CHAPTER IV

Determination of Nutritional Composition of Fish Species

IV.1. Materials and Methods

IV.1.1. Sample collection and preparation

A total of nine fish species of the *Cyprinidae* family were collected from the *Hel* river, Serfanguri, Kokrajhar, Assam of North East India based on their abundance study. The collected fish species were washed properly with tap water followed by distilled water. The fishes were first gutted and washed again. Then it was dried for 2–3 days in the hot air oven at 55^{o}C. After drying, bones were removed and the muscles were collected. The muscles were pulverized with the grinder to a powdery form, kept in the air-tight sterile vessel, and then kept in the freezer (deep) at -20^{o}C for further studies. The used chemicals and solvents were of analytical grade which were utilized without further treatment.

IV.1.2. Determination of moisture content

The moisture contents of the selected fish species were determined by following the AOAC standard procedure (AOAC, 2000). In the process, 3 g of the dried sample of each fish species was heated for 3 h in the hot-air oven at 105^{o}C. It was then cooled in the desiccator, weight noted and the moisture quantity was studied using the formula given below.

$$\text{Moisture (\%)} = \frac{\text{(Weight of sample - Dry weight)}}{\text{Weight of the sample taken}} \times 100$$

IV.1.3. Determination of total solids

Total solid contents of the nine fish species were calculated using the equation given below (James, 1995).

$$\text{Total solid} = 100 - \text{Moisture (\%)}.$$

IV.1.4. Determination of ash

Ash contents of the fishes were determined employing the standard AOAC method (AOAC, 2000). In the process, the silica crucible (empty) was first heated in a muffle furnace, was cooled in a desiccator and the empty weight was measured till the constant value is obtained. The sample of 5 g was taken in the crucible. It was heated in the furnace at 550°C for 6 h and then cooled in a desiccator, the ash was weighted and its content was determined using the following formula.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of the sample}} \times 100$$

IV.1.5. Determination of crude protein

The crude protein contents of selected fish species were determined using the Kjeldhal method (K-360, Buchi Kjelflex) following the AOAC protocol (AOAC, 2000). Briefly, 0.5 g of the sample of fish was noted and firstly, the nitrogen (N) was assessed. The total protein content was calculated from the relation given below.

Protein (%) = Nitrogen (%) × 6.25 (conversion factor).

IV.1.6. Evaluation of crude fat

The fat contents of selected fish species were calculated using the AOAC procedure (AOAC, 2000). Briefly, 3 g of each sample was taken and extracted using petroleum ether with the help of the Soxhlet apparatus for 14 h. The crude fat extracted was separated from the solvent using a rotary evaporator and the weight of fat obtained is noted. It was calculated by using the following formula.

$$\text{Crude fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of the sample}} \times 100$$

IV.1.7. Calculation of total carbohydrate content

The total carbohydrate contents of the selected fish species were determined according to the equation given below (James, 1995).

Carbohydrate (%) = 100 – [Moisture (%) + Ash (%) + Crude protein (%) + Crude fat (%)].

IV.1.8. Nutritive value

The calorific value or nutritive value (kcal/100 g) of the fish species was determined with the help of the equation given below (FAO, 2003).

$$\text{Calorific value (kcal/100 g)} = 4 \times \text{Protein (\%)} + 9 \times \text{Fat (\%)} + 4 \times \text{Carbohydrate (\%)}.$$

IV.1.9. Investigation of minerals

The mineral (metal) contents such as sodium, potassium, calcium, magnesium, manganese, iron, copper and zinc of the fishes were estimated using the Atomic Absorption Spectrometer (Analytic Jena, GF-AAS, Vario-6) at SAIF, North Eastern Hill University (NEHU), Shillong. The fish sample was prepared by processing with conc. HNO_3 and the data obtained were expressed in mg/100 g of dry weight (DW).

IV.1.10. Statistical analysis

The data of the experiments of all analyses were presented as the mean of three (3) replicates ± standard deviation. The relative significant differences among the means were resolved by the one-way ANOVA *t*-test (p<0.05). Microsoft Excel and the OriginPro 8.5 software (MA 01060 USA, Origin Lab Corporation) were employed for the statistical calculations.

