Turkish Journal of Fisheries and Aquatic Sciences 13: 487-493 (2013)
www.trjfas.org
ISSN 1303-2712
DOI: 10.4194/1303-2712-v13_3_11

Physico-chemical studies of Lipids and Nutrient contents of *Channa striatus* and *Channa marulius*

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Received 25 September 2012
Accepted 22 July 2013

Abstract

This study was carried out to determine the lipids isolated from *Channa striatus* and *Channa marulius* and to analyze the nutrient contents of both fish. The specific gravity, refractive index and viscosity co-efficients were (0.93 ± 0.03 and 0.91± 0.03 at 30°C), (1.76 ± 0.21 and 1.34 ± 0.22 at 30°C) and (438.43 ± 2.5 and 411.34 ± 1.2) for *C. striatus* and *C. marulius*, respectively. Besides, the lipids of both fish showed significant results in case of chemical characterization. On the contrary, *C. striatus* and *C. marulius* contained expected amount of carbohydrate, protein, lipid and cholesterol. The percentages of moisture, dry matter and ash were found in fair amounts. Furthermore, the minerals of *C. striatus* such as calcium and magnesium contents were higher but iron and zinc contents were lower than *C. marulius*. But, the percentages of lead, mercury and chromium of both fish were precisely in traces amounts. The fatty acids in C. *striatus* and *C. marulius* were identified respectively; lauric acid (2.73 and 5.55 %), palmitic acid (17.79 and 27.74 %), oleic acid (36.81and 31.35 %), linoleic acid (3.45 and 2.49 %) and stearic acid (25.79 and 21.81%)

Keywords: *C. striatus*; *C. marulius*, lipids, fatty acids, nutrients.

Introduction

Bangladesh is a first line littoral state of the Indian Ocean that has very good marine resources in the Bay of Bengal. Most of the people in our country are solely dependent on fish protein. It has been estimated that about 80% of the animal protein in our diet comes from fish alone (Begum *et al*., 2010). *Channa striatus* and *Channa marulius*, the fresh water snake head fish belongs to Channidae family and have long been regarded as valuable fish in Far East. In Bangladesh, *C. striatus* is known as "Shoul" and *C. marulius* is known as "Gozar". These fish species are available in the fresh water stream in South Asia.

Fish lipids are the main sources of polyunsaturated fatty acids (PUFAs) especially eicosapentaenoic acid (EPA; $C_{20:5}$) and docosahexaenoic acid (DHA; $C_{22:6}$) (Osman *et al*., 2001). These two fatty acids cannot be synthesized by 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 (Ibrahim *et al*., 2004). These fishes' *C. striatus* and *C. marulius* contain polyunsaturated fatty acids (PUFA), which play important roles in cardiovascular system to reduce the risk of heart attack.

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 lipid and they are an ideal source of animal protein for use in controlling diets (Silva and Chamul, 2000). Fish liver oil is an exceptional source of vitamins A and D. Vitamin A is necessary for healthy skin and for the development of bones. Vitamin D plays an important role in the body's use of calcium, mineral is vital for sound teeth and bones. Thus, we notice that Fat fish in particular are the prime source of vitamin D (Bechtel and Oliveira, 2006). In these studies, the fish oil was extracted from *C. striatus* and *C. marulius* and evaluated the biochemical studies as well as to determine the nutrient contents.

Materials and Methods

Fish Sample Collection and Lipids Extraction

Fresh water *Channa striatus* and *Channa marulius* fish samples were collected from Kushtia fish Market, Kushtia, Bangladesh. The weight of *C. striatus* and *C. marulius* were 3.5 kg and 4.5 kg respectively. Besides, the age of *C. striatus* was 2.5 years whereas the *C. marulius* was 2.0 years. After collection, 50 g fish was cleansed by discarding their bones, liver, stomach and viscera. The lipids were isolated by solvent extraction method using chloroform-methanol solvent through a volumetric flask (Jayaraman, 1985).

Lipids Characterization

The isolated lipids were separated from solvents at low temperature (to prevent oxidation) with the help of rotary vacuum evaporator. The lipids were stored at 4^0C in the presence of an inert gas until analyzed. The physico-chemical characterizations were determined using standard methods (Stephan *et al*., 2006).

