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Comparative Characterization of Lipids and Nutrient Contents of *Pangasius Pangasius* and *Pangasius Sutchi* Available in Bangladesh

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Abstract

The aims of this study were to characterize the lipids isolated from *Pangasius pangasius* and *Pangasius sutchi* and to analyze the nutrient contents of these fishes. The specific gravity, refractive index and viscosity co-efficient were (0.97 ± 0.01 and 0.94 ± 0.02 at 30°C), (4.88 ± 0.25 and 5.49 ± 0.30 at 30°C) and (448.96 ± 2.5 and 421.76 ± 2.1) for *P. pangasius* and *P. sutchi*, respectively. Likewise, Chemical characterization of lipids of both fishes has shown the significance results. Besides, *P. pangasius* and *P. sutchi* contained carbohydrate, protein, cholesterol and lipids of 1.89 ± 0.35% and 0.75 ± 0.11%, 35 ± 2.1% and 38.37 ± 2.5%, 17.46 ± 1.15% and 13.79 ± 0.37%, and 10.01 ± 1.11% and 6.16 ± 0.54%, consecutively. The percentages of moisture, dry matter and ash were found in fair amounts. Furthermore, the minerals of *P. pangasius* such as calcium, phosphorus and zinc contents were lower and iron contents were higher than *P. sutchi*. The fatty acids profile of *P. pangasius* and *P. sutchi* were identified as lauric acid (13.36 and 4.26%), palmitic acid (26.15 and 29.32%), oleic acid (46.07 and 59.16%) and stearic acid (14.40 and 7.24%), respectively.

Keywords: *P. pangasius*; *P. sutchi*; Lipids; Fatty acids; Nutrients

Introduction

Bangladesh is a country with hundreds of rivers and ponds and is notable for being a fish-loving nation, acquiring the name "Machh-e Bhat-e Bangali" which means, "Bengali by fish and rice". Most of the people in the developing countries are dependent on fish as a source of animal protein. It has been estimated that about 80% of the animal protein in our diet comes from fish alone [1]. *Pangasius pangasius* and *Pangasius sutchi* are fresh water fish species of Bangladesh. In Bangladesh, this fish is known as "Pangas". Bangladesh, located as it is in the delta of three mighty rivers: the Ganges, the Brahmaputra and the Meghna which has vast inland water resources in the forms of ponds, beels and haors (natural depressions), boors (ox-bow lakes), canals, rivers, flood plains, reservoirs and impounded brackish water. This fish species are available in the fresh water stream of North America, Europe and Southern part of Asia (especially in South Asia; India, Burma, Pakistan, Srilanka) and Africa. Fish lipids are the main sources of polyunsaturated fatty acids (PUFAs) especially eicosapentaenoic acid (EPA; $C_{20:5}$) and docosahexaenoic acid (DHA; $C_{22:6}$) [2]. These two fatty acids cannot be synthesized by the human body (essential fatty acid) and must be obtained from the diet. Lipids and fatty acids also play a significant role in membrane biochemistry and have direct effect on the membrane-mediated process in human such as osmoregulation, nutrient assimilation and transport [3]. The fish's *P. pangasius* and *P. sutchi* contain polyunsaturated fatty acids (PUFA), which play important roles in cardiovascular system to reduce the risk of heart attack.

Fresh fish is a central point in fish for food utilization. The knowledge of fish composition is essential for its maximum utilization. The nutritional composition of fish varies greatly from one species and individual to another, depending on age, feed intake, sex and sexual changes connected with spawning, the environment and season. Also, the fish belongs to high protein, minerals and low lipid category. They contain lower caloric content per unit of protein than do lipid and they were an ideal source of animal protein for use in controlling diets [4]. Also, Protein can be broken down into amino acids, which are essential for the growth and repair of body tissue. But some proteins are incomplete and must be supplemented with other protein foods, fish proteins are completed. Fishes are also a valuable source of vitamins, which are necessary for the body's functions. Fish liver oil is an exceptional source of vitamins A and D. Vitamin A is necessary for healthy skin and development of bones. Vitamin D plays an important part in the body's use of calcium, a mineral vital for sound teeth and bones. Fat fish in particular are a prime source of vitamin D [5]. In view of the recognized usefulness of these fishes, the present work has been under taken and the results have been reported in this communication.

