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Biochemical Composition and Heavy Metal Content of Selected Marine Fish from the Gulf of Mannar, India

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Abstract

Biochemical composition of five marine fish; grey bamboo shark, yellow margin triggerfish, pale-edged stingray, laced moray and Japanese leatherjacket fish from the Gulf of Mannar, India were evaluated. Moisture, protein, lipid and ash content for the samples ranged between 75.07-76.74, 17.99-20.97, 1-3.5 and 1.46-1.93% respectively. Profiles of heavy metal, fatty acid and amino acid data were also collected. The predominant fatty acids recorded in all five species were docosahexaenoic acid (DHA), arachidonic acid (AA) and palmitic acid. All five species contain high content of different essential and non-essential amino acids namely glutamic acid, aspartic acid, arginine, serine and lysine. Significant amount of iron (13-50 ppm), zinc (2.5-10.5 ppm) and copper (0.18-4.03 ppm) were found in all the fishes. Among heavy metals, cadmium (0.05-0.1 ppm) was detected which is below permissible limit.

Keywords: Amino acid, fatty acid, nutrient, cadmium, Gulf of Mannar

Introduction

Fish is considered as a rich source of easily digestible protein, unsaturated fatty acids, essential amino acids, minerals and trace elements. Furthermore, some nutritional components of fish (ω-3 fatty acids) have functional effects on human health. The major constituents, amino acids, are responsible for the synthesis of most body tissues, enzymes, hormones and other metabolic molecules (Metailler et al., 1981). Feeding habit, sex, species, seasonal and temporal variations and other climatic factors greatly influence the nutritional composition of individual fish species (Effiong et al., 2008; Damsgaard et al., 2006; Mayer et al., 2006; Dahl et al., 2006; Mozaffarian et al., 2006). Hence nutritional composition of fish has been widely researched.

Grey bamboo shark (*Chiloscyllium griseum*), yellow margin triggerfish (*Pseudobalisters flavimarginatus*), Japanese leatherjacket fish (*Paramonacanthus nipponensis*) are abundant benthic species along the shallow continental shelf of Indo- Pacific ocean. These are commonly used for human consumption in the coastal states of India, China, Taiwan and Thailand. Non-edible fish species like pale-edged stingray (*Dasytis zugei*) and laced moray (*Gymnothorax favagineus*) (Murugan & Namboothiri, 2012) are bycatch which are discarded by fishermen. Trace metals and organochlorine contamination level of bamboo shark from the southern waters of Hong Kong, China has been reported (Cornish et al., 2007). No other appreciable work on biochemical composition and heavy metal contamination in these species has been reported.

The present study gives a comparative account of proximate composition, amino acid, fatty acid and mineral profiles of three edible fish (grey bamboo shark, yellow margin triggerfish and Japanese leatherjacket fish) and two non-edible fish (small size pale-edged stingray and laced moray) collected from Gulf of Mannar, India. The level of cadmium in these fish species was also investigated.

Materials and Methods

Five species of fish namely, grey bamboo shark, yellow margin triggerfish, pale-edged stingray, laced moray and Japanese leatherjacket fish were collected in the month of June from Mandapam landing centre of Gulf of Mannar, India. Approxi-

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mately 5 kg of each fish species were used for the study. Muscle tissue from two individuals of each species was macerated uniformly and stored at -20°C for further studies. All reagents and solvents used in this investigation were of analytical grade. Standards like fatty acid methyl esters, amino acids standard mixture were purchased from Sigma Aldrich (Steinheim, Germany).

Moisture, fat, protein and ash content of all five samples were determined according to the AOAC (2000) method. Total lipid was extracted according to the method of Folch et al. (1957) using chloroform-methanol (2:1). Aliquot of the chloroform layer extract was evaporated to dryness under nitrogen and the lipids were quantified gravimetrically.

Total amino acid composition was determined following the method of Ishida et al., (1981) using a Hitachi amino acid analyzer equipped with an ion exchange column/Shodex CX Pak P-421S, fluorescence detector and post-column derivatization chamber. Quantification was done with the help of external standard mixture of amino acids (Sigma Aldrich, Steinheim, Germany). The results were expressed in g 100 g^{-1} of protein.

Fatty acids methyl esters (FAME) were analyzed by the method of Folch et al. (1957) with modifications as per Sankar et al. (2010). Methyl esters of the fatty acids thus obtained were separated by gas chromatography (Thermo Trace GC Ultra) equipped with a Perkin Elmer Elite 225® capillary column and a flame ionization detector. Identification and quantification were done with the help of FAME external standard mixture. Metals content of all five samples of three replicates were determined according to the AOAC (2000) method.

Results are expressed as Mean ± SD. Multiple comparisons following ANOVA were performed by Duncan's multiple comparison test. A p-value <0.05 was considered as statistically significant. All data were analyzed with the aid of statistical package program SPSS 10.0 for Windows.

