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Biochemical and mineral composition and microbial quality of some selected freshwater small indigenous species (SIS) in Meghna River of Bangladesh

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Abstract
Fish, in Bangladesh where malnutrition remains a significant development challenge, is an irreplaceable animal-source food in the diet of millions. Biochemical-composition, mineral-profile and microbial-quality of three fish species namely *Gudusia chapra* (chapila), *Xenetodon cancila* (kaika) and *Mastacembelus pancalus* (baim) inhabiting in Meghna River, Bangladesh was studied in order to evaluate their nutritional values. It was investigated that mean moisture, protein, fat, ash and TVB-N value were 76.41±0.02%, 10.53±%, 11.62±%, 1.53±% and 2.40± mgN/100g in chapila fish and 77.67±%, 14.27±%, 4.66±%, 2.94±% and 4.92±mgN/100g in kaika fish whereas 77.21±%, 15.17±%, 6.13±%, 1.72±% and 3.16± mgN/100g in baim fish respectively. The Total viable counts were in acceptable limits. The pattern of mineral composition in chapila, kaika and baim fishes, are in the order; Ca>Mg>Fe>Zn>Cu>Mn. The data showed that these species of fishes are rich in nutrient value that are not less than larger fish and meet the requirement for human nutritional needs.

Keywords: Small indigenous-species, Bio-chemical composition, Microbial-quality, Minerals

1. Introduction

Being a good source of animal protein and other nutrients fishes can help and restore as well as to maintain of healthy human body. Besides protein source, small indigenous species (SIS) of fishes are a rich source of vitamins and minerals, which is often overlooked in developing countries [1]. It also contains some bioactive compounds with therapeutic properties that are beneficial to human health [2, 3]. Fish account for 60% of the animal protein consumed by the population and also provide essential vitamins, minerals and fatty acids [4].

Majority of fish usually consists of about 70-80% of water, 20-30% of protein and 2-12% of lipid [5]. Fish meat contain the large proportion of protein, vitamin, minerals, healthy fats and in minor quantity carbohydrates which make it good and healthy diet and it is easily digested by human body [6]. Proteins are fundamental bio molecule in all aspects of cell structure and function. Nutritionally protein is the important constituent of fish.

Due to increase in the awareness about the importance and nutritive value of white meat, its demand increases all over the world even in Bangladesh. White meat, especially fish meat gained an important place in human diet due to its taste, nutritional quality and convenient price. The body nutrients composition and quality of nutrients in flesh of most fishes is reliant on their food type and feeding habit [7]. The values of these nutrients though, deviate extensively within and between species, and with size, sexual condition, feeding, time of the year and physical activity [8].

Small Indigenous Species of fish (SIS) defined as species attaining a maximum length of 25 cm [9]. In low income countries like Bangladesh, small fish are only consumed protein sources, as they are accessible, less expensive, affordable well liked, culturally acceptable and can be purchased in small quantities [9]. Small fish plays an important role in the everyday diet of Bangladesh, which are eaten along with the main staple dishes. This moment the average fish consumption is 60 g/day/person [10]. SIS have been considered as an important source of macro and micro nutrients which can play an important role in alleviating malnutrition problem that specially existing among the poor people both in urban and rural areas of Bangladesh.

Considering the nutritional benefits associated with fish consumption and the attendant risk associated with eating fish, it has therefore become important that the biochemical and mineral composition of some of the small indigenous species (SIS) of fish in Meghna river be assessed in order to ensure they meet the requirements of food regularities and commercial specifications and also establish the safety level of the fish species.

In this experiment, common freshwater lean fish chapila (*Gudusia chapra*), kaika (*Xenentodon cancila*) and baim (*Mastacembelus pancalus*) which have unique taste and have been considered as an excellent source of essential animal protein.

In view of above facts, the study was aimed to assess the Biochemical composition (moisture, protein, fat, ash contents and TVB-N value), mineral profile including Ca, Mg, Fe, Cu, Zn, Mn, Cu and microbial quality to compare the nutritional values of three selected different fishes, in order to help consumers in choosing fish based on their nutrient values which are commercially important and widely consumed.

2. Materials and methods
2.1 Sample collection
Three freshwater fish species; chapila (*Gudusia chapra*) and kaika (*Xenetodon cancila*) baim (*Mastacembelus pancalus*) was collected from fishermen of Meghna River early in the morning. Fresh mature fish samples were transported to laboratory in sterile polythene bag to avoid any type of microbial contamination.

