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Biochemical Composition of Indian Common Small Pelagic Fishes Indicates Richness in Nutrients Capable of Ameliorating Malnutrition and Age-Associated Disorders

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Abstract

Seafood is an excellent source of metabolically essential proteins, vitamins, trace elements and polyunsaturated fatty acids. There is a rising awareness of the beneficial role of fish and other marine foods in human nutrition. The knowledge regarding the nutritional significance of fish in counteracting the malnutrition and age-associated chronic diseases are relatively scanty. In this present study, an attempt has been made to examine the proximate composition, amino acid profile and fatty acid composition of small pelagic fishes (sardine, mackerel and anchovy) available in Indian south-west coast waters. The biochemical parameters were determined using standard experimental protocols. The low value small pelagic fishes are found to be rich sources of ω-3 Fatty acids [eicosapentaenoic acid (EPA) & docosahexaenoic acid (DHA)], which are very much essential for the development of brain and heart tissues. Sardine and mackerel possess all the essential and non-essential amino acids in a balanced proportion capable of ameliorating the protein deficiency disorders. The non-protein amino acid taurine essential for various physiological functions was also found to be present in rich quantities in small pelagic fishes. Presence of sulphur containing amino acids cysteine and methionine and high histidine content adds functionality to the nutritional value of small pelagic fish protein. Anchovies and sardine are valuable sources of calcium, which can effectively be used to combat calcium deficiency in children. The result of the present study has indicated that small pelagic fishes [sardine, mackerel and anchovy] are potential sources of attenuating malnutrition-related diseases and age-associated disorders.

Keywords: Proximate composition; Amino acid; Fatty acid; Small pelagic fishes; HPLC; Gas chromatography

Introduction

Undernourishment, a dominating quandary, backtrack the development of third world countries, primarily due to the lack of awareness along with awful financial status of middle class population. According to National Family Health Survey in India a significant percentage of men and women are either too lean or too fat because of unhealthy food habits. The scarcity of essential nutrients which leads to increased vulnerability to certain diseases such as common cold or diarrhoea that can kill a malnourished child. As per UN estimates 2.1 million Indian children die before reaching the age of 5 every year-4 every min-mostly from preventable infectious diseases. Researchers are in a hurry to find a solution to save our younger ones from the calamity and they are in a search to explore new resources.

Fish is an important source of animal protein which is available as inexpensive and is easily digestible compared to other animal proteins. Fish is a major source of essential fatty acids and also contains proteins, carbohydrates, lipids, vitamins and trace elements. Fish is extensively consumed worldwide by human as it provides polyunsaturated fatty acids especially ω-3 fatty acids known to support the good health. Major components of ω-3 fatty acids such as EPA and DHA are highly concentrated in the brain and required for early development of cognitive function and visual sharpness [1] and also a major component of cell membranes and precursors of the eicosanoids hormones [2]. Beneficial health effects of n-3 PUFA are well proven and include the prevention of a number of diseases, such as coronary heart diseases, hypotriglyceridemic effect, inflammation, allergies, arthritis, hypertension, autoimmune disorders, and cancer [3]. Studies with new-borns indicate that DHA is vital for the normal functional development of the retina and brain [4]. The dietary recommendations for daily intakes of ω-3 from DHA and EPA ranged between 0.5 and 1.6 g for healthy adults, infants, pregnant and lactating women published by several international scientific authorities [5]. Currently people are more conscious and gave greater concern for their nutritional and health related aspects. The small pelagic fishes commonly known as low value fishes are available sufficiently throughout the season; however the knowledge regarding the nutritional quality of fish is often limited. Generally fresh fish is consumed either cooked or dried form, and also it can be incorporated into different products in various forms. Hence the nutritional profiling is really useful for the better utilization of those fishes as useful food products, complementary foods, by products, feed ingredients, food supplements etc.