Results and Discussion

Proximate composition of all the five fish species is presented in Table 1. No significant variation in moisture content was observed, which ranged from 75.34 to 76.74%. The protein content ranged from 20.97% for pale-edged sting ray to 17.99% for yellow margin triggerfish. It was observed that pale-edged stingray contained lowest amount of lipid (1%) and highest lipid content was observed in grey bamboo shark (3.56%). There is no significant difference in ash content of all five species studied which ranged from 1.46 to 1.93%, wet weight basis. It was interesting to note that two non-edible species pale edged string ray and laced moray contained significant amount of protein and lipid and could be used for either human consumption or feed development. The observed results have good accordance with work done by Yesim (2012) and Nurjanah (2012).

Amino acid profiles of the selected fish are given in Table 2. The major amino acids noticed were aspartic acid (Asp), glutamic acid (Glu), proline (Pro), glycine (Gly), arginine (Arg), alanine (Ala), methionine (Met) and serine (Ser). Glutamic acid and aspartic acid were most prominent amino acids in all five species with values ranged from 10.3 to 18.2 and 5.47 to 12.1 g 100 g^{-1} protein respectively. Essential and non-essential amino acids such as arginine (4.63 g 100 g^{-1} protein) in yellowmargin triggerfish, serine (5.36 g 100 g^{-1} protein) in Japanese leatherjacket fish, glutamic acid (18.2 g 100 g^{-1} protein) in grey bamboo shark and methionine (4.37 g 100 g^{-1} protein) in yellow margin triggerfish. Proline which is biosynthetically derived from amino acid L-glutamate was also high in content (10.20 g 100 g^{-1} protein) in grey bamboo shark. Threonine content was significantly higher in bamboo shark (4.72 g 100 g^{-1} protein) as compared to other four other species. Japanese leatherjacket fish on the other hand was found to be high in tryptophan content (0.83 g 100 g^{-1} protein) as compared to other species.

It is notable that non-edible fish laced moray contained highest amount of lysine (7.63 g 100 g^{-1} protein) among the species studied. The efficiency of a fish diet is dependent on its balance in relation to the specific requirements of the fish species. The amino acid composition of all fishes reported in this study is in good agreement with work done by Portz & Cyrino, (2003). Fish meat is a suitable protein food resource for the healthy adult, children or even for patients during period of inflammation and wound healing, and can satisfy their needs based on their amino acids requirements (Kim & Lall, 2000).

Fatty acid composition of the five fish species is summarized in Table 3. The most abundant fatty acid in all five species was C22:6 (docosahexanoic

Table 1. Proximate composition of fishes (%)

Parameters	Grey bamboo shark	Japanese leatherjacket fish	Laced moray	Pale-edged stingray	Yellowmargin triggerfish
Moisture (%)	75.34±0.49^{a}	76.59±0.52^{bc}	76.07±0.09^{ab}	75.51±0.34^{a}	76.74±0.21^{c}
Protein (%)	20.12±0.24^{a}	19.66±0.09^{a}	18.64±0.06^{b}	20.97±0.75^{c}	17.99±0.39^{b}
Lipid (%)	3.56±0.40^{a}	1.99±0.08^{b}	2.99±0.24^{c}	1.00±0.10^{d}	2.13±0.25^{b}
Ash (%)	1.46±0.15^{a}	1.93±0.11^{b}	1.50±0.20^{ac}	1.93±0.11^{b}	1.83±0.28^{bc}

Results are mean±SD of three duplicates of samples. Values in the row with a different superscript letter (a, b, c) differ significantly ($p<0.05$) with each other.

Table 2. Amino acid composition (g 100 g^{-1} of protein)

