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Studies on nutritional composition and characterization of lipids of *Lates calcarifer* (Bhetki)

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Abstract

Physico chemical characteristics of lipids extracted from *Lates calcarifer* and nutritional composition of that fish were analyzed by standard method. The coefficient of viscosity was 447.69 millipoise at 30^{o}C and specific gravity and refractive index were 0.98 and 1.37 at 30^{o}C respectively. Saponification value, saponification equivalent, iodine value, peroxide value, acid value, ester value and reichert-meissel value were found 95.50, 286.96, 62.81, 52.59, 1.83, 193.67 and 2.94 respectively. Percentage of free fatty acid, unsaponificable matter and cholesterol were found 0.92, 1.86 and 13.74 respectively. Further, carbohydrate, protein, lipid, moisture, ash and dry matter content of *L. calcarife*r were found 0.93, 23.5, 5.7, 70, 5 and 30 % respectively. The fish was found to contain 580, 270,140, 120 and 9 mg of Na, K, Ca, Mg and Fe per 100 g respectively. However Cu and Zn were found to present in negligible amount. The fatty acid profiles *L. calcarifer* were identified by GLC method. Among fatty acid lauric, palmitic, oleic and stearic were found to present 4.49, 52.86, 36.88 and 5.75% respectively.

Keywords: *Lates calcarifer*; Lipid; Fatty acid composition; GLC; Mineral content

Introduction

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 the diet of the people of Bangladesh are contributed by fish (Hawk and Oser, 1965). *L. calcarifer* is a fresh water fish species commonly known as Barramundi and in Bangladesh, locally known as Bhetki. It is also known as Asian Seabass, is a species of catadromous fish in family Latidae of order Perciformes. The native species is widely distributed in the Indo-West Pacific region from the Persian Gulf, through Southeast Asia to Papua New Guinea and Northern Australia. Fish is inseparable part of the Bangladesh economy and it plays a vital role in nutritional balance as an important source of protein. Besides protein, fish is a good source of carbohydrate, fat, vitamin and mineral. Nutrients needed in relatively large quantities are called macronutrients and those needed in relatively small quantities are called micronutrients (Annymous, 1986). Fish liver oil is good source of vitamin A and D. Some B complex vitamins are also found in fish.

L. calcarifer contains polyunsaturated fatty acids (PUFA) enriched of omega-3 fatty acid, which play important roles in cardiovascular system to reduce the risk of heart attack (Islam, 1983). Omega-3 fatty acids are helpful to reduce cholesterol level in blood and helpful in the prevention of hyperlipidemia, secondary cardiovascular disease and high blood pressure. The nature and quantity of lipid in fish are dependent on species and habitats. 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 the human body (essential fatty acid) and must be obtained from the diet (Linko and Hayakawa, 1996). 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).

In this study, nutritional composition of *L. calarifer* was analyzed and lipid was extracted, characterized and fatty acid composition was determined by gas liquid chromatography (GLC).

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Materials and methods

Sample Collection

About 500 gm of fish *(L. calcarifer)* were collected from market of Binodpur, Rajshahi, Bangladesh. Prior to analysis, the internal organs were removed and the fish was washed to remove the residual blood. Fish fillet was obtained by cutting the fish lengthwise along the backbone to obtain maximum amount of flesh without including the backbone. The fillet was cut into small pieces.

Proximate composition analysis

The moisture, carbohydrate, protein, ash and dry matter contents of the sample were determined by the AOAC methods (AOAC, 1990). The experiments were performed in triplicate and values are expressed as mean ± standard deviation.

Mineral Analysis

Minerals analysis of fish sample were done according to the AOAC method (AOAC, 1995), using Atomic Absorption Spectrophotometer.