Determination of Fatty Acids Profile

The lipid (5 g) was taken in a round bottom flask (125 ml) and saponified with alcoholic potassium hydroxide solution (50 ml). The mixtures were then refluxed for 45 minutes on a water bath until it became clear. The reaction mixtures were allowed to cool and neutralized with HCl (5 N). Alcohol was removed from the neutralized solution by evaporation over a steam bath. Besides, 25 ml water was added to this alcohol free solution and pH 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. Therefore, Ether was 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 (Majid *et al*., 2004).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) was packed with 70-100 mesh chromosorb after impregnating with 10% diethylene glycol succinct and was used for the regular packed column GLC. The temperature programming in the oven was 130 ^{0}C to 230 ^{0}C with the rate of rising 4 ^{0}C per minute. The oven, injector and the detector temperature were 190, 200 and 205 ^{0}C respectively with a nitrogen carrier gas (flow rate 30 ml/min). 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 (Lokuruka, 2008). The area of each chromatogram peak was determined by multiplying the one-half of the height by the width of the peak. The percentages of fatty acid contributing to each peak were calculated and the results have been computed in the Table 3.

Nutritional Analysis

The nutrients of *C. striatus* and *C. marulius* were determined by the assayed as described in the volume (AOAC, 2005). All chemicals of analytical grade were used and supplied by Sigma Co. (St. Louis, USA). Each analysis was carried out in triplicates.

Estimation of Minerals by AAS

The chemical analysis for the estimation of the trace elements (Ca, Mg, Zn, Fe, Hg, Pb, and Cr) of both fish's was performed with the flame atomic absorption spectrophotometer. The technique involved the following steps: The stock standard solutions of 100 ppm were prepared by using analytical grade reagents of Ca, Mg, Zn, Fe, 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 carried out with distilled demonized water. Also, the fish sample was diluted to a known volume and analyzed by a flame atomic absorption spectrophotometer (Flame AAS). The sample was analyzed against standard solution of each element. A blank reagent was also maintained, and the absorption due to reagent was subtracted. All the glasses were Pyrex and were cleaned by detergent, 1:1 HNO_3 and demonized water to avoid any contamination before uses. 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 (Okorie, 2010).

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 the significance was defined at $p < 0.05$.

Results and Discussion

Lipids Characterization

The lipids from *Channa striatus* and *Channa marulius* were extracted by solvent extraction process using Chloform- methanol as an extracting solvent. It was found that the fish contained lipid of 2.86 ± 1.4% and 2.60± 1.2% respectively. Previous report showed that the arachidonic and docosahexaenoic acid content of the body lipid from *Monopterus albus* (a tropical freshwater fish) was 8.25 and 6.21 % respectively. While in the head oil the content of these acids were 8.77 and 6.11 % lipid respectively which was well above than our results (Razak *et al*., 2001).

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. In Table 1, the specific gravity of *C. striatus* and *C. marulius* lipids were examined in the different stages as 0.93 ± 0.03 and 0.91± 0.03 at 30^0C respectively. 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. values of the lipid of *C. striatus* and *C. marulius* were 1.76 ± 0.21and 1.34 ± 0.22 at 30^0C respectively which is well above than the ranges of earlier results for fishes (1.4 - 1.473 at 30^0C) (Abdulkadir *et al*., 2010). Besides, the co-efficient of viscosity *C. striatus* was 438.43 ± 2.5 and *C. marulius* was 411.34 ± 1.2. These values obtained in the present studies are quite similar to that reported for Hilsha fish oil (Ahmed *et al*., 2006).

The percentages of free fatty acids (above 1.5%) are the determination or indication of unsuitability of the lipid for edible purpose (Molla *et al*., 2007). In Table 2, the free fatty acids of *C. striatus* and *C. marulius* were found in the ranges of 0.88 ± 0.02% and 0.92 ± 0.04% which were just short of the above ranges. 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 *C. striatus* and *C. marulius* were 87.34± 0.64 and 83.23 ± 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 *C. striatus* and *C. marulius* were found to be 1.09 ± 0.32 and 0.97 ± 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 acids 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 (Shamsudin and Salimon, 2006). The saponification equivalents of *C. striatus and C. marulius* were 196.66 ± 3.22 and 221.09 ± 2.14, respectively which clearly indicated that the lipid mainly contained fatty acids of C_{18} molecular weight along with some palmitic acid. The reichert-meissl (R.M) value represents the amount of

Table 1. Physical constants of the lipids of *Channa striatus* and *Channa marulius*

Physical constant	*C. striatus*	*C. marulius*
Specific gravity (30^0C)	0.93 ± 0.03	0.91± 0.03
Refractive index (30^0C)	1.76 ± 0.21	1.34 ± 0.22
Co-efficient of viscosity	438.43 ± 2.5	411.34 ± 1.2

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

Table 2. Chemical constants of the lipids of *Channa striatus* and *Channa marulius*