Materials and Methods

Fish sample collection and lipids extraction

Fresh water *Pangasius pangasius* fish samples were collected from Rajbari fish Market, Rajbari. It was grown up in Padma River. Fresh water *Pangasius sutchi* fish sample were collected from our local pond, Modapur, Kalukhali, Rajbari. The weight of *P. pangasius* and *P. sutchi* were 17.5 kg and 2.5 kg respectively. Besides, the age of *P. pangasius* was 3.5 years whereas *P. sutchi* was 1.5 years. After collection, 50 g fish was cleansed by discarding their bones, liver, stomach and viscera. Then it was transferred to a volumetric flask. Then lipids were extracted by solvent extraction method using chloroform-methanol solvent [6].

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Lipids characterization

After extraction, the solvent was removed from the lipid at low temperature (to prevent oxidation) with the help of a rotary vacuum evaporator. The lipid was stored at 4°C in the presence of an inert gas until analyzed. The physico-chemical characterizations were determined using standard methods [7].

Determination of fatty acids profile

Preparation of the methyl ester mixture from the lipid for GLC: The lipid (5 g) was taken in a round bottom flask (125 ml) and saponified with alcoholic potassium hydroxide solution (50 ml). The mixture was then refluxed for 45 minutes on a water bath until it became clear. The reaction mixture was allowed to cool and then neutralized with HCl (5 N). Alcohol was removed from the neutralized solution by evaporation over a steam bath. 25 ml water was added to this alcohol free solution and pH of the solution was adjusted by adding concentrated HCl. The acidified aqueous mixture was then extracted with 20 ml ether in a separating funnel and the extraction was repeated for three times. The combined ether extract was washed with water in order to remove any adhering HCl. Ether was then removed from the extract to give the fatty acid mixture. The fatty acid mixture was then esterified with methanolic solution of sulfuric acid (0.25 M, 5 ml/g acid). After esterification, the mixture was dissolved in ether (25 ml) in a separating funnel and washed with dilute sodium carbonate solution until the effervescence ceased. It was then washed with water, dried over anhydrous Na_2SO_4 and finally ether was removed to give methyl ester mixture [8].

Gas liquid chromatographic examination of methyl esters of lipids: The experiment was carried out with a "PUE UNICAM" 4500 U model gas chromatograph equipped with a flame ionization detector. A glass coiled column (3 mm, I.D. 2.1 m) packed with 70-100 mesh chromosorb after impregnating it with 10% diethylene glycol succinate was used for the regular packed column GLC. The temperature programming in the oven was from 130°C to 230°C with the rate of rising 4°C per minute. The oven, injector and detector temperature were 190, 200 and 205°C respectively with a nitrogen carrier gas flow rate 30 ml/minute. The speed of the chromatogram was at 0.5 cm/min. The fatty acids in the mixture were identified by comparing its relative retention volume [9]. The area of each chromatogram peak was determined by multiplying the height of the peak by the width of the peak at one-half of the height. The percentage of fatty acid contributing to each peak was calculated and the results have been computed in the Table 1.

Nutrient contents

The proximate compositions of *P. pangasius* and *P. sutchi* were determined by the assayed as described in AOAC [10]. All chemicals were used of analytical grade and supplied by Sigma Co. (St. Louis, USA). Each analysis was carried out in triplicates.