Extraction and estimation of total lipids

Total lipids were extracted from fish muscle tissue samples and estimated according to Folch *et al*. (1957). Briefly, a chloroform/methanol solvent mixture (2:1, v/v) was added to samples in the ratio solvent/tissue of 20:1 (v/w). The samples were homogenized three times and each homogenization step was followed by cooling of the sample for 1h at 4 °C. The chloroform/methanol extracts were incubated overnight at 4 °C to allow the organic (containing the extract of total lipids) and aqueous layers to separate completely. The upper (aqueous) layer was removed, and the lower (organic) layer was rinsed with chloroform/methanol (2:1 v/v), then placed into a glass tube. The total lipid fraction was obtained by evaporating the lower phase. The solvent was removed in a rotary evaporator under vacuum at 40 °C. These extracts, representing the total lipids, were weighed, and results were noted. The resulting extract of total lipids was stored at 4 °C until further analysis.

Characterization of the Lipid

Physical and chemical properties of the lipid extracted from *L. calcarifer* were determined by standard method (Bockenooge, 1964, Jayaraman, 1981; Williams, 1966).

Preparation of the methyl ester

The samples lipids were converted to their constituent fatty acid methyl esters (FAME) according to standard method (Loury, 1967; Randerath, 1966). 5g lipid 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 (5N). Alcohol was removed from the neutralized solution by evaporation over a steam bath. 25mL water was added to this alcohol free solution and pH of the solution was adjusted to 1-2 drops by adding concentrated HCl. The acidified aqueous mixture was then extracted with 20 mL of 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 (0.25M, 5mL/gm acid). After esterification, the mixture was dissolved in ether (25mL) in a separating funnel and was washed with dilute sodium carbonate solution until the effervescence ceased. It was then washed with water, dried over anhydrous sodium sulfate and finally ether was removed to give methyl ester mixture.

Gas liquid chromatographic analysis of methyl esters

Analysis of methyl ester was carried out with a "PUE UNICAM" 4500 U model gas chromatograph equipped with a flame ionization detector (Kanella *et al.*, 1977; Hossain *et al*., 1988; Huq *et al,* 1979). A glass coiled column (3mm I.D. 2.1m) packed with 70-100 mesh chromosorb after impregnating it with 10% diethylene glycole 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 °C, 200 °C and 205 °C respectively with a nitrogen carrier gas flow rate 30 mL/minute. The speed of the chromatogram was at 0.5 cm/minute. The fatty acids in

the mixture were identified by comparing its relative retention volume (Gurr and James, 1975). The area of each chromatogram peak was determined by multiply the height of the peak by the width of the peak at one-half of the height.

Results and discussion

Proximate composition

Mean percentage of proximate composition of *L. calcarifer* were shown in Table I. Moisture content of *L. calcarife*r was 70 ± 2.8, which was found similar as reported by Hui, 2001. The percentage of water is a good indicator of its relative contents of energy, proteins and lipids. The lower the percentage of water, the greater the proteins and lipids contents and higher the energy density of the fish (Dempson *et al.,* 2004). Gray eel-catfish was found to contain the highest level of moisture among the demersal fish studied, with value of 81.66 + 1.47 % compared to other fish that ranged between 75 % and 80 % (Nurnadia *et al.,* 2011). Dry matter content was found 30 ± 0.8. Moisture and Dry matter content are inversely related to each other. Dry matter content was found 5 ± 0.24 %. Ash content was found 5 ± 0.24, which was similar to wild feather back fish (Naeem *et al.*, 2011) and higher than catfish (Olayemi *et al*., 2011). Protein and fat are the major nutrients in fish and their levels help define the nutritional status of the particular organism. Protein and fat content of *L. calcarifer* were found 23.5 ± 0.16 and 5.7 ± 0.3 % respectively. Fat composition exhibited seasonal variations in all species of fish. Biological values of proteins are high and it contains all essential amino acids and fish fat also contains essential fatty acids and omega-3 fatty acids. According to Ackman 1967, *L. calcarifer* is a medium fat content fish (4-8%). Protein content of Bramundi was found similar to that of Long-tailed butterfly ray and higher than that of Gray eel-catfish and Cuttlefish (Nurnadia *et al.,* 2011).

