

Nutritional properties of some freshwater fish species of Manipur, India

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ABSTRACT

Proximate composition, total free amino acid polyunsaturated fatty acid, minerals and antioxidant properties of six fresh water fishes, viz., *Amblypharyngodon mola*, *Gagata dolichonema*, *Garra abhoyai*, *Glossogobius giurius*, *Hypsibarbus myitkyinae* and *Puntius sophore* were determined. They were collected from different sites of Manipur. The highest moisture, protein and ash content were recorded in *Puntius sophore*. The lowest moisture content was recorded in *Garra abhoyai* (68.25%). The lowest protein and lipid content was found in *Glossogobius giurius* (9.42% and 2.23% respectively). The lowest ash content was recorded in *Amblypharyngodon mola* (1.39%). The highest similar lipid content (5.83%) was found in two species (*Garra abhoyai* and *Gagata dolichonema*). The total amino acid content was found highest in *Hypsibarbus myitkyinae* (487.00 mg/100g) and lowest in *Puntius sophore* (196.53 mg/100g). The highest docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid, linolenic acid and linoleic acid were found in *Amblypharyngodon mola*. The maximum Fe, Cu, Zn and Mn were found in *Amblypharyngodon mola*. The highest Mg, K, Ca and antioxidant activity were recorded in *Puntius sophore*. The fishes studied observed that they are good sources of protein, minerals, antioxidant and PUFA. Thus the consumption of the fishes should be encouraged.

Keywords: Fresh water fishes, Total amino acid, PUFA, Minerals, Antioxidant property.

Introduction

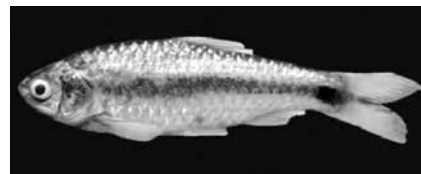
Fish is one of the important sources of animal protein and accepted to be nutritious food for their constituent of desirable component of human diet. Fish has also been an important item in the diet of the people of Manipur since time immemorial. Fish meat has received increased attention as a potential source of animal protein and essential nutrients for human diet and is regarded superior to other meats in having significantly low lipids and high digestibility. Compare to other source of protein, fish protein are known for the excellent source bioactive peptides with valuable nutraceutical and pharmaceutical potentials. The nutritional value of fish meat lies in its protein, lipid, vitamin and mineral contents and also in its caloric value (Higashi, 1962). The analysis of the major constituents (i.e., proximate composition) of fishes is necessary for providing information of the concentrations of protein, lipid, ash and moisture of the particular species (Stansby, 1962).

Beside excellent source of protein they are also an important source of vitamins, antioxidant as well as minerals including Calcium, Iodine, Selenium, Iron, etc. (Decker *et al.* 2001; Sarkar *et al.*, 2013) and also a good source of highly unsaturated fatty acid (HUFA) and polyunsaturated fatty acid (PUFA) especially omega-3 fatty acid viz.

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Huynh *et al.*, 2007). There are abundant evidences manifesting the significance of fish consumption in brain development and learning in child, protect vision and eye health and protection from cardiovascular diseases and some cancer (Sarkar *et al.*, 2013)

In Manipur, any types of big or small fishes are consumed in fresh or process (Sarojnalini, 2010). Small cobitid fish viz., *Syncrossus berdmorei* and *Lipidocephalichthys irrorata* endemic in this area are highly esteemed among the people (Sarojnalini and Vishwanath, 1988). There are some reports about the biochemical composition, minerals, antioxidant and PUFA of fishes (Chen and Decker, 1994; Sakuntala *et al.*, 1997; Vishwanath *et al.*, 1998; Larsen *et al.*, 2000; Roos *et al.*, 2003; Gokoglu *et al.*, 2004; Fatima *et al.*, 2007; Sarojnalini, 2010; Sarjubala and Sarojnalini, 2012; Sarojnalini and Sarjubala, 2014;).

So, the aim of the current study was to investigate proximate composition, minerals, antioxidant and PUFA of selected freshwater Fishes of Manipur as an extension to the previous worker on this field and to provide knowledge of nutritional value prior to human consumption.