Name	Grey bamboo shark	Japanese leatherjacket fish	Laced moray Fish	Pale-edged stingray	Yellowmargin triggerfish
Aspartic acid	12.1±0.26^{a}	5.47±0.24^{b}	10.34±0.14^{c}	6.82±0.10^{d}	7.16±0.05^{e}
Threonine	4.72±0.08^{a}	0.53±0.12^{b}	1.17±0.05^{b}	1.36±0.04^{c}	0.85±0.13^{d}
Serine	3.10±0.08^{a}	5.36±0.33^{b}	2.27±0.05^{c}	2.49±0.06^{c}	3.34±0.24^{a}
Glutamic acid	18.2±0.45^{a}	10.3±0.36^{b}	11.4±0.41^{c}	13.3±0.26^{c}	13.5±0.17^{c}
Proline	10.29±0.20^{a}	4.54±0.11^{b}	0.09±0.02^{c}	ND	5.55±0.54^{d}
Glycine	4.54±0.37^{a}	2.48±0.28^{b}	2.45±0.13^{c}	3.80±0.08^{c}	3.39±0.08^{c}
Alanine	6.13±0.11^{a}	3.12±0.09^{b}	4.2±0.17^{b}	3.09±0.10^{b}	3.71±0.38^{c}
Valine	3.07±0.03^{a}	ND	0.18±0.04^{b}	0.62±0.03^{b}	ND
Methionine	1.90±0.02^{a}	3.44±0.11^{a}	2.58±0.12^{a}	1.29±0.18^{b}	4.37±0.09^{a}
Isoleucine	5.10±0.08^{a}	0.85±0.07^{b}	1.13±0.06^{c}	2.41±0.02^{d}	1.20±0.02^{c}
Leucine	0.19±0.03^{a}	2.55±0.07^{b}	2.41±0.08^{c}	1.19±0.04^{d}	3.17±0.06^{e}
Tyrosine	3.20±0.04^{a}	4.89±0.03^{b}	6.22±0.05^{c}	4.32±0.21^{d}	6.16±0.14^{c}
Histidine	4.72±0.08^{a}	2.54±0.10^{b}	1.83±0.06^{c}	2.56±0.10^{b}	3.31±0.06^{d}
Lysine	2.18±0.04^{a}	2.72±0.24^{b}	7.63±0.07^{c}	1.23±0.07^{d}	3.42±0.18^{e}
Arginine	0.83±0.05^{a}	3.82±0.05^{b}	4.14±0.02^{c}	4.34±0.02^{c}	4.63±0.31^{d}
Tryptophan	0.14±0.07^{a}	0.83±0.07^{b}	0.72±0.14^{c}	0.12±0.10^{a}	0.61±0.30^{d}

Results are mean±SD of three duplicates of samples. Values in the row with a different superscript letter (a, b, c) differ significantly ($p<0.05$) with each other. ND-not detected

acid-DHA) ranging from 15.53 in Japanese leatherjacket fish to 28.06% in yellow margin triggerfish. The other major fatty acids were C20:5 (eicosapentanoic acid-EPA), C18:2 (linoeic acid), C20:4 (arachidonic acid-AA) and C16 (palmitic acid). EPA was highest in pale-edged stingray (6.50%) and lowest in grey bamboo shark (2.33%). AA content was highest (23.36%) in grey bamboo shark and lowest in laced moray (10.13%). Among saturated fatty acids palmitic acid was found to be highest (31.50%) in yellow margin triggerfish.

PUFA/SFA ratio gives a good indication of the quality of the fatty acid composition. In this study, it was observed that pale-edged stingray had best PUFA/SFA ratio of 2.46 and lowest for yellow margin triggerfish, 1.05. The quality of fats is determined by the balance between SFA and PUFA consumed in the diet and is an important feature that influences the risk of cardiovascular disease. Results obtained shows similarity with work done by Narcisa et al., (2011). High n3/n6 ratio of fatty acids has a positive influence on the lipid content. Highest n3/n6 ratio was observed for Japanese leatherjacket 2.65 and lowest for grey bamboo shark. Balanced n3/n6 ratio is helpful for preventing high risk of atherosclerosis and LDL levels (Harris, 2009). The results obtained are in good accordance with work done by Carolina et al., 2014. Significantly high amount of DHA, EPA and AA were present in all five fish species including the two non-edible fish species. DHA and EPA play a significant role in human health and nutrition, especially in infants. DHA and EPA are the major fatty acids in brain tissues (Beitez-Santana et al., (2007); Mourente & Tocher (1992); Florent-Béchardet al., (2007). DHA and EPA had been shown to have preventive effects on human coronary artery disease (Leaf & Webber, 2000). All species of fish studied contained arachidonic acid (C20:4), which is a precursor for prostaglandin and thromboxane biosynthesis (Pompeia et al., 2002). Oil rich in EPA and DHA is known for its positive effects on hypertriglyceridemia. PUFA content in the selected fish is in accordance with Cinzia et al. (2014) who established the importance of PUFA in growth and development of human tissues.

Mineral composition and heavy metal content of the five species is given in Table 4. The major metals taken into account were Cu, Zn, Fe, Cd and Mn. Fe content was found to be highest in pale-edged stingray (55.13 ppm) and lowest in laced moray (12.46 ppm) respectively. Amount of Zn present in different species varied significantly from 1.53 to 10.56 ppm with the highest in laced moray and lowest in pale-edged sting ray. Mn was detected at comparatively lower levels in Japanese leatherjacket fish, laced moray and yellow margin triggerfish ranging from 0.34 to 1.35 ppm. Cu was quantified

Table 3. Fatty acid composition (% fatty acids)