IV.2. Results and Discussion

IV.2.1. Proximate composition

The proximate analyses such as moisture, ash, protein, carbohydrate and fat contents per 100 g of dry weight (DW) are displayed in **Table IV.1**, which shows that all the fishes of this study have variable data of proximate composition. The lowest amount of moisture was observed in *N. hexagonolepis* (6.47 ± 1.31 g/100 g DW) and the highest being in *L. pangusia* (8.87 ± 1.46 g/100 g DW). Hei et al. (2012) reported the moisture of *L. pangusia* as 12.20 ± 0.08 g. The fresh sample of fish species showed moisture content that ranged from 73.16 ± 0.28 g to 82.58 ± 0.39 g, the lowest being in *T. putitora* and the highest in *L. pangusia*. Sarma et al. (2011) reported the moisture content of *L. pangusia* as 81.54 ± 0.78 g. Romharsha et al. (2014) and Sarma et al. (2014) also reported comparable moisture contents in their studies. The ash content varied from 1.28 ± 0.017 g to 3.20 ± 0.012 g, the lowest being in *B. barna* and the highest being in *N. hexagonolepis*, which are found to be comparable to the values of some cold-water fish species reported by Sarma et al. (2014). The ash value content is found to be similar to the results reported by Mohanty et al. (2016) and Mazumder et al. (2008). The total solid of fish species ranged from 91.13 ± 1.46 g (*L. pangusia*) to 93.53 ± 1.31 g (*N. hexagonolepis*). In this study, the amount of crude protein varied from 21.91 ± 0.012 g (lowest, *R. bola*) to 29.33 ± 0.249 g (highest, *G. gotyla*), and the crude fat quantity ranged from 13.51 ± 0.01 g in *B. barna* (lowest) to 29.85 ± 0.01 g in *C. semiplotum* (highest). Hei et al. (2012) reported higher crude protein and low-fat contents in some smoke-dried hill stream fish species compared to this study. Debnath et al. (2014) reported the higher protein content in some local fish species from Tripura, India that ranged from 39.37 % to 75.43 %. Sarma et al. (2014) reported comparable protein contents of some cold-water fish species from upland Himalaya. Linhartova et al. (2018) also reported similar protein contents in freshwater fish species of central Europe. Jena et al. (2018) reported the quantity of protein of some small indigenous fishes of Tripura, India that varied from 12.89 ± 0.14 to 16.75 ± 0.12 % of wet weight basis and fat content ranged from 1.84 ± 0.03 to 6.19 ± 0.12 % of wet weight basis. Fats and proteins are the important nutrients in fish and these compositions support in expressing the nutritional status of an organism (Jeyasanta and Patterson, 2014). Fats are needed in the diets for absorption of fat-soluble vitamins *viz*. vitamin A, D, E and K, and for regulation of metabolism of body cholesterol (Jabeen and Chaudhary, 2011). In present study, the total carbohydrate investigated was ranging from 38.55 ± 0.21 g (*C. semiplotum*) to 53.92 ± 0.57 g (*R. bola*). Jena et al. (2018) reported the carbohydrate data of indigenous fishes of Tripura, India that ranged from 0.68 ± 0.20 to 7.13 ± 0.40 % of wet weight basis. Nurnadia et al. (2011) and Teame et al. (2016) reported lower carbohydrate contents in the fish species of their studies. The maximum quantity of nutritive (energy) value of the fish species was noticed in *C. semiplotum* (521.38 ± 9.23 kcal/100 g) and the lowest quantity was noticed in *C. chagunio* (426.95 ± 4.53 kcal/100 g). Linhartova et al. (2018) reported the total energy values of freshwater fish species from central Europe that ranged from 444.57 ± 6.97 to 853.19 ± 65.32 kJ/100 g. Therefore, it was found that the proximate composition studies of different fish species of different places showed the different nutritive value of fish species and these may be due to different geographical environmental conditions, fish habited, fish feed, and various physiological conditions of the water. Boran et al. (2011) also suggested that the proximate composition of fish species greatly varies depending upon the catching season, and species having specific physiological characteristics might greatly affect the proximate composition.

Table IV.1. Evaluation of proximate composition of nine fish species per 100 g of dry weight

