Proximate Composition, Macro and Micro Mineral Elements of Some Smoke-dried Hill Stream Fishes from Manipur, India.

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Abstract : The proximate composition, macro and micro mineral element contents of some smoke- dried hill stream fishes *Neolissochilus stracheyi, Labeo pangusia, Semiplotus manipurensis, Schizothorax rechardsonii* and *Ompok bimaculatus* were determined. The range of moisture contents in all the fishes was between 9.36±0.01 % and 15.77±0.02 %. The highest protein level (71.31±3.11%) was found in *Schizothorax rechardsonii* and the lowest (30.51±2.19%) in *Ompok bimaculatus.* The highest lipid content (19.63±.88%) was found in *Ompok bimaculatus* and the lowest (6.68±.34%) in *Schizothorax rechardsonii.* The ash content was between 4.33±0.02-5.55±0.00 % in all fishes. The macro elements Ca, Mg, K, Na and P were abundant in all the fishes examined while micro elements Cu, Co, Zn, Fe, Ni and Cr were present in trace amounts. The toxic metal element Hg was absent and As, Pb and Cd were negligibly low . The P contents were most dominant in most fishes, with the highest in *Schizothorax rechardsonii* (973.15 mg/100g). The next dominant contents in most fishes were K, the highest being in *Semiplotus manipurensis* (284.24 mg/100g). Among the micro elements, Fe contents were dominant in most fishes examined. The fishes examined were good sources of minerals, proteins and other nutrients required for the balanced diet of human consumption.

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Key words: Proximate composition; macro and micro mineral elements; smoke-dried hill stream fishes; atomic absorption spectrometer.

1. Introduction

Fish are rich sources of essential nutrients required for supplementing both infant and adult diets (Abdullahi et al., 2001). Fish flesh contains most of the minerals necessary for a balanced diet. The studies of mineral elements present in living organisms is of biological importance since many of such elements take part in some metabolic processes and are known to be indispensible to all living things (Shu'lman, 1974).The body usually contains small amounts of these minerals, some of which are essential nutrients, components of many enzyme systems and metabolic mechanisms, and as such contribute to the growth of the fishes. The most important mineral elements are calcium, magnesium, potassium, phosphorus, iron and chlorine while many others are important in trace amounts. The deficiency in these principal elements induces a lot of malfunctioning, as it reduces productivity, and causes such as inability of blood to clot, osteoporosis, anemia (Shu'lman, 1974; Mills, 1980). In addition, one of the major pollution problems that poses serious health risk and environmental concern is that results from heavy metals. Generally fish has been regarded as a good source of protein and has been widely accepted to supplement the protein needs of the people especially in developing countries. Moreover, analysis of proximate composition of fishes is necessary for information of the concentrations of major component protein, lipid, ash and moisture.

Smoking is an important technique for the preservation of fish in the north eastern part of India (Vishwanath et al., 1997). Hill stream fishes are commonly found in smoke-dried forms in markets of Manipur. Fish are easily perishable after their capture and death. Due to the lack of transport and cold storage facilities in the hill areas, smoking method has been used from the early time for preserving the hill stream fishes. Methods of drying and smoking of fish vary between different countries and within the same country depending on the species of fishes used and the type of fish products desired (Opstvedt, 1988). The process may use unsalted fish or various salt addition giving salt concentration in the final products ranging from less 2% to over 20%. The variation makes it difficult to arrive at general conclusion regarding processing effects of smoking and drying on protein quality and the nutritional values of the final products (Opstvedt, 1988).

There are many reports on biochemical and

nutritional aspects of fishes by Singh et al., 1990; Lilabati & Vishwanath, 1993; Effiong et al., 2008; Ghosh et al., 2004; Luczynska et al., 2009; Pirestani et al., 2009 etc. However, there is no report so far on the hill stream fishes of Manipur. In the present paper, proximate composition, macro, and micro elements of some hill-stream fishes of Manipur are reported. So the aim of the work is to determine the mineral and proximate composition of the hill-stream fishes *Neolissochilus stracheyi, Labeo pangusia, Semiplotus manipurensis, Schizothorax rechardsonii* and *Ompok bimaculatus*.

2. Materials and methods

2.1. Reagents

Conc. HCL (suprapur), and high grade conc. HNO_3, Cu SO_4, lanthanum oxide, etc. were purchased from Merck, Germany. Deionized distilled water was obtained from Millipore system, USA. Nessler reagent was prepared using chemicals purchased from Merck, India. Standards of different elements were purchased from Merck, Germany. Standard samples were prepared according to instructions of Perkin-Elmer's methodology.