Main aim of the study was to investigate the nutritional profile of the three common small pelagic fishes namely sardine, mackerel and anchovy which are easily accessible and affordable to common people, there by stimulate the need for utilization of fish and provide information regarding the nutritional benefits of the above mentioned fishes. Regular consumption of these fishes could reduce the nutritional deficiency to a great extent.

Materials and Methods

Fish sample preparation for biochemical analyses

Fish samples were collected from fish market and stored at a temperature of -18°C in a deep freezer. Fish muscle was separated from samples like sardine and mackerel whereas anchovies as a whole were homogenized to prepare fish mince. Homogenized fish muscle tissue was used for various biochemical analyses.

Analyses

Proximate analyses: Moisture content was estimated by drying 10 g of homogenized sample in hot air oven at 105°C for overnight until a constant weight was obtained [6]. Kjeldahl method was followed to determine the crude protein content. 100 mg samples were digested in concentrated sulfuric acid along with digestion mixture until the solution become colorless. The total nitrogen content was measured and conversion factor 6.25 was used to calculate crude protein composition (AOAC, 2000). Crude fat content of tissue samples were estimated with soxhelet apparatus using petroleum ether as extraction solvent (boiling point 40-60°C) (AOAC, 2000). 1 g of dried sample was taken for analysis. Ash content was estimated by heating the sample in muffle furnace at 600°C for 6-8 h (AOAC, 2000). Sodium, Potassium and Calcium were assayed by flame emission spectrophotometry using Lan way Flame photometer [6].

Amino acid analyses

Amino acid composition of fish samples were estimated by hydrolyzing 100 mg of tissue samples in 10 mL 6N hydrochloric acid at 112°C [7]. The acid content was removed by vacuum evaporation, and then it was made up to 10 mL with 0.05 N HCl and analyzed using HITACHI HPLC (Amino acid analyzer) system equipped with cation exchange column (sulphonated polyvinyl styrene column) and fluorescence detector as shown in Figure 1. Tryptophan content of the samples was assayed spectrometrically after alkali hydrolysis [8].

Lipid extraction and fatty acid analyses

Lipid extraction from fish muscle was carried out using chloroform-methanol (2:1) mixture according to the method described by Folch et al. [9]. Around 30 g of minced fish meat was weighed and homogenized in the solvent for complete lipid extraction [9]. The extract was saponified by reflexing in methanolic NaOH (0.5N), then esterified by reflexing with 14% Boron trifluoride-methanol.

The fatty acid methyl esters (FAME) were analyzed using Perkin Elmer Autosystem XL Gas Chromatograph. The separation was performed in an Elite 225 capillary column measuring 30 m length and 0.25 mm diameter and flame ionization detector using nitrogen as carrier gas at a flow rate of 0.5 mL/min. The injector temperature was maintained at 265°C. The oven temperature was initially held at 110°C for 4 min and programmed to increase to 240°C at a rate of 2.7°C/min, held at 240°C for 3 min and then programmed to increase to 280°C and held for 5 min. Total run time was about 62.15 min.

Statistical analyses

Statistical analyses were repeated thrice and the results were expressed as mean ± standard deviation. Statistical analyses were done with Statistical software (SPSS 14.0). A level of $p<0.05$ was used to designate significant differences among the samples by following One-way ANOVA with Duncan tests.