Table I. Proximate composition of *Lates calcarifer* (Bhetki)

Parameters	Percentage (%)
Moisture	70 ± 2.8
Dry matter	30 ± 0.8
Ash	5 ± 0.24
Carbohydrate	0.93± 0.12
Lipid	5.7± 0.3
protein	23.5 ± 0.16

*Values are expressed as the mean ± standard deviation (n = 3).

Mineral Composition

The results of the percentage means of mineral elements concentrations were given in Table II. Among the mineral elements investigated, concentration of sodium (Na+) in *L. calcarifer* were observed to have appreciably dominated (580mg/100gm) following potassium, calcium, magnesium and iron. The levels of sodium found in Bhetki fish is much higher than those measured in sea bass by Abdullah *et al.* (2011). Sodium regulates the electrolyte and acid-alkali balances, the conductive capacity of the nerves, muscle contractions and the production of adrenaline and amino acids (Pirestani *et al*., 2009). The concentration of potassium (K^+) in the fish samples examined ranked second among the mineral elements analyzed. This tends to disagree with the work done by Onyia, *et al*. (2010) at Nigeria. Potassium content of *L. calcarifer was* found similar to *S. lucioperca* (Pirestani *et al.*, 2009). Ca content was found slightly higher than *R. frisii kutum* (Esmailzadeh *et al*., 2004). Bhetki can be a good source of calcium for the human body, considering the suggested daily calcium intake amount of 800 mg/day (Whithney and Rolfes, 2008). Magnesium content was found 120 mg/100gm in Bheki fish. An adequate intake of magnesium has some useful roles in a human body due to the fact that it regulates enzyme systems, helps to maintain bone

Table II. Mineral composition of *Lates calcarifer* (Bhetki)

Mineral	Content (mg/100gm)
Sodium (Na)	580 ± 0.65
Potassium (K)	270± 0.34
Calcium (Ca)	140 ± 0.19
Magnesium (Mg)	120 ± 0.45
Iron (Fe)	9 ± 0.11
Copper (Cu)	tr
Zinc (Zn)	tr

*Values are expressed as the mean ± standard deviation (n = 3). tr means traces amount

health, is required for energy metabolism, and acts as a part of the protein-making machinery in all cells of soft tissues (Whithney and Rolfes, 2008). Copper and zinc were present in trace amount. Copper and zinc are essential for good health, but very high intakes can cause health problems such as liver and kidney damage (Atsdr, 2004). The concentration of minerals in fish muscles examined could have been as a result of the rate in which they are available in the water body and the ability of the fish to absorb these inorganic elements from their diets and the water bodies where they live (Adewoye and Omotosho, 1997). Iron content of *L. calcaifer* (9 ± 0.11) was found similar to Rohu fish (9.9±0.13) as reported by Jyothirmayi *et al.* (2009). Some other fishes like bass, cod, salmon, and halibut are good source of iron containing 4.2, 9.4, 8.6 and 9.5 mg/100gm respectively (Gehring *et al*. 2011).

Fat and Fatty acid Composition

The lipid from the fish was extracted by solvent extraction process using chloroform-methanol mixture as an extracting solvent. It was found that the fish contained 5.7% lipid A number of physical and chemical tests were employed to identify the nature of oils and fats. The chemical constants are more important to characterize an oil or fat, yet the physical constants are also often capable of expressing valuable information. These constants of the lipid of *L. calcarifer* are given in Table III and Table IV.