Fish species	Moisture (g)	Ash (g)	Total solid (g)	Crude protein (g)	Crude fat (g)	Carbohydrate (g)	Nutritive value (kcal)
FS-1	7.40±0.47^{a} 79.76±0.43*	1.51±0.042^{a}	92.60±0.47^{a}	22.48±0.005^{a}	27.04±0.016^{a}	41.57±0.42^{a}	499.56±1.83^{a}
FS-2	8.74±1.13^{b} 74.38±0.83*	2.86±0.032^{b}	91.26±1.13b	22.47±0.008^{a}	14.67±0.012^{b}	51.27±1.15^{b}	426.95±4.53^{b}
FS-3	6.59±0.55^{c} 82.04±2.02*	2.18±0.074^{c}	93.41±0.55^{c}	29.33±0.249^{b}	17.93±0.012^{c}	43.97±0.34^{c}	454.55±2.44^{c}
FS-4	8.87±1.46^{b} 82.58±0.39*	2.51±0.012^{c,d}	91.13±1.46^{b}	23.35±0.033^{c}	21.30±0.017^{d}	43.96±1.43^{c}	460.94±5.97^{d}
FS-5	6.47±1.31^{c} 78.99±0.39*	3.20±0.012^{e}	93.53±1.31^{c}	23.81±0.020^{c}	14.01±0.010^{e}	52.51±1.27^{d}	431.39±5.24^{e}
FS-6	6.79±0.57^{c} 78.72±0.15*	2.37±0.008^{d}	93.21±0.57^{c}	21.91±0.012^{d}	15.01±0.010^{b}	53.92±0.57^{e}	438.38±2.26^{f}
FS-7	7.43±0.68^{a} 73.16±0.28*	2.70±0.013^{c}	92.57±0.68^{a}	25.55±0.025^{e}	22.64±0.012^{f}	41.68±0.64^{a}	472.71±2.76^{g}
FS-8	6.92±0.34^{c} 80.56±0.4*	1.54±0.033^{a}	93.28±0.55^{c}	23.13±0.094^{f}	29.85±0.010^{g}	38.55±0.21^{f}	521.38±9.23^{h}
FS-9	8.20±0.14^{d} 79.93±0.53*	1.28±0.017^{a}	91.80±0.14^{d}	23.12±0.012^{f}	13.51±0.010^{h}	53.89±0.14^{e}	429.66±0.65^{i}

FS = Fish species; FS-1 = *Barilius bendelisis*; FS-2 = *Chagunius chagunio*; FS-3 = *Garra gotyla*; FS-4 = *Labeo pangusia*; FS-5 = *Neolissochilus hexagonolepis*; FS-6 = *Raiamas bola*; FS-7 = *Tor putitora*; FS-8 = *Cyprinion semiplotum*; FS-9 = *Barilius barna*; *Moisture content of fresh sample. Data expressed as mean of 3 replicates ± standard deviation; The values with different letters in a column are significantly different from each other at $p<0.05$.

IV.2.2. Mineral contents

The mineral compositions of the nine fishes per 100 g of DW are shown in **Table IV.2**. In this study, Na, K, Ca, Mg, Mn, Fe, Cu and Zn were determined. The highest amount of sodium was observed in *T. putitora* (346.68 ± 0.07 mg/100 g DW) and the lowest in *L. pangusia* (170.15 ± 0.03 mg/100 g DW). Hei et al. (2012) reported the sodium content of fish species that ranged from 38.50 ± 0.59 to 103.12 ± 1.36 mg/100 g. Sarma et al. (2011) also reported the sodium contents of the fish species which ranged from 100 ± 4.5 to 241 ± 31.1 mg/100 g DW. The highest level of potassium was detected in *Labeo pangusia* (1102.52 ±

0.05 mg/100 g DW) and the lowest in *Garra gotyla* (500.19 ± 0.08 mg/100 g DW). Hei et al. (2012) reported the potassium content which is ranging from 121.05 ± 1.26 to 284.24 ± 0.79 mg/100 g. Sarma et al. (2011) reported the potassium values that ranged from 784 ± 23 to 1246 ± 59 mg/100 g DW. In this study, the calcium content varied from 0.30 ± 0.012 mg (*G. gotyla*) to 0.64 ± 0.020 mg (*B. barna*). Calcium is known to be very much essential for the normal functioning of muscle, nervous system and formation of strong bones. The deficiency of calcium is linked to rickets in young children and softening of bones in adults and older people (Mohanty et al., 2012). The magnesium content ranged from 0.14 ± 0.006 mg in *C. semiplotum* to 0.31 ± 0.017 mg in *B. barna*. Sarma et al. (2011) reported the magnesium contents ranging from 0.076 ± 0.002 to 0.221 ± 0.015 mg/100 g DW.

Table IV.2. Estimation of mineral contents of nine fish species per 100 g of dry weight