Estimation of minerals (Ca, Zn, Fe, P) by AAS: Chemical analysis for the estimation of the trace elements (Ca, P, Zn, and Fe) in fish were performed with the flame atomic absorption spectrophotometer. The technique involved the following steps: The stock standard solutions of 100 ppm were prepared from analytical grade reagents of Ca, Mg, Zn, Fe, P, Hg, Pb, and Cr salt with distilled demonized water. The stock standard solution were preserved in clean polythene bottles and kept in a refrigerator. Standard solutions of these metal ions were prepared by suitable dilution of the stock standard solution. Dilution was made freshly with distilled demonized water. The sample of fish were diluted to a known volume and analyzed by a flame atomic absorption spectrophotometer (Flame AAS). The samples were analyzed against standard solution of each element. A reagent blank was also maintained, and the absorption due to reagent was subtracted. All the glasses were Pyrex and were cleaned before use with detergent, 1:1 HNO_3 and demonized water to avoid any contamination. Reagents were prepared with distilled and deionized water. The water was prepared by passing distilled water through a mixed-bed ion-exchange resin column. Samples were subsequently analyzed for trace elements by "AAS-680"Atomic Absorption / Flame Emission spectrophotometer (Shimadzu, Japan). A single hollow cathode lamp for each element was used with an air-acetylene and nitrous-oxide-acetylene [11].

Statistical analysis

Values are presented as the mean ± standard deviation of triplicate determinations. Statistical analysis was carried out by one-way analysis of variance (ANOVA) using SPSS software (Version 14.0 software, SPSS Inc., Chicago, IL, USA) and significance was defined at $p < 0.05$.

Results and Discussion

Lipids characterization

The lipids from *Pangasius pangasius* and *Pangasius sutchi* were extracted by solvent extraction process using Chloform- methanol as an extracting solvent. It was found that the fish contained lipid of 10.01 ± 1.11% and 6.16 ± 0.54% respectively. Previous report showed that the arachidonic and docosahexaenoic acid content of the body oil from *Monopterus albus* (a tropical freshwater fish) was 8.25 and 6.21 % lipid respectively. While in the head oil the content of these acids were 8.77 and 6.11 % lipid respectively which was around of our results [12].

The physical and chemical characteristics of the lipids vary between certain limits and due to a small variation, they are considered to be constants. Although the chemical constants are more important to characterize the lipid, but physical constants are also often capable of giving valuable information. The specific gravity of *P. pangasius* and *P. sutchi* lipid presently examined from the different stages were 0.97 ±

Ret. Time (min)	Area	Name of fatty Acid	Rel.% in *P. pangasius*	Ret. Time (min)	Area	Rel.% in *P. sutchi*
11.54	3973	C12:0 (Lauric)	13.36	11.45	3175	4.26
15.09	7773	C16:0 (Palmitic)	26.15	14.87	21820	29.32
17.82	42801	C18:1 (Oleic)	46.07	17.66	44030	59.16
18.07	3692	C18:0 (Stearic)	14.40	17.87	5393	7.24

Results expressed as the mean (in relative% fatty acid) of duplicate or triplicate analyses
Ret. time means Retention time; Rel. % means Relative percentages

Table 1: Fatty acid composition of the fatty acid methyl ester mixture derived from the lipid of *Pangasius pangasius* and *Pangasius sutchi* (by GLC analysis).

0.01 and 0.94 ± 0.02 at 30°C respectively. The refractive index of oils or fats varies somewhat widely and is chiefly governed by the proportion and degree of unsaturated matter present. The refractive index of the oil depends to some extent on their unsaturation and the higher refractive index represents higher unsaturation. It was found that the R.I. of the oils of *P. pangasius* and *P. sutchi* fishes were 4.88 ± 0.25 and 5.49 ± 0.30 at 30°C respectively is well above than the ranges of earliar results for fishes (1.4 - 1.473 at 30°C) [13]. Besides, co-efficient of viscosity *P. pangasius* were 448.96 ± 2.5 and *P. sutchi* were 421.76 ± 2.1. These values obtained in the present studies are quite similar to that reported for Hilsha fish oil [14] (Table 2).