Result and Discussion

Proximate composition

Fish could be considered as the potential, cost-effective source to enhance micronutrient intakes and also it is capable as a complementary food for undernourished children [10]. Table 1, shows the proximate composition of sardine, mackerel and anchovy. The moisture content of the sardine, mackerel and anchovy ranged between 70.21-77.56%. Similar results were found in the studies by Nisa and Asadullah [11]; Ravichandran et al. [12]. The levels of protein content in all the three species calculated were of 21.29 ± 0.76%, 16.75 ± 0.55% and 16.95 ± 0.27% respectively. Shaji and Kannan [13] reported the protein content in sardine was 23.63% and Nisa and Asadullah [11] reported crude protein in mackerel varied from 16.65% to 20.09%, highest in June and lowest in December [11]. Ravichandran et al. [12], also reported that protein content in sardine and mackerel ranged between 17.04 - 28.01% [12]. Lakshmanan et al., [14] reported protein content in anchovy was 18.5%. Lipid content in sardine, mackerel and anchovy were 3.68 ± 0.17%, 5.03 ± 0.87% and 1.97 ± 0.14% respectively [13]. Nisa and Asadullah [11] reported fat content of mackerel varied from 3.0% to 12.0% ($p<0.05$), highest in December and lowest in January and June. Lakshmanan et al., [13] reported fat content of 0.8% in anchovy. The ash content was 3.5 ± 0.1% in sardine, 4.64 ± 0.06 in mackerel and 4.02 ± 0.08 in anchovy. Shaji and Kannan reported that Sardine consist of 3.94% of ash content [14].

	Sardine (%)	Mackerel (%)	Anchovies (%)
Moisture	70.61 ± 0.41^{a}	75.2 ± 0.5^{b}	76.97 ± 0.59^{c}
Protein	21.29 ± 0.76^{b}	16.75 ± 0.55^{a}	16.95 ± 0.27^{a}
Fat	3.68 ± 0.17^{b}	5.03 ± 0.87^{c}	1.97 ± 0.14^{a}
Ash	3.50 ± 0.1^{a}	4.64 ± 0.06^{a}	4.02 ± 0.08^{b}

Table 1: Proximate composition (%) of Sardine (*Sardinella longiceps*), mackerel (*Rastrelliger kanagurta*) and anchovy (*Stolephorous commersoni*). Mean values of triplicate determination (Mean ± SD). *Mean values within same row followed by different superscripts are significantly different ($p<0.05$).

Table 2, shows the Sodium and potassium content in sardine and mackerel were similar in quantity (4.05 ± 0.02% and 0.8 ± 0.01) compared to anchovy (0.4 ± 0.07% and 0.93 ± 0.04%). The calcium content was significantly higher in anchovy (0.93%) while sardine and mackerel contains 0.68 %.

Minerals	Sardine (%)	Mackerel (%)	Anchovy (%)
Sodium	4.05 ± 0.02^{b}	4.05 ± 0.02^{b}	0.4 ± 0.07^{a}
Potassium	0.8 ± 0.01^{b}	0.75 ± 0.01^{a}	1.89 ± 0.03^{c}
Calcium	0.68 ± 0.02^{a}	0.68 ± 0.01^{a}	0.93 ± 0.04^{b}

Table 2: Mineral compositions (g %) of Sardine (*Sardinel lalongiceps*), mackerel (*Rastrelliger kanagurta*) and anchovy (*Stolephorous commersoni*). *Mean values of triplicate determination (Mean ± SD).

Mean values within same row followed by different superscripts are significantly different ($p<0.05$).

Amino acid composition

Table 3 show the amino acid composition of Sardine (*Sardinella longiceps*), mackerel (*Rastrelliger kanagurta*) and anchovy (*Stolephorous commersoni*). These species contain higher amount of essential amino acids which is important for the normal human health care. The most abundant essential amino acid in the three species were leucine (9.29 ± 0.40% in sardine, 9.62 ± 0.33% in mackerel and 7.99 ± 0.35 in anchovy) followed by lysine, isoleucine and phenylalanine. The predominant non-essential amino acid among the species were glutamic acid (15.71 ± 0.05 in sardine, 15.91 ± 0.16 in mackerel and 14.77 ± 0.23 in anchovy) and aspartic acid (15.56 ± 0.21 in sardine, 15.91 ± 0.16 in mackerel and 11.71 ± 1.02 in anchovy) followed by glycine and alanine.

