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RESEARCH ARTICLE

STUDY OF MINERAL CONSTITUENTS, PROXIMATE COMPOSITION OF FISH FLESH AND PHYSICO-CHEMICAL CHARACTERISTICS OF FISH LIPID IN *PUNTIUS GONIONOTUS*

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ABSTRACT

The present investigation was carried out to know the proximate composition of flesh of the fish *Puntius gonionotus*. Lipid from fish flesh was extracted and some analytical tests of the crude lipid were carried out to ascertain some of its physical and chemical characteristics of the fish. The fish was found to contain moisture (71.51±0.93) %, protein (22.21±0.92) mg/100 gm, lipid (5.24±0.64) mg/100 gm, ash (1.02±0.5) mg/100 gm. This study also revealed that the minerals Sodium (76±2.16 mg), Potassium (294±2.00 mg), Magnesium (48.1±1.83 mg), Calcium (276±4.54 mg), and Phosphorus (210±1.80 mg) content of this fish are relatively richer than other small indigenous fishes. The physical and chemical characteristics viz. density (g/cc), refractive index, iodine value (I_2/100 g), saponification value (mg/KOH), saponification equivalent, acid value (mg/KOH), ester value, peroxide value (mEq/Kg), % of free fatty acids (as oleic acid), total cholesterol (mg/dl in lipid) of lipids have also been reported.

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INTRODUCTION

Minerals and vitamins are important micronutrients to sustain normal life and they help to provide a normal life and other chemical reactions in the body. Minerals regulate osmotic balance and aid in bone formation and integrity. In tropical countries, addition of sodium chloride is of great importance because of the loss of NaCl in sweat (Deb, 1990).On an average, a man excretes daily about 20 to 30 gms of minerals salts and these out puts must be made by the intake through food stuffs. *Puntius gonionotus* is preferred by the people of our country for their delicious tastes. Many minor and trace elements such as sodium, potassium, calcium, iron, iodine, zinc, magnesium and phosphorus may be present in this fish species(Hossian*etal*.,1999).

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Thelipids are important constituents of the diet because of their higher energy value and also because that the fat-soluble vitamins and the essential fatty acids are found within the fat of the normal food stuffs (Corinne, 1980). In the human body, fat serves as efficient source of energy which is stored in the adipose tissues. High accumulation of fat in body develops heart disease, breast's cancer and obesity (Steven and Helfrich, 2002). Fishes are the good natural sources of dietary fat, protein, vitamins and minerals. ω-3 poly unsaturated fatty acids (the type of fat found in fish oil) have been used to kill or slow down the growth of cancer cells in the culture media and model animals and to increases the effectiveness of cancer chemotherapeutic drugs (Robinson, 1980, William, 1966). There is substantial evidence that diets rich in polyunsaturated fatty acids (FUFAs) such as those found in fish oil (eicosapentaenoic acid and docosahexaenoic acid) protect against colon carcinogenesis. Polyunsaturated fatty acids (FUFAs) especially linoleic acid is necessary for the proper functioning of many metabolic processes (Murry and Burt,

1969, Murray *et al.*, 1996.). In view of these recognized usefulness of this fish with respect to lipid characteristics, the present investigation was undertaken. The present work reported the proximate composition of fish flesh; extraction of lipid from fish flesh and to carried out analytical tests of the crude lipid to ascertain some of its physical and chemical characteristics of the fish *Puntius gonionotus*. The need for through and long-term investigation on the nutritional value of this fish is urgently needed. Considering the importance, this study was under taken to assess the nutritional value of this fish, available in Bangladesh.

MATERIALS AND METHODS

Standard medium sized alive *Puntius gonionotus* were collected from the local market of Shaheb Bazar, Rajshahi, Bangladesh. Keeping into ice, the samples of fish were brought into the laboratory for analysis within hours of collection. Finally, they were cleansed by discarding their scales, bones, stomach, viscera, fins etc. and only the body flesh of the fishes were preserved in frozen condition in a refrigerator.