2.2. Equipment

Muffle furnace used was of Tempo Instruments & Equipments Pvt. Ltd., Mumbay, India. Crucibles and Microjeldhal flasks are obtained from India. UV–spectrometer was of Systronics company,India. Atomic absorption spectrometer was Perkin- Elmer, Model- AA200, USA, Washington.

2.3. Collection and Sampling

The smoked dry hillstream fishes *Neolissochilus stracheyi* (in standard length, 29-34.5cm), *Labeo pangusia* (18.5-22.5cm), *Semiplotus manipurensis* (27-28.5), *Schizothorax richardsonii* (21-20cm) and *Ompok bimaculatus* (18-21.5 cm) were purchased from the markets of hill districts of Manipur during the month of March to June. The collected fishes consisted of six fishes of each species. Each species of the fish sample was oven-dried in an electric oven at 60^{O}C until the sample had constant weight. Because of variations in composition of different regions, they were homogenized separately without including any bones and used for all analysis.

2.4. Analysis of Proximate Composition

The values of proximate composition were determined by the methods of AOAC(1957). Nitrogen was estimated by colorometrically in spectrophotometer. Protein values were calculated multiplying the nitrogen values of the fishes by 6.25. Lipid was determined following the method of Folch et al. (1957).

2.5. Mineral Analysis

2.5.1. Sample preparation

Samples for mineral analysis were prepared according to recommendations of Perkin Elmer's procedures of Atomic Absorption Spectrometer (1996)**.** 2gm muscle tissue of each fish sample was taken and dried at 135^0C for 2 hours and weighed. It was heated up to 500-550^0C in a muffle furnace and ashed overnight, cooled to room temperature. It was added 2 ml H_2NO_3 and evaporated to dryness and again heated to 500^0C for 1 hr to get clean carbon free ash. 10 ml H_2NO_3 was added and dissolved ash by heating continuously on a hot plate. It was transferred to a volumetric flask (50ml) and added HCl as necessary and diluted to volume with deionized distilled water (Millipore). All care was taken for cleanness and non-contamination.

2.5.2. Atomic absorption spectrometer analysis

Analysis of mineral elements Na, K, Ca, Mg, Mn, Cu, Co, Ni, Cr, Zn ,Fe Hg, Pb, Cd and As were done by Atomic Absorption Spectrometer (USA) with analyst version'06, 2007, PerkinElmer Inca., following the methods of Perkin-Elmer (1996). Most of the mineral elements Na, K, Ca etc. were done with hollow cathode lamps (HCL). Lanthanum oxide was used for Na, K to depress chemical interferences. Heavy elements like Hg, Pb, Cd and As were determined with electrodeless discharge lamps (EDL). Acetylene was used as fuel in the determination of elements with hallow cathode lamps. Phosphorus (P) content was determined using a spectrophotometer (Systronics company, India) using the ammonium molybdate method (AOAC, 1995).

2.6. Statistical Analysis

Three samples were used for each determination. The data were subjected to one way-ANOVA and the significant means were compared by Duncan's multiple range tests using SPSS version 12 .0 and the data are presented as means ± standard deviations.

3. Results

The proximate composition of the smoke-dried hill stream fishes *N. stracheyis, Labeo panguisa, Semiplotus manipurensis, Schizothorax richardsonii,* and *Ompok bimaculatus* are shown in table 1. The highest protein level (71.08±3.11%) was found in *Schizothorax richardsonii* which was followed by *Labeo pangusia.*

Medium levels of lipid content were found in fishes examined with highest level (19.63 ±0.88 %) in *Ompok bimaculatus.* The ash content varied between 4.33-5.65% in all fishes.

Table 1. Proximate composition of *Neolissochilus stracheyi, Labeo panguisa, Semiplotus manipurensis, Schizothorax richardsonii* and *Ompok bimaculatus*

Name of fishes	Moisture%	Protein %	Lipid %	Ash%
Neolissochilus stracheyi	15.77±.02e	57.373±3.18b	7.512±0.50c	4.43±0.02a
Labeo pangusia	12.20±0.08c	70.08±0.63d	8.487±0.07d	5.65±0.01d
Semiplotus manipurensis	13.40±0.50b	69.00±1.25c	7.348±0.14b	5.43±0.01c
Schizothorax rechardsonii	9.36±0.01a	71.08±3.11d	6.568±0.34a	5.44±0.028c
Ompok bimaculatus	15.54±0.04d	30.02±2.19a	19.63±0.88e	5.33±0.02b

Values are mean of three replicates.
Means (±SD) within a row followed by the different letters are significantly different (P≤0.05).

Table 2. Mineral Composition of macro elements (mg/100g).