	Sardine (%)	Mackerel (%)	Anchovy (%)
Essential amino acid			
Threonine	1.98 ± 0.12^{b}	1.06 ± 0.02^{a}	5.50 ± 0.17^{c}
Valine	6.86 ± 0.16^{c}	6.66 ± 0.20^{b}	6.95 ± 0.21^{a}
Methionine	0.98 ± 0.04^{a}	1.61 ± 0.16^{c}	1.44 ± 0.21^{b}
Isoleucine	3.97 ± 0.04^{b}	3.29 ± 0.31^{a}	5.03 ± 0.31^{c}
Leucine	9.62 ± 0.33^{c}	9.29 ± 0.40^{b}	7.99 ± 0.35^{a}
Tyrosine	0.90 ± 0.01^{b}	1.01 ± 0.06^{a}	0.57 ± 0.35^{a}
Phenylalanine	3.52 ± 0.11^{a}	3.70 ± 0.16^{a}	3.74 ± 0.20^{a}
Histidine	4.85 ± 0.11^{b}	5.69 ± 0.27^{c}	3.49 ± 0.29^{a}
Lysine	6.95 ± 0.72^{b}	6.78 ± 0.19^{a}	7.66 ± 0.24^{c}
Tryptophan	0.86 ± 0.05^{a}	0.85 ± 0.05^{a}	1.72 ± 0.27^{b}
Σ EAA	40.49 ± 0.84^{b}	39.94 ± 0.82^{a}	44.09 ± 1.30^{c}
Non-essential amino acids			
Aspartic acid	15.56 ± 0.21^{c}	14.60 ± 0.34^{b}	11.71 ± 1.02^{a}
Serine	2.90 ± 0.16^{b}	2.69 ± 0.16^{a}	5.97 ± 0.26^{c}
Glutamic acid	14.71 ± 0.05^{a}	15.91 ± 0.16^{c}	14.77 ± 0.23^{b}
Glycine	11.48 ± 0.14^{b}	11.93 ± 0.06^{c}	11.04 ± 0.42^{a}
Alanine	10.56 ± 0.49^{c}	10.35 ± 0.17^{b}	8.87 ± 0.46^{a}
Proline	0.12 ± 0.01^{a}	0.21 ± 0.03^{b}	1.51 ± 0.23^{c}
Arginine	3.71 ± 0.18^{c}	3.65 ± 0.22^{b}	0.60 ± 0.44^{a}
Cysteine	0.32 ± 0.01^{a}	0.54 ± 0.21^{c}	0.37 ± 0.30^{b}
Σ NEAA	57.36 ± 0.72^{b}	59.88 ± 0.68^{c}	54.84 ± 1.68^{a}

Table 3: The amino acid composition (%) of Sardine (*Sardinella longiceps*), mackerel (*Rastrelliger kanagurta*) and anchovy (*Stolephorous commersoni*). *Mean values of triplicate determination (Mean ± SD). Mean values within same row followed by different superscripts are significantly different ($p<0.05$).