The fish flesh of *Puntius gonionotus* was homogenized in a micro cutter and then mixed uniformly with pre-cooled petroleum in a homogenizer and filtered through a clean muslin cloth. The process was repeated at least twice in order to obtained lipid free homogenate. Finally, filtrate was clarified further by centrifugation at 8×10^3 rpm for 10 minutes. Then these precipitate was air dried at room temperature. Ash was prepared from this defatted fish sample by dry ash method. For this purpose, defatted homogenate fish sample (about 5g) was heated in pre-weighted crucible to a white ash 600^{o}C. This was done for four hours in a muffle furnace. The ash was dissolved in a minimum quantity of concentrated HNO_3 and warm water. The diluted sample was then filtered and adjusted to a known volume (100ml) preferably, so that the final HNO_3 concentration is about 1%. Small portion of this solution was taken for Na, K, Ca, Mg, Cu and Zn estimation. To determined the amount of Phosphorus, wet oxidation was performed by adding acid mixture (HNO_3: H_2SO_4 : $HClO_4$ = 5 : 1 : 2) into fish flesh (2.02g). Then it was allowed to predigest at least for two hours and again digest until the content became colorless (AOAC, 1995). Atomic absorption spectrophotometer method was used for the determination of Na, K, Ca, Cu and Zn contents in the species and Fe and P were determined by spectrophotometric method (Sharma, 1997-98). Metal free (deionised water) water was used for preparing all reagents and calibration standards and as dilution water. Lipid was extracted from *Puntius gonionotus* by Bligh-Dyer method (Bligh and Dyer, 1959). A mixture of chloroform and methanol (2:1 v/v) was used in this process. Standard methods were used for determining moisture, ash, dry solid substance, crudefibre of this fish flesh. The protein content of this fish was estimated by Micro-kjeldahl's method. The estimation of nitrogen was done by kjeldahl method which depended upon the fact that organic nitrogen when digested with sulphuric acid in presence of catalyst (copper sulphate) was converted to ammonium sulphate. Ammonia liberated by making the solution alkaline was distilled into a known volume of a standard acid. Collected ammonia was then measured by back titration. The percentage of nitrogen was calculated from the following formula:

$$\% N = \frac{(V_2-V_1) \times 0.01401}{W} \times 100$$

Where,

V_2 = Volume in ml of standard HCl used in the actual titration.
V_1 = Volume in ml of standard HCl used in the blank titration.
N_A = Normality of the HCl and
W = Weight of the sample in gram.

The total nitrogen content when multiplied by a factor 6.25, afforded the amount of total protein in the sample, the physical and chemical contents of the lipid was determined by standard methods (William,1966, Lehninger *et al.*, 1993).

RESULTS AND DISCUSSION

The macro and micro minerals constituents in this fish are show in Table-I and Table-II. The study revealed that the nutritional components of this fish were relatively rich in minerals like calcium, potassium, magnesium, phosphorus, sodium, iron, copper and zinc. Utilization of this fish in our diet may be considered as an important strategy to ensure the supply of minerals particularly to the communities who do not have enough purchasing capacity. The fresh fish flesh contained 76.01±2.16 mg/100g sodium which implies that this fish has a role in regulating plasma volume, acid base balance, nerve and muscles function and many other purposes within the cell (Gangong, 1991). Sodium content per 100g fish is varied from 30-150 mg for different fish (Gosal, 1997). Potassium plays an important role in the regulation of acid-base balance in the cell and also regulates the transmission of the nerve impulse and the contraction of the muscle (Deb, 1990). The amount of potassium found in *P. gonionotus* fish potassium was 294±2.01 mg. Potassium content in different fish species is varied from 250-500 mg (Gosal, 1997). Thus this fish may be considered as a great supplement source of potassium. Calcium along with phosphorus is essential for the formation and development of bones and teeth. Calcium in normal ratio with potassium maintains the normal activity of muscle (Deb, 1990). An estimated 99% of the body calcium resides in the teeth and bones. It is seen that *Puntius gonionotus* contained 276±4.54 mg calcium. From this result it is seen that this fish species is a rich source of calcium. Phosphorus is required for the formation of phospholipids, nucleic acid and phosphoproteins. It is also essential for utilization of calcium in the body and in the assimilation of carbohydrate and fat (Deb, 1990). Phosphorus content in *Puntius gonionotus* was found at 210±1.80 mg which is within FAO range of 19-502 mg/100g. Magnesium is a component of bones. Magnesium content of this fish was found to be 48.10±1.83 mg. The magnesium content in different fish is between 10-60 mg, within the FAO range of 4.5-452mg/100g. The recommended daily intake of magnesium for adults is 220-260 mg (FAO, 2001).

