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BIOCHEMICAL VARIATION AMONG SOME SPECIES OF POND FISHES

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Abstract

Eight various species of fishes were collected from local market to evaluate biochemical composition and enzymatic activities. Biochemical analysis is an index of nutritive value only because the fraction it isolates are correlated with some of the properties of organism that are nutritionally significant. Fish is one of the most important sources of animal protein and has been widely needed as a good source of protein and other elements for the maintenance of healthy body. The result showed that protein, carbohydrate, fat, ash, moisture, methionine and tryptophan content was respectively highest in Rohu (19.33% ± 0.01%), Mangoor (25.00±1.32), Pehna(35.00±1.39), Kawai (1.30±0.22), Mangoor (6.00±1.36), Kawai(1.55±0.76), Nanauti (2.00±0.76). Antioxidant enzyme activities of fish were determined to establish environmental impact of toxic effect on anthrogenic pollution on pond. The result indicate the among then 8 fishes species, the activity of enzyme vis SOD (5.034±2.43) in Bamm, Catalase (652.0±17.8) in Mangoor, PPO (424±20.11) in Botha, POD (3.36±2.56) in pehna.

Therefore, proximate biochemical composition of a species helps to assess its nutritional and edible value in terms energy units compared to other species.

Keywords: *Pond Fish, Enzyme, Nutrition*.

1. Introduction

All over world, it is well accepted that fishes are good sources of animal protein and other elements for the maintenance of healthy body (Andrew, 2001). Fish is widely accepted because of its high palatability, low cholesterol and tender flesh. It is the cheapest source of animal protein and other essential nutrients required in human diet (Sadiku and Oladimeji, 1991). Fish flesh contains up to 15-25% protein, 80% water, 1-2% mineral matter (CSIR, 1962). FAO 1991 report shows that fishes contain 72% water, 19% protein and 5% calcium. In terms of weight of food consumed, fish ranks third after rice and vegetables (Minkin *et al.,* 1997 and Hels *et al.,* 2002). Many nutrients such as vitamin A and C, iron, calcium, zinc and iodine which are not found in rice and have to be obtained from other sources. Small fish which are usually eaten whole with the organs and bones contain large amounts of calcium and possibly iron and zinc and indigenous fishes also contain large amount of vitamin A. (Thilsted *et al.,* 1997).

The fishes were sun dried or oven dried, which are also method of preservation. Quality of the oven-dried fish was better than that of the sun-dried fish, but sun-drying process is easy and can be used in large scale. The fish powder remained in good condition for 7-9 months at normal room temperature, but at -18°C the powder was in good condition throughout the year. (Sabina *et al.,* 2011) As the supply continues to decrease, it is becoming more difficult to meet the food requirements of the world's population. As human population size continues to increase, there is concomitant increase in over-fishing and habitat destruction, thus resulting in dwindling fish stocks. Over the years, fishermen have adopted the use of more efficient and illegal methods of fishing. Fish catch has stagnated and in some cases, declined in the inland fisheries (Adeleye, 2000). The exact composition of fish depends on the species of fish, (which is related to its feeding habit) and source of fish (habitat). The fish reared under the extensive and semi intensive conditions has higher nutritive value for human consumption than the ones found in the wild. (Ahmad *et al.,* 2012). Therefore, this study was designed to investigate the nutritional value of different varieties of pond fishes.

2. Material and Methods

2.1 Fish Species Used

Fishes, Rohu (*Labeo rohita*), Pehna (*Eutropiichthys vacha*), Mangoor (*Clarious batrachus*), Nanauti (*Puntius chonius*), Chelwa (*Oxygaster bacaila*), Kawai (*Etroplus surastensis*), Botha (*Glossogobius giuris*), Bamm (*Mastacembelus pancalus*) were taken from local Market of Allahabad and identified by Dr.(Mrs.) Sashya Nagar, Department of Biological Sciences, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad, India.

2.2 Preparation of Biological Sample for Analysis

Fishes were washed carefully with tap water, their fins and scales were removed and abdomens were cut open to remove the gut. Fishes were then cut into small pieces and were kept in oven for complete drying at 50-60^{0}C temperature

for 6-8 hours. After complete drying all the samples were removed from the oven and kept in separate airtight glass containers with proper labelling. Before the start of biochemical analysis the samples were ground to fine powder by electric blender.