Fish species	Na (mg)	K (mg)	Ca (mg)	Mg (mg)	Mn (mg)	Fe (mg)	Cu (mg)	Zn (mg)
FS-1	299.61±0.05[a]	836.44±0.03[a]	0.59±0.06[a]	0.31±0.08[a]	2.19±0.02[a]	8.98±0.02[a]	1.89±0.03a	1.06±0.04[a]
FS-2	222.50±0.02[b]	649.56±0.07[b]	0.60±0.02[a]	0.22±0.02[a]	1.63±0.10[b]	21.89±0.09[b]	1.90±0.02[a]	3.32±0.03[b]
FS-3	220.40±0.09[c]	500.19±0.08[c]	0.30±0.12[a]	0.14±0.09[a]	6.50±0.04[c]	13.26±0.42[c]	1.92±0.02[a]	1.56±0.06[a]
FS-4	170.15±0.03[d]	1102.52±0.05[d]	0.62±0.10[a]	0.20±0.06[a]	Nd	17.27±0.02[d]	1.50±0.04[a,b]	2.90±0.03[c]
FS-5	269.63±0.08[e]	834.41±0.21[e]	0.52±0.02[a]	0.18±0.03[a]	1.11±0.07[b]	21.44±0.08[b]	2.91±0.09[c]	1.46±0.07[a]
FS-6	227.17±0.06[f]	593.26±0.04[f]	0.44±0.02[a]	0.22±0.03[a]	1.06±0.09[b]	13.70±0.02[c]	0.523±0.03[d]	4.51±0.02[d]
FS-7	346.68±0.07[g]	982.32±0.13[g]	0.53±0.09[a]	0.24±0.07[a]	0.59±0.06[d]	19.98±0.08[d]	1.07±0.03[b]	3.57±0.05b
FS-8	292.25±0.12[h]	746.28±0.17[h]	0.60±0.02[a]	0.14±0.06[a]	0.82±0.01[a]	10.29±0.05[e]	1.49±0.01[b]	2.91±0.03[c]
FS-9	299.24±0.03[a]	665.66±0.21[i]	0.64±0.20[a]	0.31±0.07[a]	1.07±0.05[b]	5.56±0.07[f]	1.11±0.09[b]	1.90±0.09[a]

FS = Fish species; FS-1 = *Barilius bendelisis*; FS-2 = *Chagunius chagunio*; FS-3 = *Garra gotyla*; FS-4 = *Labeo pangusia*; FS-5 = *Neolissochilus hexagonolepis*; FS-6 = *Raiamas bola*; FS-7 = *Tor putitora*; FS-8 = *Cyprinion semiplotum*; FS-9 = *Barilius barna*. Data expressed as mean of 3 replicates ± standard deviation; The values with different letters in a column are significantly different from each other at $p<0.05$. Nd = Not detected.

In this study, the highest level of manganese was found in *Garra gotyla* (6.50 ± 0.04 mg) and the lowest being in *Tor putitora* (0.59 ± 0.06 mg). The highest iron content was found in *C. chagunio* (21.89 ± 0.029 mg) and the lowest was in *B. barna* (5.56 ± 0.071 mg). The levels of iron of 10 fish species from North Eastern Himalayan reported by Sarma et al. (2014) varied from 1.4 ± 0.2 to 21.0 ± 0.7 mg/100 g. Hei et al. (2012) reported the iron content in smoke-dried hill stream fish species of Manipur (India) that varied from 1.7105 ± 0.36 to

8.375 ± 0.03 mg/100 g. Debnath et al. (2014) also reported the iron content of some local fishes of Tripura (India) and it was found to vary from 9.18 mg to 99.77 mg per 100 g which is higher compared to the investigation of this study. The daily intake recommendation for iron is 8 and 18 mg for adult males and females, respectively (Islary et al., 2016). Iron is necessary for the synthesis of haemoglobin in red blood cells which is required for oxygen transportation to all parts of the body. Iron deficiency is linked to anaemia, impaired brain function and poor learning ability in children, and it also causes immune system dysfunction which is associated with an increased risk of infection (Mohanty et al., 2012). The highest level of copper was found in *N. hexagonolepis* (2.91 ± 0.029 mg) and the lowest in *R. bola* (0.523 ± 0.034 mg). Cu can play a very significant role in the process of biological electron transport and is indispensable for generation of enzyme in the body. The deficiency of Cu can cause abnormal glucose and cholesterol metabolism, reduced energy production, and increased oxidative damage (Islary et al., 2016). The highest zinc concentration was observed in *R. bola* (4.51 ± 0.029 mg) and the lowest was found in *B. bendelisis* (1.06 ± 0.044 mg). The zinc concentration found in this study was almost similar to that of the fishes of Bangladesh reported in the works of Bogard et al. (2015). Hei et al. (2012) also reported the zinc concentration of smoke-dried hill stream fish species of Manipur (India) that varied from 0.4375 ± 0.00 mg/100 g to 3.750 ± 0.00 mg/100 g. Sarma et al. (2011) reported the zinc contents which are ranging from 0.769 ± 0.006 to 1.497 ± 0.005 mg/100 g DW. The recommended dietary allowance of zinc for adult women is 8 mg per day and 11 mg per day for adult men (Islary et al., 2016). Zinc is an essential element for human growth which also increases resistance to infection, and excess intake of zinc and other heavy metals has been reported to be toxic (Salama and Radwan, 2005; Lilly et al., 2017). So, the knowledge of mineral contents is very essential for maintaining body function and the present study can help the local people to know about the nutritional compositions of fish species.

Conclusion

This study showed that the nine fish species from the *Hel* river of Assam, India are good sources of fats, proteins, high nutritive values and minerals. Hence, consumption of these species can be an alternative source of nutrients and can be included as a healthy human diet.