Table III. Physical constants of the lipid of *Lates calcarifer*

Physical constant	Value
Specific gravity at 30 ^{o}C	0.98
Refractive index at 30 ^{o}C	1.37
Co-efficient of viscosity (milipoise)	447.69

Table IV. Chemical constants of the lipid of *Lates calcarifer*

Chemical constants	Value
Saponification value	95.50
Ester value	193.67
Iodine value	62.81
Peroxide value	52.59
Reichert-Meissel value	2.94
Acetyl value	15.94
Saponification equivalent	286.96
Percentage of free fatty acid	0.92
Unsaponifiable matters (%)	1.86
Acid value	1.83
Cholesterol value (%)	13.74

The refractive index of fats and oils depends to some extent on their unsaturation (Peach and Tracy, 1995) and the higher refractive index represents higher unsaturation. The refractive index of fish *L. calcarifer* (137 at 30 ^{o}C) is slightly lower than the range of standard value of 1.4 - 1.473 for fishes (Abdulkadir *et al*., 2010). The specific gravity of particularly all fats and oils lies between 0.90 - 0.95. The specific gravity obtained in the present study (0.98 at 30 ^{o}C) is slightly higher than the normal range.

Iodine value gives an estimate of the degree of unsaturation and so of the relative amounts of unsaturated fatty acids in the triglyceride molecules of the fat. Iodine value of Bhetki fish was found 62.81, which was lower than *Lepidocephalus guntea* (96.05) and Soybean oil (129.00) (Islam, 2008). The percentage of free fatty acid (above 1.5%) is a determination or indication of unsuitability of the lipid for given edible purpose. So the fish lipid might be suitable for edible purpose.

Table V. Fatty acid percentages derived from methyl ester mixture of *Lates calcarifer* (by GLC analysis)

Ret. Time	Area	Name of fatty Acid	Rel.%
9.71	412	C12:0 (Lauric)	4.49
13.07	4842	C16:0 (Palmitic)	52.86
15.97	3378	C18:1 (Oleic)	36.88
16.46	527	C18:0 (Stearic)	5.75

The unsaponifiable matter amounting to 0.45-2.0% represents a mixture of several lipid classed, viz, sterols, tocopherols, hydrocarbons, higher aliphatic and terpenoid alcohol. The unsaponifiable matter in the lipid was found to be 1.86%, which indicates that lipid, also contained sterols, tocopherols, hydrocarbons etc. The higher the saponification value, the lower the average molecular weight (O'Brien, 1998). 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 (Carrol and Noble, 1957). The present result (286.96) clearly indicated that the lipid contained mainly fatty acids of C_{18} molecular weight along with some palmitic acid.

Fatty acid analysis of the lipid was carried out by GLC after trans-esterification of the glycerides to their methyl esters. The refractive index of fats oil depends to some extent on their unsaturation (Peach and Tracy, 1995) and the higher refractive index represents higher unsaturation. 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). 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 acid. The fish lipid contained highest amount of palmitic acid (52.86%), while lauric acid, oleic acid and stearic acid were found 4.49, 36.88 and 5.75% respectively.

From the findings of the present investigation, it may be suggested that the lipid is suitable for edible purpose as it contained higher amount of unsaturated fatty acids. These unsaturated fatty acids always play important roles in the metabolism of living organism (Hawk and Oser, 1965). High level of unsaturated fish lipid is found to inhibit the activity of HMG-CoA reductase (Ide *et al,* 1978; Siscovick *et al* 1995) which is the regulatory enzyme in cholesterol biosynthesis. Due to the biosynthesis of cholesterol in liver, PUFA plays an important role in maintaining the blood cholesterol level