2.3 Biochemical Analysis

2.3.1 Estimation of Moisture Content

Moisture content was determined by the method of Hart and Fisher (1971), firstly drying the known amount of sample in an oven at 60-70^{0}C for 8-10 hours and obtained by subtracting dry weight from fresh weight and it was expressed as percent fresh weight.

2.3.2 Estimation of Carbohydrate

The total carbohydrate content of the fish was estimated by anthrone reagent (Sadassivam and Manickam, 1992). The anthrone reaction is basic and convenient method for the determination of hexoses, aldopentoses and hexuronic acids either free or present in polysacharides. Carbohydrates are dehydrated by conc. H_2SO_4 to form furfural. Furfural condenses with anthnone (10-keto 9, 10dehydro- anthrone) to form a blue green complex which is measured calorimetrically at 630 nm.

2.3.3Determination of Protein by Lowry's Method

The Lowry's reaction for protein estimation is an extension of the biuret method. The method developed by Lowry et al., (1951). A 10% (w/v) homogenate of samples was prepared in DW, centrifuged and the supernatants were used for protein estimation. Added 4ml of alkaline copper solution to 0.1ml of supernatants mixed well and incubated at RT for 10 min. After that 0.4mL of FCR was added and after 30min of incubation at RT, absorbance was read at 720nm. A standard was prepared using different concentrations of BSA. Calculate the protein using a calibration curve; the results were expressed in µg ml^{-1}.

2.3.4 Fat Content

Soxhelt method (A.O.A.C. 1970) was used for the estimation of fish fat in biomass. Accordingly, 5 g dried sample was taken in a Soxhlet flask and about 40 ml petroleum ether (b.p. 40-60^{0}C) was add to it. This was then refluxed for 7-8 hr. Thereafter the traces of petroleum ether were removed from the flask by keeping it in an oven at 105^{0}C for 30 minutes. Fish fat was expressed as percent on dry weight basis.

2.3.5 Total Ash Content

Total ash content was estimated with method as described by Hart and Fisher (1971). 1 g dried sample which was dried at 70^{0}C temperature was transferred to ash less filter paper. The ignition of sample was carried out on non luminous flame in pre weighed silica crucible. The crucible was placed in to muffle furnace maintained at 525- 550^{0}C for about 5-6 hours to density the organic matters. After expiry of period, the crucible was transferred to a desiccator for cooling to avoid absorption of moisture by the ash. The cold ash along with silica crucible was weighted and the result was calculated and reported on moisture free basis.

2.3.6 Tryptophan Content

Tryptophan content was estimated by the methods of Spies and Chamber (1949) 0.2 g homogenized sample was transferred in 100 ml of conical flask and 10 ml 19 NH_2 SO_4 was added. The content of conical flask was kept for 12 hours in dark place. After expiry of period 1 ml distilled water l ml p- dimethyl amino benzaldehyde 80 mg dissolved in 100 ml 2 N H_2SO_4 and 0.1 ml of sodium nitrite solution (0.45% in water) were added. This was kept for 30 minutes for colour development. This intensity of colour was measured in photoelectric colorimeter (Sepectronic-20) at 620 nm. The calculation was done by standard curve.

2.3.7 Estimation of Methionine

Methionine content was estimated by the methods of Horn *et al.,* (1946).

2.3.8 Catalase (EC.1.11.1.6) Assay

Catalase activity was assayed by estimating the residual H_2O_2 in the reaction mixture, which was then determined by oxidation with $KMnO_4$ titrimetrically by modified Barber (1980) method. Expressed the enzyme activity as unit ml^{-1}. One unit of enzyme activity is defined as that amount of enzyme which breaks down 1µmole of H_2O_2 under the assay condition.