Fig. 1 : *Amblypharyngodon mola*Fig. 2 : *Gagatado lichenema*Fig. 3 : *Garra abhoyai*Fig. 4 : *Glossogobius giuris*Fig. 5 : *Hypsibarbus myitkyinae*Fig. 6 : *Puntius sophore*

Materials and Methods

Sample collection

Some fresh water fish species (*Amblypharyngodon mola*, *Gagata dolichonema*, *Garra abhoyai*, *Glossogobius giurinus*, *Hypsibarbus myitkyinae* and *Puntius sophore*) were collected from different sites of Manipur (Fig. 1-6). The respective site of their collection has been shown in Table 1. And the length and weight of the fish species were also shown in Table 2. The fish samples were brought to the Fishery Research Laboratory, Department of Life Sciences, Manipur University, Canchipur with proper caring and were prepared for further analysis.

Table 1: Fish species and their respective collection sites:

Species	Local Name	Collection site
<i>Amblypharyngodon mola</i>	Mukanga	Ima Market
<i>Garra abhoyai</i>	Ngamusangum	Itok river, Yairipok
<i>Gagata dolichonema</i>	Ngarang/Ngayek	Iril river
<i>Glossogobius giurinus</i>	Nilongamu	Moirang Market
<i>Hypsibarbus myitkyinae</i>	Heikaknga	Moreh Market
<i>Puntius sophore</i>	Phabounga	Moreh Market

Table 2: Length-weight of the selected fish species:

Species	Total Length (cm)	Weight (gm)
<i>Amblypharyngodon mola</i>	04.46-4.90	01.46-01.51
<i>Gagata dolichonema</i>	07.90-09.80	05.31-07.00
<i>Garra abhoyai</i>	06.01-07.52	08.21-10.04
<i>Glossogobius giurinus</i>	13.20-14.60	20.35-24.38
<i>Hypsibarbus myitkyinae</i>	12.15-13.53	22.87-24.32
<i>Puntius sophore</i>	04.80-5.90	03.48-05.55

Proximate compositions

Proximate compositions (moisture, protein, lipid and

ash) were determined by using the methods of AOAC, 1995. Total protein values were obtained by multiplying the nitrogen value with 6.25 (Osborne and Voogt, 1978).

Total Free amino acids

Total amino acid were determined by the method described by Moore and Stein (1948). Calculation of the amount of total free amino acids was done by using standard curve prepared from leucine by pipetting out 0.1-1.0 ml (10-100 µg range) of working standard solution. Results were expressed in percentage equivalent of leucine.

Polyunsaturated fatty acids (PUFA)

The polyunsaturated fatty acids content were determined following the method of AOAC, 1995. 2ml of the sample solution was transferred to a reaction tube and the solvent was evaporated. Absolute ethanol and KOH glycol reagent are added and mixed thoroughly. The air in the tube was removed by heating at 180°C. A tube with only reagent and ethanol, which serves as a blank, was run with each set of samples.

Mineral Analysis

Mineral (Fe, Zn, Ca, Cu, Mg, Mn, K and Na) analysis was done following the method of Perkin-Elmer, 1996. Ash of respective sample was digested in HNO₃ making carbon free and make up to the volume of 50 ml distilled water and subjected to analysed the dissolved metal content.

Determination of Antioxidant activity

The antioxidant activity of the fishes extract was examined by comparing it to the activity of known antioxidants such as ascorbic acid by scavenging of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity. The free radical scavenging capacity of the extracts was determined using DPPH (Cuendet *et al.* 1997). Ascorbic acid was used as reference antioxidants.

Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA) and the significant differences between means of experiments were determined by post hoc Duncan's multiple range test. A significance level of 0.05 was chosen. Data were analysed using SPSS package (Version 17.0). Differences were considered significant at $P < 0.05$ (Sokal and Rohlf, 1974).