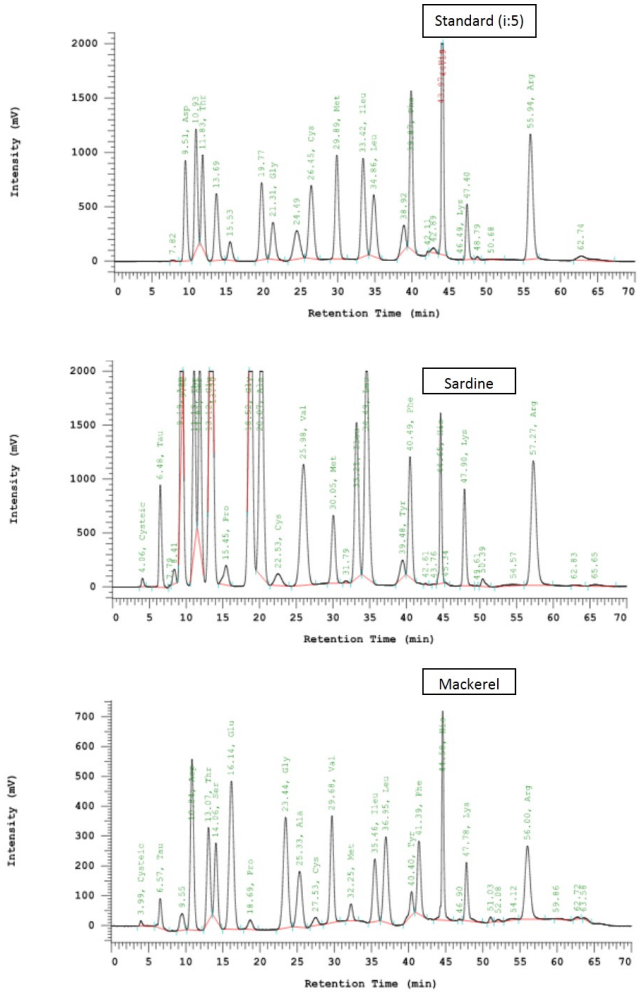

Figure 1: Chromatograms of the amino acid analysis of small pelagic fishes standard (1:5), sardine, and mackerel respectively using HPLC.

Fatty acid composition

Table 4 show the fatty acid composition of sardine, mackerel and anchovy. Compared to saturated fatty acids, unsaturated fatty acids are present in higher amount in all the three species. Similar results were found in the studies by Nisa and Asadullah [11]; Marichamy et al. and Sahena et al. [15,16]. The predominant saturated fatty acid found among these species were palmitic acid (sardine 16.73 ± 0.36%, mackerel 16.75 ± 0.18% and anchovy 30.34 ± 0.74%) ranged between 16.73-30.34% followed by myristic acid (7.77 ± 0.21% in sardine, 2.82 ± 0.05% in mackerel and 4.76 ± 0.55% in anchovy) and stearic acid (8.56 ± 0.49% in mackerel 5.77 ± 0.44% in sardine and 10.87 ± 0.54% in anchovy).

Amid the SFA, palmitic acid (C16: 0) was the major fatty acid which showed seasonal variation like the one reported by Chitra and Radhakrishnan [17]. Bandarra et al., [18] and reported similar findings and concluded that C16: 0 was a key metabolite in fish and did not seem to be influenced by diet [18].