The percentages of free fatty acids (above 1.5%) are a determination or indication of unsuitability of the lipid for edible purpose [15]. The free fatty acids of *P. pangasius* and *P. sutchi* were found in the ranges of 0.87 ± 0.03% and 1.14 ± 0.05% which were just shorter than the above range. So, these lipids might be suitable for edible purpose. Likewise, the iodine value gives an estimation of the degree of unsaturation. In the present investigation, the lipids contained higher amount of unsaturated fatty acid as the iodine values of *P. pangasius* and *P. sutchi* were 83.85 ± 0.64 and 85.36 ± 1.20 respectively. The unsaponifiable matter amounting to 0.45-2.0% represents a mixture of several lipid classes e.g., sterols, tocopherols, hydrocarbons, higher aliphatic and terpenoid alcohol. The unsaponifiable matters in the lipids of *P. pangasius* and *P. sutchi* were found to be 2.09% ± 0.36 and 2.37% ± 0.12% respectively which indicated that the lipid contained higher amount of sterols, tocopheros, hydrocarbons etc. Saponification equivalent 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, palmitic acids, a little unsaponifiable matter and a low free acidify generally have a saponification equivalent around 290.80. Higher value indicates the presence of appreciable quantity of higher acids [16]. The saponification equivalents of *P. pangasius and P. sutchi* were 217.66 ± 3.22 and 240.09 ± 2.24, respectively which clearly indicated that the lipid contained mainly fatty acids of C_{18} molecular weight along with some palmitic acid. The reichert-meissl (R.M) value represents the amount of volatile and water soluble acids components. The R.M value of *P. pangasius* was 3.631 ± 0.22 whereas it was 2.54 ± 0.31 for *P. sutchi* which indicated that *P. pangasius* contained higher amounts of volatile and water soluble acids than *P. sutchi.* On the other hand, the lipids of *P. pangasius* contains higher amounts of ester than *P. sutchi* because the ester value of *P. pangasius* were 255.48 ± 4.10 whereas the ester value of *P. sutchi* were 238.36 ± 3.15. Also, the acetyl value (17.09 ± 0.64) and cholesterol (17.46 ± 1.15%) of *P. pangasius* were just over than those of *P. sutchi* (13.63 ± 0.45 and 13.79% ± 0.37%) respectively. Previous report showed that the saponification value, saponification equivalent, iodine value, peroxide value and acetyl value of the lipid were found to be 220.325, 254.624, 96.05, 1.993 and 11.32, respectively. Also, the acid value, percentage of free fatty acid as oleic and unsaponifiable matter present in the lipid were found to be 2.005, 1.008 and 0.593, respectively which have similarities of our reports [17] (Table 3).

Determination of fatty acids profile

Fatty acid profile of the lipid was carried out by GLC after trans-esterification of the glycerides to their methyl esters (Figure 1). The stationary phase used in the column was the polar polyester 10% DEGS (diethylene glycol succinate) with its packing materials (gas chromp. 100-120 mesh).

Physical constant	*P. pangasius*	*P. sutchi*
Specific gravity (30°C)	0.97 ± 0.01	0.94 ± 0.02
Refractive index (30°C)	4.88 ± 0.25	5.49 ± 0.30
Co-efficient of viscosity	448.96 ± 2.5	421.76 ± 2.1

Values are given as mean ± S.D. of triplicate experiments

Table 2: Physical properties of the lipids of *Pangasius pangasius* and *Pangasius sutchi.*

