higher content of essential amino acids. Similarly, the content of histidine, an amino acid of nutritional significance in children, was higher in mrigal. The lysine content in these species was lower than that in marine fish. Aromatic amino acids formed 8 to 10% of the total amino acids, which was slightly higher than that of marine fish. The increase is related to the higher tyrosine content associated with these fish. Proline content of freshwater carps was lower than that of marine species indicating the lesser connective tissue content of these fishes. However, there are no marked differences in the amino acid pattern between the small and big fish in all the three species.

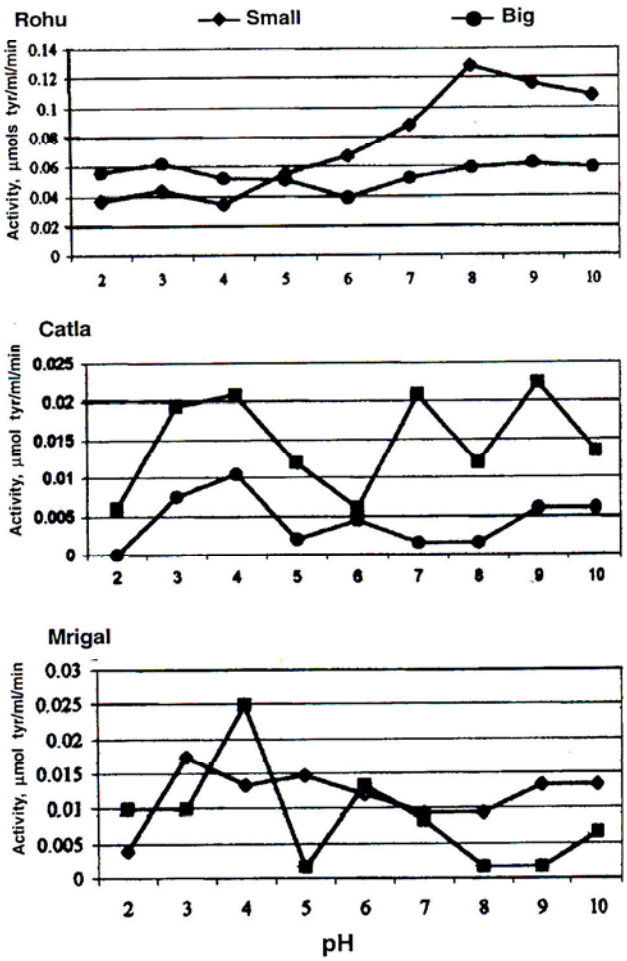

Fig. 1. Autolytic activities in small and big size rohu, catla and mrigal at different pH

The autolytic activity was significantly higher in small fish in all the three species studied (Fig 1). Among the smaller fish, catla showed several times higher activity at alkaline, neutral and acid pH ranges. Mrigal followed, with activities at almost all pH ranges while rohu showed lowest activity among the three fish, with maximum activity in the alkaline range. Rohu-small showed low activity at lower pH ranges (pH 2-5) but rohu - big showed marginally higher activity. However, above pH 5, there was an increase in autolysis and at pH 8 the activity was more than double the activity at pH 5. Above pH 8, the activity decreased marginally. Catla showed high activity at pH ranges 3, 4, 7, 9 and 10, moderate activity at pH 5 and 8 and low activity at pH 6. In big fish, higher activity was noticed in the acid and neutral pH ranges.

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