2.2 Place of experiment
The whole experiment was carried out at the laboratory of Fish Technology and Food Microbiology Section of the Institute of Food Science and Technology (IFST) of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka and only mineral work was done in Center for Advanced Research in Science (CARS).

2.3 Preparation of fish samples
The experimental fish was quickly carried to the laboratory as early as possible to avoid any spoilage during that period. At first, the collected chapila fish was discaled while kaika and baim fish was beheaded. Then three fish samples were gutted and washed properly with clean water.

2.4 Sampling procedure
3 or 4 slice of 3 experimental fish species was taken randomly which represented the parts from whole body of the fish. Then the slices were chopped with skin and bone and finally ground with an electric blender to make a homogenous sample before being sampled for analysis. Triplicate experiments were conducted for this analysis.
Analytical methods were applied for the determination of biochemical composition of the raw fishes on experimental basis.

2.5 Biochemical analysis
Biochemical compositions of fish were determined by conventional method of AOAC (Association of Official Analytical Chemicals) on weight basis [11].

2.5.1 Estimation of moisture
About 5 gram of previously prepared fairly minced samples were taken into each known weight basin and weighed in a digital balance (Toledo, Switzerland). The samples were allowed to dry into the oven (Memmet 854 Schwabach) at 105^{0}C for 24 hours in order to remove the moisture until constant weight. After that, the basins are taken out of the oven, cooled in a desiccators and were weighed in a digital balance.

Calculation

$$\% \text{ of Moisture} = \frac{\text{Weight Loss}}{\text{Original Weight of Sample Taken}} \times 100 \quad (1)$$

2.5.2 Estimation of protein
The protein content of the fish was determined by micro-kjeldahl method [12]. It involves conversion of organic nitrogen to ammonium sulphate by digestion with concentrated sulphuric acid in a microkjeldahl flask. The digest was diluted, made alkaline with sodium hydroxide and distilled. The liberated ammonia was collected in a boric acid solution and was determined titrametrically.

Calculation

The percentage of nitrogen in the sample was calculated by the following equation:

$$\frac{\% \text{ of } N_2 (\text{titration reading - blank reading}) \times \text{strength of acid} \times 0.002 \times \frac{100}{5} \times 100}{\text{weight of sample taken}} \quad (2)$$

For most routine purposes the percent of protein in the sample is then calculated by multiplying the % of N_2 with the protein conversion factor of 6.25 for fish.

% of protein = % of total $N_2 \times 6.25$

2.5.3 Estimation of fat
About 5 g of the homogenous sample was taken into conical flasks and 10 ml of folch reagent (Chloroform: Methanol = 2:1) was added into the sample and homogenized properly and kept in air-tight condition for 24 hours. Fat contents of the fish muscle react with that solvent and remains in the solution. After 24 hours the solution of the flask was filtered in another weighed conical flask through a filter paper. Then these flasks were given in a hot water bath to dry up and removed the solvent. After that the flasks were kept into an oven for an hour to get the actual fat content. Then the flasks were weighed in an electronic balance to get the amount of fat content.

Calculation

$$\% \text{ of Fat} = \frac{\text{Weight of the residue}}{\text{Weight of sample taken}} \times 100 \quad (3)$$

2.5.4 Estimation of ash
About 4-5 g fish sample was weighed into a pre-weighed crucible. The crucible with the contents was heated first over a long flame till all the material was completely churned. Then it was transferred in the Muffle Furnace held at dark red at a rate of 600^{0}C for 5 hours until the residue become white. The crucible were cooled in desiccators and weighed. Finally the % of ash content was calculated.

Calculation

$$\% \text{ of ash} = \frac{\text{Weight of fish}}{\text{Weight of sample taken}} \times 100 \quad (4)$$

2.5.5 Estimation of TVB-N (Total Volatile Base Nitrogen)
TVB-N has been used as an index for the determination of freshness of fish [13, 14]. Volatile nitrogenous bases increase in concentration during the spoilage of fish [15]. The TVB-N measurement can be used as a parameter for the determination of microbiological and enzymatic spoilage of fish product. TVB-N value was determined by using Conway modified micro-diffusion technique [16]. Samples that were in the different levels of acceptability from highly acceptable condition to unacceptable condition had been selected for

TVB-N analysis. 25 ml of 10% Trichloro Acetic Acid (TCA) was added to 2 gm of ground fish sample and kept overnight and then filtrated with known volume. 2% boric acid, TCA, K2CO3 and the solutions made from the fish samples were taken into the Conway dishes. After the addition of Potassium Carbonate (K2CO3), each dish was covered by a piece of glass that was stacked with glue (Paraffin soft white) initially. Then it was kept for 24 hours. The samples and Potassium Carbonate (K2CO3) reacts to form NH3 which was absorbed by the boric acid and then the solution of each Conway dish had been titrated by N/70 H2SO4 with the help of a micro-burette. Finally TVB-N was calculated.