Chemical constants	*C. striatus*	*C. marulius*
Acid value	1.76 ± 0.03	1.87 ± 0.05
Free fatty acid (%)	0.88 ± 0.02	0.92 ± 0.04
Saponification value	205.7 ± 2.34	196.66 ± 3.15
Saponification equivalent	196.66 ± 3.22	221.09 ± 2.14
Iodine value	87.34± 0.64	83.23 ± 1.20
Peroxide value	42.24 ± 2.32	43.32 ± 2.10
Ester value	255.48 ± 4.10	238.36 ± 3.15
Unsaponificable matter (%)	1.09 ± 0.32	0.97 ± 0.12
Reichert-Meissel value	2.54 ± 0.21	1.14 ± 0.32
Acetyl value	16.07 ± 0.32	12.53 ± 0.45

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

volatile and water soluble acids components. The R.M value of *C. striatus* was 2.54 ± 0.21 whereas it was 1.14 ± 0.32 for *C. marulius* which indicated that *C. striatus* contained higher amounts of volatile and water soluble acids than *C. marulius.* On the other hand, the lipids of *C. striatus* contains higher amounts of ester than *C. marulius* because the ester value of *C. striatus* was 255.48 ± 4.10 whereas the ester value of *C. marulius* was 238.36 ± 3.15. Also, the acetyl value (16.07 ± 0.32) of *C. striatus* was just over than those of *C. marulius* (12.53 ± 0.45) 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 the similarities of our reports (Islam *et al*., 2008).

Determination of Fatty Acids Profile

Fatty acid profile of the lipid was carried out by GLC after trans-esterification of the glycerides to methyl esters. The stationary phase in the column was the polar polyester 10% DEGS (diethylene glycol succinate) with its packing materials (gas chromp. 100-120 mesh).The identification of fatty acid components from GLC analysis were carried out on the basis of relative retention time and were quantified by measuring the peak area in comparison with standard fatty acids. The methyl ester of fatty acids mixture obtained from the lipid of *C. striatus* and *C. marulius* were identified as oleic acid, linoleic 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.

The analytical data is summarized in the Table 3. It is evident that the lipid of *C. striatus* contained higher amount of oleic acid (36.81%) than (31.35%) of *C. marulius.* The percentage of palmitic acid (17.79%) was also lower than the palmitic acid (27.74%) of *C. striatus*. On the contrary, the percentage of linoleic acid (3.45%) and stearic acid (25.79%) were higher than the linoleic acid (2.49%) and stearic acids (21.81%) of *C. marulius*. Also, the lauric acid (2.73%) of *C. striatus* was lower than the lauric acid (4.55%) of *C. marulius*.

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 (Khoddami *et al*., 2009).

Nutritional Analysis

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 (Potter and Joseph 1996). The moisture contents of *C. striatus* and *C. marulius* were tabulated in Table 4. The moisture content of *C. striatus* was 70.80 ± 1.1% whereas it was 69.5 ± 1.2% for *C. marulius*. The ash contents of both fishes are ranges 1.97 ± 1.7% to 2.20 ± 1.3% which were maximum in *C. marulius* and minimum in *C. striatus*. Besides, the percentages of dry matter contents of both fishes were almost same. The carbohydrate percentage of *C. striatus* was 1.10 ± 0.31% which is higher than 0.84 ± 0.10% of *C. marulius*. Both fishes contain higher amount of protein. However, the protein contents of *C. striatus* (18.24 ± 1.3%) were lower than *C. marulius* (20.5 ± 1.2%).

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 (Sutharshiny and Sivashanthini, 2011).

Estimation of Minerals 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.

Table 3. Fatty acid percentages derived from methyl ester mixture (by GLC analysis)

Ret. time	Area	Name of fatty Acid	Rel.% in *C. striatus*	Ret. time	Area	Rel.% in *C. marulius*
3.89	973	Lauric Acid	2.73	3.68	1611	4.55
8.12	6294	Palmitic Acid	17.79	7.63	9815	27.74
9.21	9125	Stearic Acid	25.79	8.16	7716	21.81
9.98	13023	Oleic Acid	36.81	9.76	11091	31.35
12.78	1220	Linoleic acid	3.45	11.80	880	2.49

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

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 tooth formation. Deficiency of calcium leads to rickets, osteomalacia and osteo porosis (Anderson, 1982). The total amount of calcium in the body of *C. striatus* and *C. marulius* are 5279 and 165 mg/kg respectively. Both fishes, Calcium contents were found well above than the previous reports (Esmailzadeh *et al*., 2004).