The function of iron is mainly in the transport of oxygen to the tissues (haemoglobin). It is also involved in the process of cellular respiration (Deb, 1990). A well balanced diet for growing children or for adults should contain sufficient amount of iron to meet the iron deficiency of the body and to allow for possible regional and seasonal variation in the iron contents of food. *Puntius gonionotus* contains 1.70±0.16 mg iron. The recommended nutrient intake of iron for female adults between the ages of 19-50 years is 24 mg/day. From the literature review it was found high variation in the concentration of iron from country to country. Copper is required for melanin formation and also for haemoglobin, phospholipids and

collagen synthesis. Again, Zinc is a part of many enzymic biomembranes and is involved in RNA transcription, among other activities. Zinc deficiency is linked to stunted grown and delayed sexual maturation (EPA U. S., 1999). From this study, it is found that *Puntius gonionotus* contains (1.01±0.20) mg copper and (3.80±0.82) mg zinc. For adults, the daily requirements of Copper and Zinc were 1.5-3.0 mg and 12 mg,

Table 1: The macro minerals (mg/100g) constituents in *Puntius gonionotus* fish flesh

Macro minerals	Value (mg/100g)
Ca	276.00±4.54
K	294.00±2.01
P	210.00±1.80
Na	76.01±2.16
Mg	48.10±1.83

Table 2. The micro minerals (mg/100g) constituents in *Puntius gonionotus* fish flesh

Micro minerals	Value (mg/100g)
Fe	1.70±0.16
Cu	1.01±0.20
Zn	3.80±0.82

Table 3. Proximate compositions of the fish flesh of *Puntius gonionotus*

Moisture content	**(71.51±0.93)%**
Protein content	(22.21±0.92)%
Lipid content	(5.24±0.64)%
Ash content	(1.02±0.05)%
Crude fiber content	(4.655±0.32)%
Dry matter content	(28.50±0.65)%

Table 4. The physical constants of *Puntius gonionotus* fish lipid

Physical constant	value
Density (g/cc)	0.9516
Refractive index	1.4612-1.4635

Table 5: The chemical constants of *Puntius gonionotus*

Chemical constants	Values
Iodine value (I_2/100g)	108.72
Saponification value (mg KOH/g)	186.52
Saponification equivalent	296.52
Peroxide value (m Eq/Kg)	1.78
Ester value	235.29
Acid value (mg/KOH)	1.96
% of free fatty acids (as oleic acid)	1.24
Total cholesterol (mg/dl in lipid)	1.25

respectively (Murray *et al.*, 1996). In the present investigation the lipid, protein, proximate compositions and other related substances in the fish fillet of *Puntius gonionotus* were recorded. The results have been shown in the following Tables-III. Moisture is the essential for most of the physiological reactions in the plant and animal tissue and in absence of moisture, life does not exist. The moisture content of this fish was found to be 71.51±0.93% and this value is less than other common fishes in Bangladesh. Protein is much more expensive feed ingredient than lipid. Protein content of fish varies widely, depending on facts such as natural feeding habits and availability of feed, fasting during spawning, migration etc. (Lilabati and Vishwanath, 1996.). The protein content of *Puntius gonionotus* in the present study was found to be 22.21±0.92. The protein content showed that the species is very rich as a source of protein as compared to the average value of other different fish species like *Anguilla bengalensis*, *Rita rita* (Molla *et al.*, 2003) etc.