Name of fishes	Ca	Mg	K	Na	P
N. stracheyi	9.35±0.75a	68.75±0.256b	236.25±2.15b	60.87±1.35b	106.48±0.69a
Labeo pangusia	9.70±0.55a	56.57±0.65a	234.25±1.77b	58.87±2.60b	322.22±30.12b
Semiplotus manipurensis	10.75±0.25b	81.00±0.66c	284.24±0.79c	65.87±0.88c	973.15±11.21e
Schizothorax rechardsonii	10.05±0.23b	97.50±0.25d	280.50±2.35c	38.50±0.59a	369.59±3.93c
Ompok bimaculatus	24.25±0.54c	106.75±1.86e	121.05±1.26a	103.12±1.36d	483.33±0.33d

Values are mean of three replicates.
Means (±SD) within a row followed by the different letters are significantly different (P≤0.05).

Table 2 shows the values of macro elements of the smoke-dried fishes. The highest level of calcium (24.25±0.54 mg/100g) was found in *O. bimaculatus* and lowest (9.35±0.75 mg/100g) in *N. stracheyi*. The highest and lowest K contents were 280.50±2.35mg/100g and 121.05±1.26 mg/100g in *Schizothorax rechardsonii* and *Ompok bimaculatus*. The highest magnesium level (106.75±1.86 mg/100g) was in *O. bimaculatus* and the lowest (59.57±.65 mg/100g) in *Labeo pangusia*. Sodium was highest (103.12±1.36 mg/100g) in *O. bimaculatus* and lowest 38.50±0.58 mg/100g in *Schizothorax rechardsonii*. Among the macro elements phosphorus content was most dominant in most fishes and the highest value (973.15±11.21 mg/100g) was in *Semiplotus manipurensis* and the lowest 106.48±0.69 mg/100g *in N. stracheyi*.

In the table 3, the values of the micro element contents of the hill stream fishes are shown. The trace element contents in the fishes examined recorded very low in trace amounts. Some trace elements were present below the level of detection in some fish samples. Fe content among the trace elements was dominant in most fishes. Fe content was highest (8.000±0.08 mg/100g) in *N. stracheyi* and lowest (1.711±0.36 mg/100g) in *O. bimaculatus*. Cobalt (Co) was present in all the fishes with the highest (4.868±0.06 mg/100g) in *Ompok bimaculatus* and the lowest (1.416±0.07 mg/100g) in *Schizothorax rechardsonii*. The values of chromium (Cr) were much lower than those of other trace elements in table 3. Lowest chromium content (0.035±0.00 mg/100g) was detected in *Labeo pangusia*. In some samples, the trace elements were not detected.

Table 3. Micro elements (mg/100g).

Name	Cu	Co	Mn	Zn	Fe	Ni	Cr
Neolissochilus stracheyi	2.565±0.01d	4.125±0.03e	1.000±0.01e	0.4375±0.00a	8.000±0.08e	1.375±0.00bc	0.200±0.01b
Labeo pangusia	0.500±0.00c	3.875±0.00c	0.500±0.00c	3.750±0.00e	5.969±0.53c	1.800±0.00d	0.035±0.00a
Semiplotus manipurensis	0.875±0.01e	2.187±0.025b	ND	2.062±0.00c	8.375±0.03d	1.500±0.00c	ND
Schizothorax rechardsonii	0.291±0.01b	1.416±0.07a	0.666±0.02d	2.229±0.01d	4.2708±0.04b	1.208±0.01a	0.200±0.00b
Ompok bimaculatus	ND	4.868±0.06d	0.1315±0.00b	0.921±0.01b	1.7105±0.36a	2.500±0.00e	1.115±0.00c

ND-Not Detected
Values are mean of three replicates
Means (±SD) followed by the same letter are not significantly different (P ≤0.05).

In table 4, toxic elements like Hg was below the detection level in all the fishes. Other toxic elements like As, Pb, Cd were present in very low detectable level.

Table 4. Toxic metal elements (mg/100g).

Name of fishes	Hg	As	Pb	Cd
Neolissochilus stracheyi	ND	0.011±.001a	0.130±.003b	0.030±.002c
Labeo pangusia	ND	0.005±.002a	0.015±.001a	0.014±.000b
Semiplotus manipurensis	ND	0.007±.001a	ND	0.008±.001a
Schizothorax rechardsonii	ND	ND	0.010±.000a	0.003±.0025a
Ompok bimaculatus	ND	0.012±.001a	0.170±.001b	0.015±.003b

ND- Not Detected
Values are mean of three replicates
Means (±SD) followed by the same letter are not significantly different (P ≤0.05).