The mineral contents of these fish species are shown in Table 5. Iron is a macronutrient. It is essential as a life supporting element for animal and human being. Besides, the fish species 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 transportation 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 *C. striatus* (4.93 mg/kg) was just below than of *C. marulius* (5.26 mg/kg). Iron content of both fishes was found near to Rohu fish (9.9 ± 0.13) as reported by (Jyothirmayi, *et al*., 2009). 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 were practically around to our results (Gehring *et al*., 2011).

Magnesium is an extremely important and valuable mineral, whose value for good health is just being recognized by conventional physicians. In the USA, magnesium supplementation is dramatically under utilized by conventional physicians and is more important in patient therapy than most physicians realize. There are over 200 published clinical studies documenting the need for magnesium. In fact, at the 1992 American College of Cardiology annual meeting, a limited biography on magnesium was the most often requested item at the National Council on Magnesium and Cardiovascular booth. Increased use of oral and injectionable magnesium, along with a diet rich in magnesium, should greatly improve therapeutic results for many patients (Baker, 1991-92). In this study, the magnesium conc. of *C. striatus* (276 mg/kg) was just below than of *C. marulius* (216 mg/kg). Previous report shows that the magnesium status of juvenile rainbow trout (*Salmo gairdneri*) feed (semi-purified diets) containing the graded levels of magnesium (36–2120 mg/kg diet) was about the same to our results. (Shearer, 1989)

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. It is essential for the synthesis of protein, RNA and DNA. Zinc helps in the transportation of vitamin ‘A`. This increased ratio of zinc to copper then causes an increased concentration of cholesterol in plasma, and presumably, results in increased risk of coronary heart disease. 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

Table 4. Fish fillet analysis of *Channa striatus* and *Channa marulius*

Name of composition (in %)	*C. striatus*	*C. marulius*
Moisture content	70.80 ± 1.1	69.5 ± 1.2
Dry matter content	49.85 ± 1.3	50.34 ± 1.6
Ash content	1.97 ± 1.7	2.20 ± 1.3
Carbohydrate content	1.10 ± 0.31	0.84 ± 0.10
Lipid	2.86 ± 1.4	2.60± 1.2
Protein	18.24 ± 1.3	20.5 ± 1.2
Cholesterol value	15.40 ± 1.21	12.45 ± 0.41

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

Table 5. The percentages of mineral content in *Channa striatus* and *Channa marulius.*

Serial No.	Parameters	*C. striatus* (Conc. in mg/kg)	*C. marulius* (Conc. in mg/kg)
1.	Calcium (Ca)	5279	165
2.	Iron (Fe)	4.93	5.26
3.	Magnesium (Mg)	276	216
4.	Zinc (Zn)	8.30	10.32
5.	Lead (Pb)	0.06	0.13
6.	Mercury (Hg)	0.01	0.02
7.	Chromium (Cr)	0.01	0.03

impaired sexual development, skin changes, loss of appetite, white opaque spots on finger nails (Molla, 1991). Zinc contents *C. striatus* and *C. marulius*, were 8.3 and 10.32 mg/kg whereas the Zn content of the rainbow trout was found to be 9.68 mg/kg which was quite similar to our reports (Nalan *et al*., 2004).

Besides, epidemiological studies have established positive associations between chronic exposure (occupational or environmental) to these metals and adverse health effects. The reviews presented in this issue were focused on the following toxic metals: Pb, Cr, Hg. Oxidative stress has been recognized as underlying the mechanism in toxicity associated with numerous metals. Metals either undergo redox cycling Cr or deplete cell's antioxidant pool (Pb, and Hg) and induce oxidative stress (Editorial. 2008). Lead, Mercury and Chromium contents *C. striatus* were 0.06, 0.01 and 0.01 respectively and *C. marulius* were 0.12, 0.02 and 0.03 respectively. The previous report showed that the axial muscles from 10 species each of freshwater and marine fish purchased from markets in Hong Kong contained 0.09 to 0.36 mg/kg Cr, 0.07 to 0.34 mg/kg Hg, 0.11 to 0.52 mg/kg Pb which were almost the same to our results (Cheung, 2008).

Conclusion

Regarding the suitable amount of lipids and nutrient contents, it appears that *C. striatus* and *C. marulius* could be used as human diet or as supplementary food for other animals as an excellent source of good quality fatty acids, especially PUFA under higher body weights. Besides, both fish 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 the potential industrial material for possible utilization for different food products.

Acknowledgement

We are grateful to the Faculty of Science & Technology, Islamic University, Kushtia, Bangladesh for supporting this research project. Moreover, we are also grateful to Organic Chemistry Laboratory, University of Dhaka, Bangladesh for supporting GLC analysis.

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