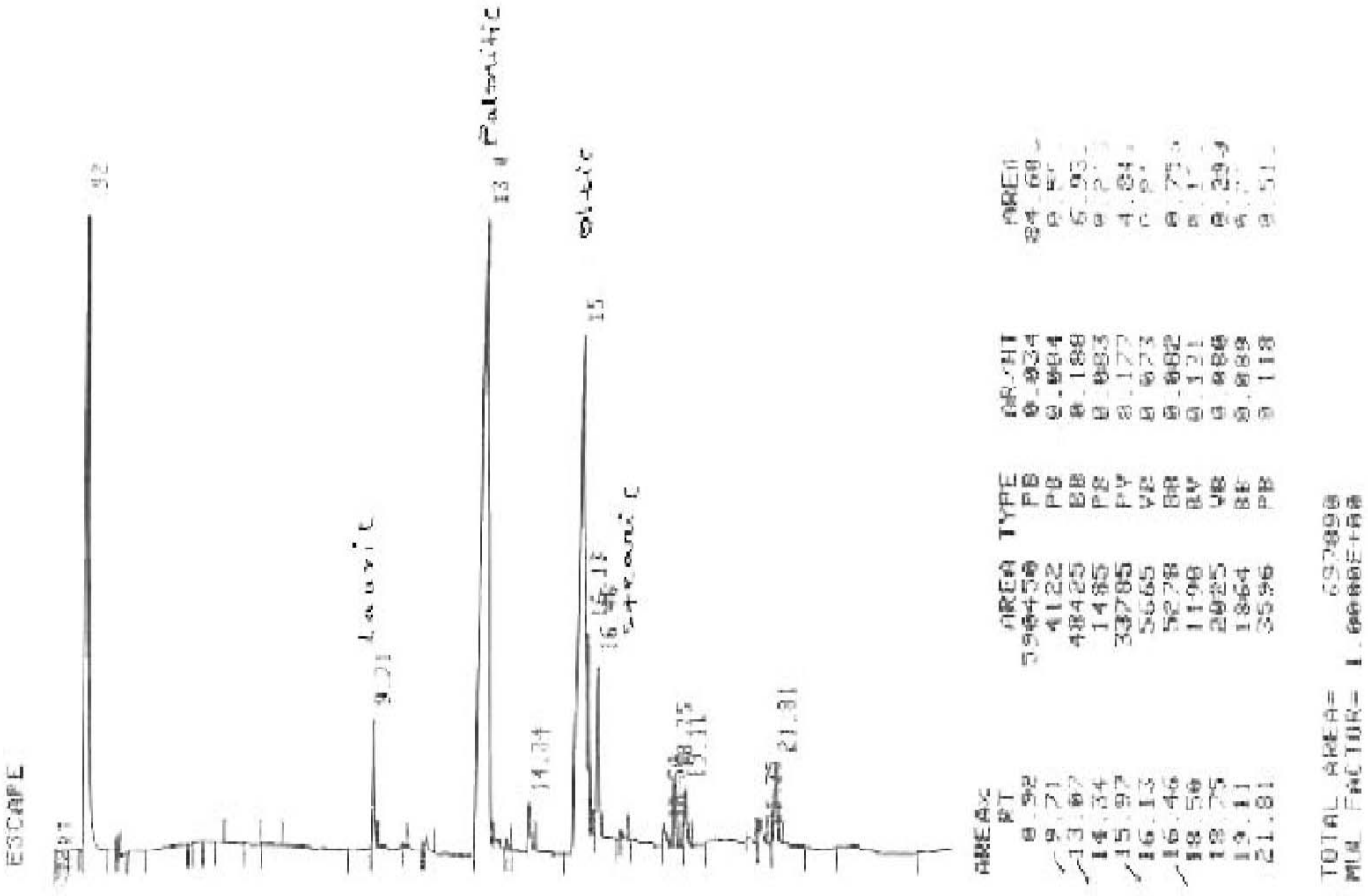

Fig. 1. Fatty acid composition of the methyl ester mixture derived from the lipid (by GLC analysis)

normal (Nestel, 1990). Lipid content and fatty acid composition of marine fishes are differ among species, sex, age, size, reproductive status, geographic location and season (Pigott and Tucker, 1990).

Conclusion

Barramundi are catadromous fish and is native to the tropical waters of northern Australia, Southeast Asia, and southern China. From the current study it can be concluded that due to the presence of appreciable amount of protein, fat, mineral and fatty acid, this fish and 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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