Calculation

$$\text{TVN} = (\text{titration reading} - \text{blank reading}) \times \text{Strength of acid} \times 0.2$$

$$\times \frac{\text{Volume of extract}}{\text{Volume of extract taken}} \times \frac{100}{\text{Weight of sample taken}}$$

2.6 Microbiological analysis

Enumeration of bacterial load was done using plate count agar by pour plate techniques. 10 g of the sample was mixed with 90 ml of previously sterile ringer solution. Appropriate dilutions of homogenate-fish samples was then transferred to petri dish and mixed with plate count agar and incubated at 37°C for 24 hours and the colonies were counted for total Plate count and the count was expressed as CFU/g [17].

2.7 Mineral determination

Samples for mineral analysis were prepared according to recommendations of Perkin Elmer's procedures of Atomic Absorption Spectrometer [18].

Statistical analysis

Data were analyzed by using SPSS for windows-20 statistical programme. Significance was established at $p<0.001$ [19].

3. Results & Discussion

3.1 Bio-chemical Composition: Bio-chemical composition of the fresh experimental fish, chapila (*Gudusia chapra*), kaika (*Xenentodon cancila*) and baim (*Mastacembelus pancalus*) is given Table-1.

Table 1: Biochemical composition and other features of the fresh experimental fish, chapila (*Gudusia chapra*), kaika (*Xenentodon cancila*) and baim (*Mastacembelus pancalus*)

Parameters	Chapila	Kaika	Baim
Moisture (%)	76.41±0.02^{a}	77.67±0.03^{b}	77.21±0.08^{c}
Protein (%)	10.53±0.03 ^{a}	14.27±0.03^{b}	15.17±0.03^{c}
Fat (%)	11.62±0.03 ^{a}	4.66±0.01^{b}	6.13±0.03^{c}
Ash (%)	1.50±0.03 ^{a}	2.94±0.04^{b}	1.72±0.03^{c}
TVB-N (mgN/100g)	2.40 ±0.03 ^{a}	4.92 ± 0.04^{b}	3.16 ± 0.02^{c}

Values are shown as mean of triplicate determination on ± standard deviation; a, b, c: Means significant differences between groups. Values with different superscript letter along a row are significantly different (p< 0.001).

3.1.1. Moisture (%) content

It was observed that the major component of the fishes were moisture. The moisture contents of chapila, kaika and baim fish in fresh raw condition was recorded as 76.41±0.02%, 77.67±0.03% and 77.21±0.08% respectively which are in accordance with the findings that moisture content of fresh water fish ranged from 70-80% [20]. Begum and Minar found the moisture content of G. *chapra, C. soborna, C.* (*Pseudentropius*) *atherinoides* is 76.01%, 77.91% and 76.60% respectively which were SIS and similar to the recent study [21].

3.1.2 Protein (%) content

In fresh raw condition the protein value (%) of chapila, kaika and baim 10.53±0.03%, 14.27±0.03% and 15.17±0.03% respectively. The variation of protein contents among the studied fish is not so high and it is due to mainly for species variation. The variation of protein content (except chapila) is more or less coincides with the findings of Mazumder *et al.* in *P. chola* and *P. atherinoides* [22]. The protein content in fish range with species due to certain factors such as the season of the year, effect of spawning and migration, food availability etc [23]. The percentage of protein in edible muscles of 20 Indian species was ranging from 8 to 20% which agreed well with the present findings [24].

3.1.3 Fat (%) content

The fat content of the experimented chapila, kaika and baim fish was 11.62±0.03%, 4.66±0.01% and 6.13±0.03% respectively. Fish can be grouped into four categories according to their fat content: lean fish (< 2 %), low fat (2 to 4 %), medium fat (4 to 8%), and high fat (> 8%) [25]. Kaika and baim can thus be classified as medium fat fish whereas the relatively high fat content observed for chapila. Among three freshwater experimental fishes, chapila has high fat content which is similar with the findings of Zurani *et al.* [26]. The percentage of lipids in aqua cultured sea bass (*Dicentrarchus labrax*) and sea beam (*Sparus aurata*) ranging from 6 to 15% respectively which is also in good agreement with the present findings [27]. The differences in fat levels in the fish tissues could have been due to the impact of food [28].