Fatty acids	Grey bamboo shark	Japanese leatherjacket fish	Lacced moray	Pale-edged stingray	Yellowmargin triggerfish
C14	0.54±0.05[a]	ND	0.83±0.05[b]	ND	4.55±0.06[c]
C16 (palmitic acid)	16.30±0.45[a]	13.23±0.45[b]	17.56±0.32[c]	12.50±0.32[d]	31.50±0.3[e]
C16:1 (Palmitoleic acid)	1.30±0.21[a]	0.73±0.04[b]	1.51±0.12[c]	7.12±0.31[a]	ND
C17 (Margaric acid)	1.07±0.05[a]	0.68±0.04[b]	1.33±0.03[c]	1.25±0.07[d]	ND
C18:0 (Stearic acid)	14.63±0.15[a]	7.06±0.04[b]	9.33±0.51[c]	8.10±0.33[d]	11.46±0.09[e]
C18:1n9 (Oleic acid)	9.50±0.26[a]	6.93±0.09[b]	5.43±0.2[c]	5.66±0.22[d]	8.50±0.39[d]
C18:2n6 (Linoleic acid)	2.46±0.13[a]	0.62±0.06[b]	1.09±0.18[c]	2.19±0.10[d]	ND
C18:3n3 (alpha-Linolenic acid)	0.46±0.15[a]	1.10±0.11[b]	0.83±0.06[bc]	1.56±0.07[c]	0.96±0.05[d]
C18:3n6 (gamma-Linolenic acid)	0.63±0.05[a]	0.81±0.03[b]	ND	0.81±0.04[a]	0.67±0.02[b]
C20:4 (Arachidonic acid)	23.36±0.50[a]	11.63±0.08[b]	10.13±0.49[bc]	18.40±0.15[c]	10.76±0.2[d]
C20:5n3 (Ecosapentaenoic acid)	2.33±0.21[a]	5.50±0.77[b]	4.50±0.16[c]	6.50±0.36[d]	3.73±0.25[e]
C22:6n3 (Docosahexaenoic acid)	23.16±0.25[a]	28.06±0.77[b]	24.30±0.73[c]	27.06±0.45[d]	15.13±0.20[e]
C23:0 (Tricosilic acid)	2.71±0.02[a]	3.20±0.07[b]	2.54±0.07[c]	1.12±0.05[d]	ND
PUFA/SFA	1.48±0.05[a]	1.93±0.03[b]	1.29±0.04[a]	2.46±0.05[c]	1.05±0.01[a]
n3/n6 ratio	0.98±0.01[a]	2.65±0.08[b]	2.64±0.06[b]	1.64±0.05[c]	1.73±0.03[c]

Results are mean±SD of three duplicates of samples. Values in the row with a different superscript letter (a, b, c) differ significantly ($p<0.05$) with each other. ND-not detected

Table 4. Mineral composition and heavy metal levels (in ppm)

Minerals (ppm)	Grey bamboo shark	Japanese leatherjacket fish	Laced moray	Pale-edged stingray	Yellowmargin triggerfish
Zn	2.55 ± 0.05^{a}	6.63 ± 0.50^{b}	10.56 ± 0.39^{c}	1.53 ± 0.35^{d}	7.48 ± 0.39^{e}
Cu	0.18 ± 0.06^{a}	1.53 ± 0.41^{b}	0.70 ± 0.07^{c}	0.19 ± 0.58^{a}	4.03 ± 2.02^{d}
Cd	0.05 ± 0.03^{a}	0.11 ± 0.02^{b}	0.09 ± 0.01^{c}	ND	ND
Mn	ND	0.34 ± 0.05^{a}	0.35 ± 0.02^{a}	ND	1.35 ± 0.07^{b}
Fe	17.84 ± 0.32^{a}	13.00 ± 0.4^{b}	12.46 ± 0.58^{b}	55.13 ± 1.00^{c}	20.26 ± 0.45^{d}

Results are mean±SD of three duplicates of samples. Values in the row with a different superscript letter (a, b, c) differ significantly ($p<0.05$) with each other. ND-not detected

in all five species studied and the values ranged from 0.18 ppm (grey bamboo shark) to 4.03 ppm (yellow margin triggerfish) with significant variation. Cd values ranged from 0.05 to 0.1 ppm and values were below the permissible limit.

High amount of important minerals (such as Fe, Mn, Zn and Cu) were observed in all the fish species studied. Fe is a vital mineral required for optimal health, especially for women. Fe, Zn and Mn are an integral part of biological metabolism. Cd detected was negligible, which indicates the safety of the species collected from Gulf of Mannar, as diet or for fish feed development.

The data generated gives valuable information on nutritional value and contaminant levels of commonly available fishes from Gulf of Mannar. All the species were observed to contain good protein content, healthy PUFA/SFA and n-3/n-6 ratio and also found to contain all essential amino acids in significant proportion. Heavy metals were observed to be below permissible limits.

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