2.3.9 Peroxidase (EC.1.11.1.7) Assay

The activity of peroxidase was assayed by measuring the oxidation of guaiacol to form tetraguaiacol in the presence of H_2O_2 according to the method given by Chanda and Singh (1997). Pippetted 1ml each of the potassium phosphate buffer, 1mM H_2O_2, 4mM guaiacol and enzyme extract. The change in absorbance at 470 nm due to the oxidation of guaiacol to form tetraguaiacol in the presence of H_2O_2 was measured.

$$\text{Volume activity (U ml}^{-1}) = \frac{A_{470}\ \text{min}^{-1} \times 4 \times V_t \times \text{dilution factor}}{\varepsilon \times V_S}$$

Vt=final volume of reaction mixture (ml) = 4.00
Vs= sample volume (ml) = 1.0
ε = µmolar extinction co-efficient of tetraguaiacol (cm^2/µmol) = 25.5
4 = derived from unit definition & principle

2.3.10 Polyphenol oxidase (EC.1.14.18.1) Assay

The polyphenol oxidase activity was measured by the increase in absorbance at 420nm with the oxidation of catechol as substrate according to the method given by Liu *et al.,* (2005). The reaction mixture contained 1.0 ml of 0.1M catechol, 1.9 ml 0.1M potassium phosphate buffer (pH 7.0), and 0.1 ml of enzyme extract, and incubated for 10 min at 30 ^{0}C . Measured the increase in absorbance at 420 nm with a spectrophotometer.
1 Unit of PPO activity was expressed as = $0.001\Delta A_{420}$ min $^{-1}$g $^{-1}$ fresh weight.

2.3.11 Superoxide dismutase (EC.1.15.1.1) Assay

The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) according to the method given by Calatayud *et al.,* (2002). Take 1ml each of potassium phosphate buffer, 10mM methionine, 57µM NBT, 1.0µM riboflavin, 0.025 % (v/v) Tween-20 and enzyme extract. A control without enzyme extract was prepared. The reaction mixture was thoroughly mixed and illuminated for 5min with 60 Watt electric bulb placed 20cm away. Absorbance was recorded at 560 nm after the illumination period. In this assay, 1 unit of SOD was defined as the amount of enzyme necessary to produce a 50 % inhibition of the NBT photo reduction.
SOD = ($O.D._{control} - O.D._{sample}$) x Volume of reaction x Dilution
The specific activity of each enzyme was also calculated using the formula: Enzyme activity mg $^{-1}$protein.

2.3.12 Statistical Analysis

All data recorded as MEAN ± S.D. of triplicate experiments.

3. Result and Discussion

In table 1 results show the Moisture content in the range of (1.18-6%). The highest value was found in species in Mangoor (6.00±1.36%) followed by Kawai (1.18±0.03%) and other species and lowest moisture content was obtained in species Kawai. Similar results were also reported by Salam *et al.,* 2002 and Harris *et al.,* 1996. The carbohydrate content in the range of (18.96-25.00%). The highest value was obtained in specie in Mangoor (25.00±1.32%) followed by Rohu(18.96±1.75%) and other species and lowest carbohydrate content was obtained in species Rohu (18.96±1.75) similar result were reported by Ravichandran *et al.,* 2011 and Omotosho *et al.,* 2011. The protein was in range (13.00-19.33%). The highest value was achieved in species in Rohu (19.33±2.50%) followed by Botha (13.00±1.22) and other species and the lowest protein contain was obtained in species Botha similar result were reported by Rustad *et al.,* 2003 and Afrnesen *et al.,* 2007. The fat content ranged from (12.53-35.00%). The highest value was achieved in species in Pehna (35.00±1.39%) followed by Nanauti (12.53±0.22%) and other species and the lowest fat content was obtained in species Nanauti similarly result were reported by Hossain et al., 1999 and Rahman *et al.,* 1989. The ash content was in the range of (0.99-1.30%). The highest value was found in species in Kawai (1.30±0.22%) followed by Nanauti (0.99±0.65%) and other species and the ash content was lowest in species Nanauti. Similar results were found by Gopalan *et al.,* 1987. The tryptophane was in the range of (0.90-2.00%).The highest value was found in species in Nanauti (2.00±0.76%) followed by Botha (0.90±0.33%) and other species and the lowest tryptophane content results were reported by Gopalan *et al.,* 1987 and Zambon *et al.,* 1992. The content of methionine was in range of (0.95-1.55%). The highest value was obtained in pseices in Kawai (1.55±0.76%) followed by Rohu (0.95±0.11%) and other species and the lowest Methionine content was obtained in species Rohu similar result were reported by Rahman *et al.,* 1994 and Stans *et al.,* 1962.