	Carbon length	Sardine (%)	Mackerel (%)	Anchovy (%)
Saturated fatty acids				
Myristic acid	C14	7.77 ± 0.21^{c}	4.82 ± 0.05^{b}	4.76 ± 0.55^{a}
Pentadeeyelic acid	C15	0.878 ± 0.10^{a}	1.88 ± 0.01^{b}	1.78 ± 0.12^{b}
Palmitic acid	C16	20.73 ± 0.36^{a}	23.75 ± 0.18^{b}	30.34 ± 0.74^{c}
Margaric acid	C17	0.81 ± 0.14^{a}	2.95 ± 0.01^{c}	1.35 ± 0.33^{b}
Stearic acid	C18	9.77 ± 0.44^{a}	13.56 ± 0.49^{b}	10.87 ± 0.54^{c}
Others		1.77 ± 0.01^{a}	2.31 ± 0.05^{c}	1.88 ± 0.43^{b}
∑ SFA		41.73 ± 0.63^{a}	49.27 ± 0.79^{b}	50.95 ± 1.36^{c}
Monosaturated fatty acids				
Palmitoleic acid	C16:1	8.57 ± 0.27^{c}	3.88 ± 0.01^{a}	5.26 ± 0.42^{b}
cis-10-pentadecenoic acid	C17:1	1.03 ± 0.21^{c}	0.98 ± 0.11^{b}	0.11 ± 0.15a
Oleic acid	C18:1n9	9.24 ± 0.54^{a}	11.89 ± 0.11^{c}	10.16 ± 0.81^{b}
Gadoleic acid	C20:1	0.97 ± 0.01^{c}	0.12 ± 0.01^{a}	0.27 ± 0.15^{b}
Others	-	1.02 ± 0.03^{b}	2.65 ± 0.01^{c}	0.79 ± 0.38^{a}
∑ MUFA	-	20.83 ± 1.06^{c}	18.52 ± 0.25^{b}	16.59 ± 0.96^{a}
Polyunsaturated fatty acids				
Linoleic acid	C18:2n6	1.32 ± 0.09^{a}	0.16 ± 0.01^{a}	1.24 ± 0.09^{a}
Linolenic acid	C18:3n3	0.65 ± 0.01^{b}	0.03 ± 0.05^{a}	2.17 ± 0.17^{c}
Eicosedienoic acid	C20:2	0.18 ± 0.02^{c}	0.25 ± 0.07^{a}	0.50 ± 0.1^{b}
Arachidonic acid	C20:4	5.42 ± 0.41^{b}	6.75 ± 0.03^{c}	2.03 ± 0.27^{a}
Eicosapentaenoic acid	C20:5n3	20.05 ± 0.31^{c}	18.06 ± 1.03^{b}	2.87 ± 0.76^{a}
Decosahexaenoic acid	C22:6n3	5.44 ± 0.29^{b}	3.47 ± 0.21^{a}	13.83 ± 0.95^{c}
Others	-	1.95 ± 0.86^{c}	1.89 ± 0.68^{b}	1.86 ± 0.03^{a}
∑ PUFA	-	35.01 ± 0.99^{c}	30.61 ± 1.07^{b}	24.50 ± 1.19^{a}

Table 4: Fatty acid composition (%) of Sardine (*Sardinella longiceps*), mackerel (*Rastrelliger kanagurta*) and anchovy (*Stolephorous commersoni*). *Mean values of triplicate determination (Mean ± SD). Mean values within same row followed by different superscripts are significantly different ($p<0.05$).

The major MUFAs observed were oleic & palmitoleic acid. The percentage of Oleic acid in sardine, mackerel and anchovy were 9.24 ± 0.54%, 11.89 ± 0.11% and 10.16 ± 0.81% respectively. Variation was observed in palmitoleic acid content in sardine (8.57 ± 0.27%), mackerel (3.88 ± 0.01%) and anchovy (5.26 ± 0.42). Presence of the Poly unsaturated fatty acid, DHA in sardine, mackerel and anchovy were 5.44 ± 0.29%, 3.47 ± 0.21% and 13.83 ± 0.95% respectively. The EPA content of mackerel, sardine and anchovy were 20.05 ± 0.31%, 18.06 ± 1.03% and 2.87 ± 0.76% respectively. Sufficient intake of EPA and DHA is vital in maintaining an individual's health. A total intake of EPA plus DHA (1200 mg/day) is recommended through the diet that comes from fish/marine sources in a considerable portion. According to Kinsella (1986), an increase in PUFA content will be followed by the decrease in the cholesterol content [19].

Conclusion

The results of the present study revealed that the small pelagic fishes available in Indian waters contain significant amount of essential amino acids and n-3 fatty acids, represent a very precious essential nutrient choice for the maintenance of a healthy body and also indicating their capability in the amelioration of malnutrition and age-associated disorders. Significant values of EPA, DHA and essential micronutrients are demonstrating the nutritional importance of these fishes and their consumption during pregnancy is essential for the normal development of brain, retina and bone tissues of the fetus. Small pelagic fishes are capable of playing a vital role not only in attenuation of malnutrition related problems of young children, but also in counteraction of age-associated aberrations in adults. Hence, it is necessary to include these low value fishes in the diet on a regular basis for human healthcare. Apart from that, the data derived from the present study can be used by various nutrition, health and medicinal groups especially in planning interventional programs and also for initiating long term campaigns towards the consumption of local fishes as major protein, EPA and DHA source.

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