The lipid content of the fish *Puntius gonionotus* was (5.24±0.64) %. The physical and chemical characteristics of the oils and fats varied within a small so that they seem to be constant. Although the chemical constants are more important to characterize a lipid, but physical constants are also often capable of giving valuable information. The ash figure can be regarded as a general measure of quality and often is useful criterion in identifying the authenticity of the quality of a food. The ash value of *Puntius gonionotus* was found to be 1.02±0.05 %. The ash content is of significance in measuring of mineral contents of the sample suggests the presence of an inorganic adulterant (Boekenoogen, 1964). The percentage of dry matter and crude fiber of this fish were estimated and found to be (28.50±0.65) % and (4.655±0.32) % respectively. The physical and chemical constants of the fish lipid are given in Tables IV and V. The density of the oils varies with their type and temperature and also treatment process. The density of this fish lipid was 0.9516 g/cc and was less dense than water whose density is 1.00 g/cc. The specific gravity and apparent density of each kind of oil lie within a fairly narrow range if the determinations are made at a standard temperature and the figures are thus of diagnostic value in assessing the degree of purity of an oil (Willam, 1966). The refractive index of fats and oils depends to some extent on their unsaturation (Peach and Tracy, 1955). The refractive index of the investigated *Puntius gonionotus* fish lipid was found to be 1.4635 which is much closed to that of other edible oils (Tasic *et al.*, 1999).

Iodine value gives an estimation of the degree of unsaturation and the relative amounts of unsaturated fatty acids in the triglyceride molecules of the fat. It may be suggested that the oil under investigation may be contained higher amounts of unsaturated fatty acids as its iodine value was calculated to be 108.72 I_2/100 g of the sample. The saponification value of the lipid of *Puntius gonionotus* was found to be 186.52 mg KOH/g, when the saponification equivalent of the fish lipid was observed to be 296.52. It is directly proportional to the average chain length of fatty acid present. Fats or oils consisting largely of C_{18} fatty acids, along with some myristic acid, palmitic acid, a little unsaponifiable matter and a low free acid, generally have a saponification equivalent of around 296.52; a higher value indicates the appreciable quantity of fatty acids (Molla *et al.*, 1994). The present result found for *Puntius gonionotus* was 296.52 indicated that the fish lipid contained mainly fatty acids of C_{18} molecular weight along with some longer chain fatty acids. The unsaponifiable matter in this fish lipid was found to be 1.85% which indicates that the fish lipid also contained sterols, tocopherols, hydrocarbons etc. The peroxide value of the lipid of *Puntius gonionotus* was determined and was found to be 1.78 mEq/kg where as the standard value of fish oil is less than 10 mEq/Kg (Boekenoogen, 1964). Oils with high peroxide values tend to have strong bad odour and taste, so can be oil with low quality. The specific limits of high quality oils are peroxide value of less than 3 when the oil leaves factory. The peroxide value was less than 5 before it is encapsulated and less than 10 following encapsulation (Pokorny *et al.*, 1973).The peroxide value indicates that the fish lipid was in fresh condition. The ester value of the fish lipid was found to be 235.29. The acid value and percentage of free fatty acid (as oleic) of the lipid of *P. gonionotus* were estimated and the amounts were found to be 1.96 mg/KOH and 1.24% respectively which were within the standard value of 5 mg/KOH and 0.5-1.5 respectively. The low percentage of free fatty acid (bellow 1.15%) is an indication of suitability of the lipids for edible purpose.

Cholesterol is an important constituent of living cells and is needed for digestion of fat. The amount of cholesterol of the lipid of this fish was determined and was found to be 1.25% (1250 mg/dl). Form this result, it can be suggested that the lipid of *Puntius gonionotus* is more suitable for edible purposes with the existing cholesterol level.

Conclusion

Lipids are used for long-term energy requirements during periods of extensive exercise or during periods of inadequate food and energy intake. The percentage of lipid yield from *Puntius gonionotus* is lower in fat than most of other common fishes in Bangladesh. The proximate composition of fish flesh, extraction of lipid from fish flesh and analytical tests of the crude lipid were investigated and most of the analytical results obtained were tolerable to the standard values. Based on the improved characteristics of the lipid, it could be suitable for applications in pharmaceutical and food industries and there is every reason to believe that this lipid could be used as an edible one, since it has properties very similar to those of other edible fish oils. The macro and micro mineral constituents of the fish can be used to eliminate micro nutritional deficiency and malnutrition problem among the common people.

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