Table 1: Biochemical content of different species of fishes

Fish species	Moisture % (Dry weight)	Carbohydrate % (Dry weight)	Protein % (Dry weight)	Fat % (Dry weight)	Ash % (Dry weight)	Methionine % (Dry weight)	Tryptophane % (Dry weight)
Rohu (*Labeo rohita*)	1.90±0.52	18.96±1.75	19.33±2.50	22.00±0.92	1.20±0.25	0.95±0.11	1.20±0.45
Pehna (*Eutropiichthys vacha*)	2.20±0.32	19.50±1.03	17.34±2.23	35.00±1.39	1.10±0.32	1.20±0.46	1.30±0.56
Mangoor (*Clarious batrachus*)	6.00±1.36	25.00±1.32	14.70±1.23	25.66±0.32	1.21±0.56	1.23±0.23	0.99±0.86
Nanauti (*Puntius chonius*)	4.54±1.26	21.52±1.26	13.55±1.33	28.12±0.47	1.00±0.34	1.12±0.98	2.00±0.76

Chelwa (*Oxygaster bacaila*)	3.08±1.46	20.22±2.10	13.75±1.76	18.43±0.37	0.99±0.65	0.99±0.65	0.98±0.23
Kawai (*Etroplus surastensis*)	1.18±0.03	20.23±1.34	16.55±1.89	19.76±0.32	1.30±0.22	1.55±0.76	1.11±0.45
Botha (*Glossogobius giuris*)	3.90±1.69	20.11±1.22	13.00±1.22	12.53±0.22	1.11±0.43	1.26±0.33	0.90±0.33
Bamm (*Mastacembelus pancalus*)	2.94±0.45	21.11±1.23	18.25±2.03	14.30±0.33	1.20±0.78	1.22±0.67	1.02±0.11

All data expressed in ±SD of triplicate experiments

The polyphenol oxidase was in the ranged of (224-236) upper (table 2). The highest value was found in (224±22.83activity/mg protein) in nanauti, kawai (236±12.87) activity/mg protein) followed by botha and lowest polyphenol oxidane was obtained in species botha. Smilar results were reported by Bitterlich *et al.,* 1985. The peroxidase content in the ranged of (0.38-3.36) upper. The highest value was found in species in pehna (3.36±2.56) unit/mg protein) and other species and the lowest value was found in species in botha(0.38±0.11)followed by botha and lowest peroxidase was obtained in species botha. Similar results were reported by the Rehbein *et al.,* 1992. The Catalase was in the range of (128-652). The highest value was found in species in Mangoor (652.0±17.8unit/mg protein) followed by bam (128.0±12.5unit/mg protein) and other species and lowest catalase was obtained in species bamm. Similarly results were reported by Fish, *et al.,* 1960. The SOD was in the range of (1.608-5.034). The highest value was obtained in species in botha (5.034±2.43 unit/mg protein) and other species and lowest protein content was obtained in species nanauti (1.608±1.84) similarly results were reported by Faber *et al.,* 1993.

Table 2: Enzymes activity of different fish spicies

Fish spicies	Catalase Specific activity (activity/mg protein)	Peroxidase Specific activity (activity/mg protein)	Polyphenol oxidase (activity / mg protein)	superoxide dismutase Specific activity (activity/mg protein)
Rohu (*Labeo rohita*	2234.78±43.8	25.00±1.12	5826.08±321.65	48.18±2.65
Pehna (*Eutropiichthys vacha*)	2234.77±32.91	48.69±1.61	5130.43±218.98	33.76±3.23
Mangoor (*Clarious batrachus*)	11050.84±54.12	28.30±1.22	5796.61±376.76	38.84±3.43
Nanauti (*Puntius chonius*)	3745.45±38.13	41.81±2.11	7527.27±289.12	54.21±4.65
Chelwa (*Oxygaster bacaila*)	4000.00±32.84	19.23±2.61	8233.00±333.86	31.22±3.65
Kawai (*Etroplus surastensis*)	1753.42±21.33	30.54±3.12	4538.46±231.32	53.34±3.22
Botha (*Glossogobius giuris*)	2336.36±45.23	48.93±1.68	3452.05±321.65	62.63±2.76
Bamm (*Mastacembelus pancalus*)	3961.53±34.21	17.30±2.74	6424.24±221.11	96.80±2.12

All data expressed in ±SD of triplicate experiments

4. Conclusion

In current finding it is concluded that pond fishes are the good source of protein, amino acid and some antioxidants. It is well understood from the current study that each habitat group of fishes has its own nutritional value parameters with sense to their different food preferences.

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