4. Discussion

The moisture levels in all the fishes examined were below 20% which is good/ acceptable for smoked dried fishes (Lilabati, 1996). The relatively high to moderate percentage of crude protein could be attributed to the fact that fishes are good sources of pure protein, but the differences observed in the obtained values may also be attributed to fishes's consumption or absorption capability and conversion potentials of nutrients from their diet or local environment into such biochemical attribute needed by the organism's body (Burgress, 1975; Adewaye and Omotosho, 1997).

The ash content gives a measure of the total mineral content in the tissue (Nair and Mathew, 2001).The variability in the body composition of the fish has been attributed to several factors such as environment, age, size, diet and species (Reinitz, 1983).The composition of a particular species often appears to vary from one fishing ground to another, and season to season, but the basic causes of change in composition are usually variation in the amount and quality of food it eats and in the amount of movement it makes (Murray and Burt, 2009).

Calcium, magnesium, potassium, sodium, and phosphorus are abundant in all the fishes studied. The present study showed that hill stream fishes are good sources of mineral elements.

The present studies results are in agreement with the results of previous work. Akinneye et al. (2007) reported the values of the major elements were obtained in the decreasing order K>Na>Mg>Ca in *Sardinella* (oven dried), *H. niloticus*, (oven dried), *H. niloticus* (smoke dried) and *Sardinella sp* (smoke dried). In smoke dried samples, lower values of major elements were reported compared with other drying methods. Nutrition data

(2011) shows that the values of major elements of smoked heat dried carp were in the decreasing order P> K>Na>Mg>Ca. Gopakumar (2000) reported the values of major elements of Indian fishes in the above same order in most fishes. Their abundant presence may be due to the facts that the body needs these macro elements in more amounts than the micro elements in the structure and function of the body. In fish, calcium and phosphorus together accounts for 60-70% of the minerals in the skeleton. Apart from being a constituent of the skeleton, phosphorous has many roles in fish. It is present in adosine polyphosphates, the key substances for energy release and also in phospholipids (Nair and Mathew, 2001).

The highest values of the micro elements in these hill-stream fishes are in the decreasing order Fe>Co>Zn>Ni>Cu>Mn>Cr. In the results reported by Fafioye et al. (2008) of traditionally smoke- dried fresh water fishes, the order of magnitude of the three trace elements are Fe>Zn>Cu and the same order of magnitude was reported by Kinsella et al. (1977) in fillets of several species of fresh water fishes. Fawole et al. (2010) reported decreasing order of Zn>Fe>Ni>Cu>As in the studies of some fresh water species. The results of some fishes reported by Ghosh et al. (2004) and Nurulla et al. (2003) were in the decreasing order Fe>Zn>Mn>Co>Cu. Other researchers have similar observation with regards to lack of agreement of different reports on the order of magnitudes of mineral contents of a given species of fishes (Akinneye et al., 2007).

It may be right to say that mineral elemental contents of each species is function of the availability of these elements in their local environment, diet absorptive capability and as well as their preferential accumulation. The variation recorded in the concentration of minerals in fish muscles examined could have been a result of the rate in which they are available in the water body.

The functions of inorganic elements include the formation of skeleton structure, electron transfer, regulation of acid base equilibrium and osmoregulation. Minerals also are important components of hormones, enzymes and vitamins. They activate complex biochemical mechanisms, control, and regulate the uptake, storage and excretion of various inorganic elements allowing fish to live in a dynamic equilibrium with their aquatic medium (Committee on animal nutrition, 1993). The functions and values of these elements are many and varied and their deficiency causes diseases in the body. Iron has the longest and best history among all the micronutrients. It is key elements in the metabolism of almost all living organisms. Cobalt is known for its component of the vitamin B- complex. Numerous aspects of cellular metabolism are zinc dependent.

The toxic elements were in negligible levels. There was no detection of mercury(Hg) in the samples. This indicates the pure and clear water of water of hill streams ecosystems and absorption level of the elements by the fishes from the environment though these heavy metals pose sometimes serious health risks and environmental concern. There is no report of mercury pollution in these hill stream fresh water ecosystems though there were reports of mercury pollution in marine environments.

The studies therefore showed that smoke- dried hill stream fishes were still good sources of macro and micro mineral elements in spite of the processing effects of smoking. It may be noted that the mineral elements content of each species is a function of the availability of these elements in their local environment, diet absorptive capability and as well as their preferential accumulation (Fawole, 2007). However, it was discovered that micro elements recorded very low values, this may be due to the fact that the body demands them in trace amounts and that their concentration in the water body is very low. Moreover some minerals might have been lost during processing of the fishes.

5. Conclusion

The present studies has added information that the smoke-dried hill stream fishes are a good sources of protein, lipid, macro and micro mineral elements that may contribute to health, growth and development of human beings and a safe food from environment concern due to negligible level of toxic elements.

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