Results

The Proximate composition for the above selected fish samples were shown in Table 3. The moisture content was found to be highest in *Puntius sophore* ($76.35 \pm 0.09\%$) and lowest in *Garra abhoyai* ($68.25 \pm 0.39\%$). The crude protein content ranged from $9.42 \pm 0.47\%$ in *Glossogobius giurius* to $20.50 \pm 0.08\%$ in *P. sophore*. The relative lipid content varied from one fish species to another. The highest lipid content was recorded in *G. abhoyai* and *Gagata dolichonema* ($5.83 \pm 0.15\%$) and lowest in *G. giurius* ($2.23 \pm 0.15\%$). The ash content was found high in all the fish species except in *Amblypharyngodon mola* ($1.39 \pm 0.01\%$). The highest ash content was recorded in *P. sophore* ($4.38 \pm 0.03\%$).

The Total amino acid content of the fish species were shown in Fig. 7. The highest total amino acid was recorded in *Hipsibarbus myitkyinae* ($487.00 \pm 0.2 \text{ mg/100g}$) and lowest was in *Puntius sophore* ($196.53 \pm 0.14 \text{ mg/100g}$).

Further, polyunsaturated fatty acid (PUFA), minerals and antioxidant property were analysed for two species (viz., *Puntius sophore* and *Amblypharyngodon mola*). The polyunsaturated fatty acid (PUFA) consists of Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA), Arachidonic acid (AA), lenolenic acid and lenoleic acid. The polyunsaturated fatty acid content of fresh *Puntius sophore* and *Amblypharyngodon mola* are shown in Table 4. The highest DHA (2.9 mg/100g), EPA (0.12 mg/100g), lenolenic acid (1.4 mg/100g) and linoleic acid (2.3 mg/100g) were found in *A. mola*.

The mineral element content of *Amblypharyngodon mola* and *Puntius sophore* is shown in Fig. 8. The highest Fe (482 mg/100g), Cu (4.8 mg/100g), Zn (109 mg/100g) and Mn (35 mg/100g) were found in *A. mola*. Whereas, the highest Mg (228 mg/100g), K (208 mg/100g), Na (82 mg/100g) and Ca (902 mg/100g) were recorded in *P. sophore*.

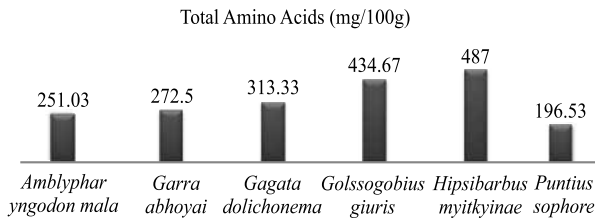


Fig. 7: Graph showing the Total amino acid content of the fish species.

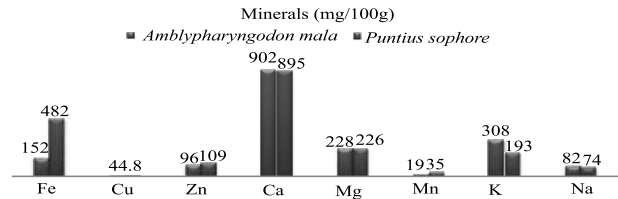


Fig. 8: Mineral content of *Amblypharyngodon mola* and *Puntius sophore* (mg/100g)

Table 3: Proximate composition of the selected fresh water fish species.

Species	Moisture %	Protein %	Lipid %	Ash %
<i>Amblypharyngodon mola</i>	74.69 ± 0.00	14.11 ± 0.00	5.82 ± 0.00	1.39 ± 0.01
<i>Gagata dolichonema</i>	72.52 ± 1.27	12.42 ± 0.84	5.83 ± 0.15	3.73 ± 0.74
<i>Garra abhoyai</i>	68.25 ± 0.39	10.56 ± 0.21	5.83 ± 0.15	3.35 ± 0.11
<i>Glossogobius giurius</i>	76.21 ± 1.11	9.42 ± 0.47	2.23 ± 0.15	3.77 ± 0.03
<i>Hipsibarbus myitkyinae</i>	74.12 ± 1.78	14.16 ± 0.57	5.00 ± 0.62	3.49 ± 0.13
<i>Puntius sophore</i>	76.35 ± 0.09	20.50 ± 0.08	2.46 ± 0.14	4.38 ± 0.03

The results are mean \pm S.D. of the samples taken in triplets.