Chemical constants	*P. pangasius*	*P. sutchi*
Acid value	2.26 ± 0.04	1.72 ± 0.02
Percentage of free fatty acid	0.87 ± 0.03%	1.14 ± 0.05%
Saponification value	257.7 ± 2.54	233.66 ± 3.35
Saponification equivalent	217.66 ± 3.22	240.09 ± 2.24
Iodine value	83.85 ± 0.64	85.36 ± 1.20
Peroxide value	58.24 ± 2.32	43.90 ± 2.20
Ester value	255.48 ± 4.10	238.36 ± 3.15
Unsaponificable matter (%)	2.09% ± 0.36	2.37% ± 0.12%
Reichert-Meissel value	3.631 ± 0.22	2.54 ± 0.31
Acetyl value	17.09 ± 0.64	13.63 ± 0.45
Cholesterol value (%)	17.46% ± 1.15%	13.79% ± 0.37%

Values are given as mean ± S.D. of triplicate experiments

Table 3: Chemical properties of the lipids of *Pangasius pangasius* and *Pangasius sutchi.*

The identification of fatty acid components from GLC analysis was carried out on the basis of relative retention time and was quantified by measuring the peak area in comparison with standard fatty acids. The fatty acids methyl ester mixture obtained from the lipids of *P. pangasius* and *P. sutchi* were identified as oleic acid, lauric acid, stearic acid and palmitic acid by comparison with standard methyl ester of fatty acids profile in different retention time where the areas under the peaks were proportional to the concentration of those components (Figure 2).

The analytical data were summarized in the Table 1. It was evident that the lipid of *P. sutchi* contained higher amount of oleic acid (59.16%) than (46.07%) of *P. pangasius.* The percentage of palmitic acid (29.32%) is also higher than the palmitic acid (26.15%) percentage of *P. pangasius*. Also, the lauric acid (4.26%) and stearic acid (7.24%) of *P. sutchi* were lower than the lauric acid (13.36%) and stearic acid (14.40%) of *P. pangasius*.

On the contrary, the previous report expressed that the predominant fatty acids in sardine wastes were palmitic (C16:0; 27.80-35.56 %), stearic (C18:0; 5.90- 9.30 %), oleic (C18:1; 15.47- 21.79 %) which were just about our results [18].

Nutrient contents

The nutrients such as proteins are essential to all life. In animals, they help form supporting and protective structures such as cartilage, skin, nails, hair and muscles. They are major constituents of enzymes, antibodies, many hormones, and body fluids such as blood, milk and egg white [19].

The moisture contents of *P. pangasius* and *P. sutchi* were tabulated in Table 4. The moisture content of *P. pangasius* was 52.08 ± 1.8% whereas it was 54.38 ± 2.1% for *P. sutchi*. The ash contents of fishes were ranges 1.012 ± 0.1% to 1.275 ± 0.12% which were maximum in *P. sutchi* and minimum in *P. pangasius*. Besides, the percentages of dry matter contents of both fishes are just about same. The carbohydrate percentage of *P. pangasius* was 1.89 ± 0.35% which is well above than

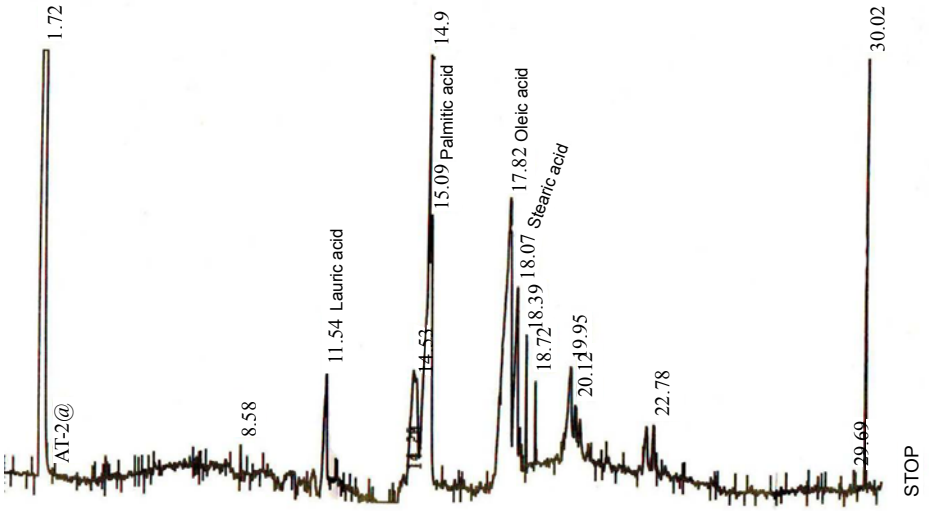

Figure 1: Chromatogram of the fatty acid composition of *Pangasius pangasius*.