3.1.4 Ash (%) content

Ash is a measure of the mineral content of the fish species. It is the inorganic residue that is left after the organic matter has been burnt off [29]. Present findings revealed slightly lower value of ash content of the fish species as reported by Kamal *et al.* [30]. The value of ash in chapila, kaika and baim fish was 1.50±0.03%, 2.94±0.04% and 1.72±0.03% respectively. Srivastava and Balachandran who has got percent of ash ranged from 0.4-3.0 respectively which is similar with the present findings [31, 32]. The values of ash in chapila and baim were nearer to the values obtained by Mazumder *et al*. in *G. chapra* and *A. mola* [22]. The values of ash in kaika was similar to the findings of Begum and Minar in *P. monodon* [21] .The observed range of ash content in chapila, kaika and baim (1.50±.03%-2.94±.04%) indicates that the species are a good source of minerals such as calcium, magnesium, iron zinc copper and manganese.

3.1.5 Total Volatile Base Nitrogen (TVB-N)

TVB-N levels were monitored as the main parameter of fish muscle freshness. TVB-N is produced by decomposition of proteins into simpler substances (ammonia, trimethylamine, creatine, purine bases and free amino acids) [33]. TVB-N contents of fresh chapila, kaika and baim *fish* were 2.40±0.03, 4.92±0.04 and 3.16±0.02 mgN/100g respectively. According to EEC the TVB-N value of raw fish was much lower than the acceptable upper limits of 25-35 mg/100 g for some fish species [34]. This is in agreement with the initial TVB-N values of these three experimental fishes.

3.2 Microbial quality (TVC)

Bacterial growth is the main cause of fish spoilage; therefore it is logical to use bacterial number as an index of fish quality. In this study the Total Viable Count (TVC) of bacteria for fresh chapila, kaika, and baim fish were 3.1×10^5 CFU/g, 4.1×10^5 CFU/g and 5.8×10^4 CFU/g respectively (Table- 2). The acceptable limit for bacterial count is 5×10^5 CFU /g for fresh fish [35]. The total viable count of bacteria in this experiment with three fish species showed the values within the range of acceptable limit. For high quality fresh fish, the number of bacteria present on the surface varies from 10^3– 10^4 CFU/g which is in harmony with the present findings [36].

Table 2: Total viable count (CFU/g) in experimental fresh fishes, chapila (*G. chapra*), kaika (*X. cancila*) and baim (*M. pancalus*)

Chapila (*G. chapra)* CFU/g	Kaika (*X. cancila*) CFU/g	Baim (*M. pancalus*) CFU/g
3.1×10^5	4.1×10^5	5.8×10^4

3.3 Mineral composition

Minerals are important for vital body functions such as acid, base and water balance [37]. Mineral (mg/100g of Fish) composition in three experimental fish in fresh condition is presented in Table 3.

Table 3: Important minerals (mg/100g of Fish) in experimental fresh fishes, chapila (*G. chapra*), kaika (*X. cancila*) and baim (*M. pancalus*)

Important minerals	Ca mg/100g	Mg mg/100g	Fe mg/100g	Zn mg/100g	Cu mg/100g	Mn mg/100g
Chapila (*G. chapra*)	37	10	2.07	1.05	0.47	0.22
Kaika (*X. cancila*)	20	12	2.52	1.45	0.55	0.12
Baim (*M. pancalus*)	15	9	1.95	1.42	0.65	0.04

The mineral composition showed variable values in all the fishes analyzed; with Ca, Mg recording the most abundant while Fe, Zn, Cu and Mn recording the trace amounts. All the fish samples examined in this study contained appreciable concentrations of major elements (mineral) like calcium(Ca), Magnesium(Mg) and minor elements like Iron (Fe), Zinc(Zn), Copper (Cu) and Manganese (Mn) suggesting that these fishes could be used as good sources of minerals. Ca and Mg were observed to dominate other minerals in all fish samples.