Table 4. Polyunsaturated fatty acid content of fresh *Puntius sophore* (mg/100g).

Fish species	DHA	EPA	AA	Lenolenic Acid	Lenoleic Acid
<i>A. mola</i>	2.91 ± 0.00^d	0.12 ± 0.00^a	0.13 ± 0.00^b	1.4 ± 0.00^b	2.3 ± 0.00^d
<i>P. sophore</i>	0.75 ± 0.00^b	0.04 ± 0.00^a	0.06 ± 0.00^a	0.91 ± 0.00^a	2.19 ± 0.00^d

DHA=Docosahexaenoic acid; EPA=Eicosapentaenoic acid; AA=Arachidonic acid

Values are shown as mean \pm standard error of triplicates.

Values within the same row have different superscripts are significantly differences ($P < 0.05$).

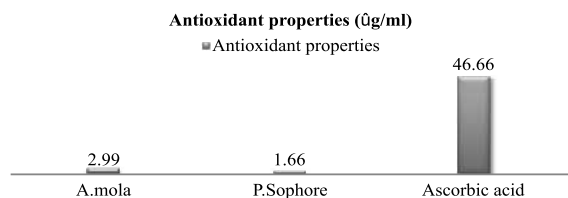


Fig. 9: Antioxidant properties of *Amblypharyngodon mola* and *Puntius sophore* ($\mu\text{g/ml}$).

The antioxidant activity of two fishes was shown in Fig. 9. The inhibition at 50% (IC_{50}) value of reference ascorbic acid was $46.66\mu\text{g/ml}$ and higher the IC_{50} value lesser the antioxidant properties. The antioxidant activity was highest in *Puntius sophore* ($1.66\mu\text{g/ml}$) and lowest in *Amblypharyngodon mola* ($2.99\mu\text{g/ml}$).

Discussion

Proximate composition

The moisture content in the present study is comparable with the values as reported by Sarojnani and Vishwanath (1988). They reported that moisture content was 71.00 to 80.00% in some fresh water fishes of Manipur. The relative lipid content varied from one fish to another. This might be due to their ecological niche and feeding habits. In many species, there is a build-up of fat during feeding season and its proportion decreases substantially after spawning (Nair and Mathew, 2000). The protein content in the present work was found in moderate to high percentage. The relatively high to moderate percentage of protein could be attributed to the fact that fishes are good sources of pure protein, but the differences observed in the present values might be due to the fishes' consumption or absorption capability and conversion potential of nutrients from their diet or local environment in to such biochemical attribute needed by the organisms body (Burgess, 1975). The higher ash content in the fresh water fish species might be due to its higher bony consistency and high scaly nature. Such fish offer minerals in their edible forms more abundantly than large-sized fish do (Higashi, 1962). And the total amino acid content is related with the increased or decreased in the availability of foods.

Polyunsaturated fatty acid content

Fish lipids are mostly distributed among liver, muscle and mesenteric fat. The results show the polyunsaturated fatty acid content of these two fishes. The present study shows that the total PUFA level varies among the species. In two samples 5 fatty acids were identified. Of the polyunsaturated fatty acids viz., DHA, EPA, AA, linolenic and linoleic acid were found varied. The most significant

($p < 0.05$) highest content of DHA, EPA, AA, linolenic and linoleic acid were observed in *Amblypharyngodon mola*. The EPA value was found lower in the two fishes than Tra catfish ($0.76\text{mg}/100\text{g}$) and Atlantic salmon ($61.12\text{mg}/100\text{g}$). This might be due to the species and also a number of factors can influence in fatty acid composition, such as water temperature, time of capture, salinity and feed type etc (Fatima *et al.*, 2007). The polyunsaturated fatty acids composition may vary among species of fish, even among fresh water and marine fish. The compositions of fatty acids are influenced by season, species, age, food availability and life cycle (AbdRahman *et al.*, 1995). The changes in amounts of EPA and DHA observed in the results were caused by changes in amounts of water and lipid content. The changes in lipid extractability had also some influence on the amount of measured EPA and DHA, what in turn directly affected percentages and composition of fatty acids (Chen and Zhang, 2006 and Mottram, 1998).