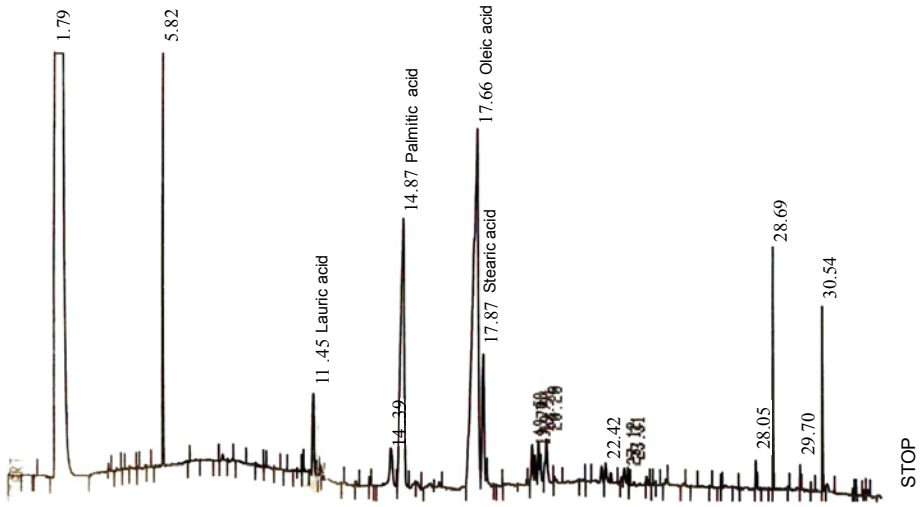

Figure 2: Chromatogram of the fatty acid composition of *P. pangasius* and *Pangasius sutchi*.

0.75 ± 0.11% of *P. sutchi*. Both fishes contain higher amount of protein. However, the protein contents of *P. pangasius* (35 ± 2.1%) were just below than *P. sutchi* (38.37 ± 2.5%). On the other hand, previous report showed that the highest moisture content was present in *S. lysan* (75.67%) and *S. commersonianus* (72.57%). The ash content estimated in *S. lysan*, *S. tol* and *S. commersonianus* were 1.42, 1.49 and 1.6%, respectively. Carbohydrate was present in very low level (<0.3%) in all fish species. Protein content was estimated as 19.47 ± 0.16%, 18.99 ± 0.51% and 21.68 ± 0.65% in *S. lysan, S. tol* and *S. commersonianus* which was approximately close to our reports [20].

Estimation of minerals (Ca, Zn, Fe, P) by AAS

Calcium is the most abundant mineral in the human body (2% of the body weight) and the fifth most abundant metallic element in the earth's crust. Calcium is essential for the growth, bone formation, blood coagulation, milk formation, vitamin D, absorption, etc. It is an inert inorganic mineral usually associated with bones and tooth formation. Deficiency of calcium leads to rickets, osteomalacia and osteo porosis [21]. The total amount of calcium in the body is about 1200 g. content of *P. pangasius* and *P. sutchi* were 110 and 1879 mg/kg respectively. Both fishes, Calcium contents were found well above than the previous reports [22].