Calcium is an essential mineral and it is present in the structure of bone. Calcium is present in a healthy human body at about 2% of body weight [38]. It is good for growth and maintenance of bones, teeth and muscles [39]. Normal extra cellular calcium concentrations are necessary for blood coagulation and for the integrity, intracellular cement substances [40]. As small indigenous species (SIS) of fishes are mostly eaten whole with bones, they are also a very rich source of highly bioavailable calcium. So, the amount of available calcium is high in chapila, kaika and baim fish .The concentration of Ca was found 37 mg/100g, 20 mg/100g and 15 mg/100g in fresh chapila, kaika and baim fish respectively.

Magnesium is an essential mineral element for organisms for oxididative phosphorrylation and activates many enzymes [38]. It is also maintain the electrical potential in nerves [41]. Magnesium content of chapila, kaika and baim was determined as 10 mg/100g, 12mg/100, 9mg/100g respectively.

A lot of minerals are necessary in low concentration for human, because they are essential elements such as Fe, Zn, Cu and Mn.

Iron content of fish is very low compared to that of mammals [42]. Fish is the major source of Fe in adults and children and its deficiency causes anaemia [43] .Iron has the longest and best history among all the minor elements. It is an important trace mineral for the metabolism of almost all living organisms, but its high concentration is toxic and inhibits the enzymatic function [43]. Fe was detected in all selected freshwater fish species and its concentration varied as 2.07 mg/100g in chapila, 2.52 mg/100g for kaika, 1.95 mg/100g in baim.

Zinc is another important mineral content and it is present active sites of many enzymes. It is also a natural component of many sea foods [44]. The presence of Zn in the fishes could mean that the fishes can play valuable role in the management of diabetes, which results from insulin malfunction [40]. The value of zinc was reported 1.05 mg/100g, 1.45 mg/100g and 1.42mg/100g in fresh chapila, kaika and baim respectively.

In present study, fresh chapila, kaika and baim fish had Cu content of 0.47 mg/100g, 0.55 mg/100g & 0.65 mg/100g; Mn content of 0.22 mg/100g, 0.12 mg/100g, 0.4 mg/100g respectively. Copper is essential trace elements for fish metabolism and important micronutrients in human diet [45]. Cu is a cofactor in a wide range of enzymes including cytochrome oxidase, superoxide dismutase and lysyl oxidase [42]. Among the three fishes highest Cu was found in baim (0.65 mg/100g) whereas lowest was found in chapila (0.47 mg/100g).

From the overall findings it was discovered that Mn and Cu recorded very low values in each species, 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. However 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 an as well as their preferential accumulation.

Variations in the concentration of minerals in fish muscles could be due to their concentration in the water bodies where they live, the fish physiological state and or the ability of the fish to absorb the elements from their diets and the water bodies [46, 47, 48]. This is supported by the findings of Ricardo *et al*., Adewoye *et al.* and Fawole *et al.* [49, 50, 7].

In this research work the order of mineral concentrations in chapel, kaika and baim were Ca>Mg>Fe>Zn>Cu>Mn. The highest values of micro elements in hill-stream fishes are in the decreasing order Fe>Co>Zn>Ni>Cu>Mn>Cr reported by Hei and Sarojnalini which is similar to the present findings [51]. The order of magnitude of the three trace elements are Fe>Zn>Cu in fillets of several species of freshwater fishes which was also in harmony with the present study [52].

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 [53]. The functions and values of these elements are many and varied and their deficiency causes diseases in the body.

4. Conclusions

The consumption survey confirmed that fish is an important part of diet for majority people in rural parts of Bangladesh. The results of present study implies that despite of some little variations, small indigenous species (SIS) of fishes like chapila, kaika and baim fish in Meghna river of Bangladesh, exhibited adequate quantities of protein, fat, ash and minerals. In countries like Bangladesh, where milk and milk-products make up only small amounts of the diet, small fish can be an important source of calcium. The enormous number of people in Bangladesh is poor and unable to afford large fish to their daily diet due to hickey price. SIS on the other hand is cheap and easily available. Consequently, it can be suggested that small fish species might be a good alternative in mass poor people of Bangladesh to meet their daily nutritional requirement in improving their health status. There is limited information on the nutritive value difference of fish species in Bangladesh. It is necessary to formulate guideline for common people to help them to plan better nutritional diet for good health. The outcomes of this study provide valuable information about biochemical composition of local food fishes to distinguish their nutritional value and make a choice based on that information from consumer point of view. Therefore further research has to be done to find out the nutritional values of these small food fishes for making a healthy society and for enhancing the nutritional awareness among the villager.

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