Mineral content

The Ca contents of two fishes ranged from $895\text{--}902\text{mg}/100\text{g}$. This value is higher than that reported by other authors Gokoglu *et al.*, 2004 and Lall, 1995. The Magnesium (Mg) contents of the fish ranged from $226\text{--}228\text{mg}/100\text{g}$. This value is higher than that reported by other author (Zamil *et al.*, 1992). The Fe contents of the fish ranged from $152\text{--}482\text{mg}/100\text{g}$. This value is higher than that reported by Hoffman *et al.*, 1994 and Gokoglu *et al.*, 2004. The Cu contents of the fishes were between $4\text{--}4.8\text{mg}$ and 100g . This value is higher than that reported by other authors (Hoffman *et al.*, 1994, Rosa *et al.*, 2007 and Gokoglu *et al.*, 2004). The Zn contents of the fish ranged from $96\text{--}109\text{mg}/100\text{g}$. This value is higher than that reported by Zamil *et al.*, 1992 in Flat needle fish ($0.41\text{mg}/100\text{g}$). The high levels of Zn might be due to contamination (Zamil *et al.*, 1992). The Na content of the raw fish was $74\text{--}82\text{mg}/100\text{g}$. This value is higher than that reported by Zamil *et al.*, 1992 in Flat needle fish ($72.8\text{mg}/100\text{g}$). The Mn contents of the raw fishes ranged from $19\text{--}35\text{mg}/100\text{g}$. This value is higher than that reported by Zamil *et al.*, 1992. The K content of the raw fish ranged between $193\text{--}208\text{mg}/100\text{g}$. This value is higher than that reported by other author (Beyza and Ozeren, 2009). However the present value was lower than that of Zamil *et al.*, 1992. Data from the literature indicate that the contents of macro- and microelements in fish depends on species and feeding type (Gladyshev *et al.*, 2001). The bioaccumulation of minerals (Zn, Cu, Mn, Fe) also depends on fish weight and body length (Anan *et al.* 2005) and age (Farkas *et al.*, 2003). Windom *et al.*, 1987 attributed such variations to the chemical forms of the elements and their concentration in the environment. The mineral content of fish makes fish unavoidable in the diet as it is a good source of different minerals that contribute greatly to good health.

The minerals were recorded variation in their concentration of sample (Eyo, 2001).

Antioxidant properties

The antioxidant properties of the raw fishes ranged from 1.66-2.99µg/ml. These values are lower than the ascorbic acid. Many proteins have been shown to have antioxidative activity against peroxidation of lipids or fatty acids. Kawashima *et al.*, 1979 investigated the effects of many synthetic peptides on lipid oxidation and found that some peptides having branched-chain amino acids (valine, leucine and Isoleucine) showed antioxidative activity. The peptides containing basic amino acids are electron acceptors that take electrons from radicals formed during the oxidation of unsaturated fatty acids (Chen and Decker, 1994). Shailaja *et al.*, 2012 reported the seer fish protein has exhibits the antioxidant activity. Amino acids are also suggested to have antioxidant properties as reaction products with carbonyls from oxidizing lipids. Various studies have shown that reactions between oxidized lipids and amino acids produce many non-enzyme browning reaction compounds, which exert antioxidative properties (Alaiz *et al.*, 1995; Alzaiz *et al.*, 1996 and Ishtiaque *et al.*, 1996).

Conclusion

The results suggest that the studied fresh water fishes have high protein, low lipid, high mineral content, antioxidant activity and high PUFA. The Fatty acids like DHA, EPA have beneficial effects on diseases such as coronary heart disease and may also contribute to various health benefit, growth and development for human. It also provides the importance of Fresh water fishes for their high nutritive value ahead of consumption. And in many low-income states like Manipur, fish is important for livelihoods, income and as food for the rural poor who suffer from malnutrition, including micronutrient deficiencies. So, it can play role in nutritional security for the rural people of low-income groups. Further works are still undergoing in the nutritional importance of the fresh water fishes and its contribution to human health and economy of the state.

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