Phosphorous is a major constituent of all animal cells, phosphorous is present in all natural foods. Most of the phosphate in the body is present in bones which contain from 600 to 900 g P. Bone ash was a component of many ancient and mediaeval remedies and later glycerol phosphates have had a great vogue as a tonic. Now with all other tonics it is in disrepute. Phosphate metabolism may be disturbed in many types of disease, notably those affecting the kidneys and bones [23]. In this study, the *P. sutchi* contained higher amount of phosphorous (2128 mg/kg) than *P. pangasius* (551 mg/kg) which was well above than the earlier reports [24] (Table 5).

Name of composition	*P. pangasius*	*P. sutchi*
Moisture content	52.08 ± 1.8%	54.38 ± 2.1%
Dry matter content	47.92 ± 1.4%	47.55 ± 1.7%
Ash content	1.012 ± 0.1%	1.275 ± 0.12%
Carbohydrate content	1.89 ± 0.35%	0.75 ± 0.11%
Protein	35 ± 2.1%	38.37 ± 2.5%

Values are given as mean ± S.D. of triplicate experiments

Table 4: Fish fillet analysis of *Pangasius pangasius* and *Pangasius sutchi.*

Sample Name	Parameters	Concentration in (mg/kg)
Pangasius pangasius (River pangas)	Ca (Calcium) P (Phosphorous) Zn (Zinc) Fe (Iron)	110 551 4.3 11.2
Pangasius sutchi (Pond pangas)	Ca (Calcium) P (Phosphorous) Zn (Zinc) Fe (Iron)	1879 2128 5.3 8.7

Table 5: The minerals concentration in *Pangasius pangasius* and *Pangasius sutchi.*

Iron is a macronutrient. It is in essential in life supporting element for animal and human being. Besides, the fishes are sufficient source of iron. Iron plays an important role in cellular metabolism as an active component of various enzymes, especially those associate with the respiration chain of mitochondria. Iron function mainly in the transport of oxygen to the tissues (hemoglobin). It is also involved in the processes of cellular respiration. Iron deficiency anemia is widely prevalent among children, adolescent girls and nursing mothers. In this study, the iron conc. of *P. pangasius* (11.2 mg/kg) was higher than of *P. sutchi* (8.7 mg/kg). Iron content of both fishes was found similar to Rohu fish (9.9 ± 0.13) as reported by [25]. Some other fish like bass, cod, salmon, and halibut are good source of iron containing 4.2, 9.4, 8.6 and 9.5 mg/100gm respectively which was around our results [26].

Also, zinc is essential element for animals, humans and plants. Although zinc represents only 0.003 percent of the human body, it is an intrinsic part of at least 110 metalloenzymes (alcohol dehydrogenate lactate dehydrogenate, glutamate dehydrogenate, carboxy peptidases A and B, carbonic anhydrate, etc.) and other cellular components. If is essential for synthesis of protein, RNA and DNA. Zinc helps in the transport of vitamin 'A`. Dietary zinc to copper ratio is important for disease increases. Zinc levels in the bloods decreases for two to three days after heart attack. Studies have shown that raising the blood level of zinc protects heart damage after a heart attack. The most prominent sings of zinc deficiency are growth retardation, anemia, and impaired sexual development, skin changes, loss of appetite, white opaque spots on finger nails [27]. Zinc contents of P. *pangasius* and *P. sutchi*, were 4.3 and 5.3 mg/kg whereas the Zn content of the rainbow trout was found to be 9.68 mg/kg which was well across than our reports [28].

Conclusion

The results obtained from this study suggest that the lipids of *P. pangasius* and *P. sutchi* could be used in the human diet or as supplementary food for other animals as it is an excellent source of good quality fatty acids, especially PUFA under higher body weights. Moreover, It could be concluded that both fishes can be referred to as high protein, minerals and PUFA which can be utilized by food processors in fish canning and other value added fish products such as fish burger, fish cake and fish crackers and also for use in controlling diet while the wastes recovered can be used for fish meal or silage production for animal feeds. Hence, they are suitable as potential industrial material for possible utilization for different products.

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