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PROXIMATE COMPOSITION, AMINO ACID AND FATTY ACID PROFILES OF COMMERSON'S ANCHOVY (*STOLEPHORUS COMMERSONII*) AND INDIAN MACKEREL (*RASTRELLIGER KANAGURTA*)

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ABSTRACT – Proximate composition, amino acid profile, fatty acid composition and mineral status of Commerson's anchovy (*Stolephorus commersonii*) and Indian Mackerel (*Rastrelliger kanagurta*) were examined. It is observed that the amino acid composition and fatty acid profile of low value Commerson's anchovy (*Stolephorus commersonii*) is comparable to that of Indian Mackerel (Rastrelliger kanagurta). It is found to be rich in all essential amino acids and polyunsaturated fatty acids in balanced proportions. It is also containing minerals, macro elements and trace elements in significant proportions. The results of the present study indicate that both Commerson's anchovy and Indian Mackerel contain all nutrients in balanced proportions essentially required for the growing children and pregnant women.

***Key word*:** Proximate composition, amino acid profile, polyunsaturated fatty acids, *Stolephorus commersonii, Rastrelliger kanagurta.*

INTRODUCTION

The marine fish landings of India estimated during 2010 was 3.07 million tonnes. The second important resource in terms of contribution towards total landings is Indian mackerel (*Rastrelliger kanagurta*) accounting for 7.9% of total landings. Stolephorus sp. accounted for 79127t in 2010 (CMFRI annual report 2010-11). Pelagic fish, including Indian mackerel and anchovy, are an important marine resource for human consumption. Anchovies are small saltwater fish that grow up to 20 cm and prefer the warmer waters around the world. Commerson's anchovy (*Stolephorus commersonii)*, is one of the commercially important pelagic fish species in India. Indian mackerel (*Rastrelliger kanagurta*) have long bodies with a rather thick appearance.

The role of fish human nutrition is of great importance, as it is a source of high quality, balanced and easily digestible proteins and other important nutrients. The anatomy of fish muscle is different from the anatomy of terrestrial mammals, as the fish lacks the tendinous system connecting muscle bundles to the skeleton of the animal (Huss, 1995). Fish muscle is known to contain an excellent amino acid composition (Venugopal *et al.,* 1996; Yanes *et al.,* 1976) and is a unique source of physiological amino acids (arginine, histidine, lysine, taurine; Marshall, 1994, Metzner *et al.,* 2001). Its proteins and amino acid profiles are quite similar to the muscle of terrestrial animals, but the fish bodies are supported by a mass of water so the muscle fibres require less structural support than the muscles of land animals (Hultin, 1985).

The beneficial effect of fish consumption onhuman health has been related, among other factors tothe high content of μ-3 fatty acids, especially eicosapentaenoic acid (C20:5n -3) and docosahexaenoic acid (C22:6n - 3). The effect of these fatty acids is well documented in numerous investigations, as were reviewed by Horrocks and Yeo (1999) and Leaf *et al* (1999).It has been found that ω -3 fatty acids have a protective effect on heart and tissue related diseases in particular, and they have beneficial effects on hypertension, diabetes, and brain growth and cancer in infants and that its deficiency results in disorders such as skin diseases, anaemia and defective eyesight (Dyberg and Bamg, 1979; Kinsella *et al,* 1990; Carlson and Werkman, 1996; Simopoulos, 2002; Tapiero *et al,* 2002; Sidhu, 2003; Mahaffey, 2004). It is very much important to analyze the biochemical and nutrient property properties of Indian fish species for human health benefits.

Minerals constitute important components of enzymes, enzyme activators and hormones. Lack of essential minerals (e.g., Sodium, Magnesium, Chlorine, Phosphorus, Sulphur, Potassium, Calcium, Manganese, Iron, Copper, Zinc, Selenium) leads to improper enzyme-mediated metabolic functions and results in organ malfunctions, chronic diseases and ultimately death. The metals must

be taken up from water, food, or sediment for the normal metabolism of fish. However, similar to the route of essential metals, nonessential ones are also taken up by fish and accumulate in their tissues (Canlý & Atlý 2003).

In this study we have compared the biochemical component of nutrient profiling of Commerson's anchovy (*Stolephorus commersonii*) with Indian Mackerel (*Rastrelliger kanagurta*) with respect to the proximate, amino acid and fatty acids composition.

MATERIALS AND METHODS

Sample collection

The Commerson's anchovy (*Stolephorus commersonii*) and Indian Mackerel (*Rastrelliger kanagurta*) used in the experiments were collected from fish landing center at Vishakapatanam, India. Fish samples were obtained under iced condition in insulated styrofoam boxes, and transported to the laboratory. The samples were cleaned with distilled water and surface water was blotted with filter paper and the edible meat was homogenized to form mince. The proximate composition, fatty acid profiles amino acid profiles and minerals, were determined.

Chemicals

All reagents and solvents used in this investigation were of analytical grade. Standards like fatty acid methyl esters, amino acids, etc were purchased from Sigma–Aldrich GmbH (Steinheim, Germany).

Determination of moisture

Moisture of the fish samples was determined according to the AOAC (2000) method by drying in a hot air oven at 105°C. 10g of homogenized sample until a constant weight was obtained. Results were expressed as percentage of wet weight.

Ash and Mineral analysis

Ash content was determined by burning sample for 12 h in a furnace at 525°C according to the AOAC (2000) method. Results were expressed as percentage of wet weight. Minerals were assayed using AOAC method. Macro elements were determined by flame photometry using working standards in the range of 10-40 ppm for each element (Na, K and Ca). Trace metals (Cu, Fe, and Zn) were determined by Varian Spectra-220 AA atomic absorption spectrophotometer. Samples were aspirated into the flame and the corresponding absorption of the characteristic radiation by each element was recorded. Values are expressed in ppm.

Table 1: Comparison of proximate composition of Commerson's anchovy and Indian mackerel.

Compositions (% wet wt.)	Mackerel	Anchovy
Protein	19.2 ± 0.18	17.68 ± 0.14
Lipid	2.64 ± 0.05	1.81 ± 0.07
Moisture	76.17 ± 0.26	77.37 ± 0.27
Ash	1.99 ± 0.04	3.14 ± 0.05

Values are expressed as mean±SD for three replicates

Determination of Protein

Total protein content in the homogenized samples (0.2g) was determined using Kjeldahl method (AOAC, 2000). A conversion factor (6.25), by which the concentration of nitrogen is measured was used to convert total nitrogen to crude protein. Results were expressed as percentage of wet weight basis.

Determination of Lipid content

The estimation of crude fat content was done by continuous extraction of fat with petroleum ether according to AOAC method (2000). Total lipids were extracted according to the method of Folch *et al.,* (1957) using

Table 2: Comparison of Amino acid (%) composition of Commerson's anchovy and Indian mackerel.

Amino acid	Mackerel	Anchovy
Asparatate	8.58±0.39	9.11±0.51
Threonine	3.08±0.14	4.66±0.30
Serine	2.05±0.13	6.83±0.72
Glutamic Acid	3.71±0.41	10.3±0.31
Proline	1.07±0.01	2.80±0.20
Glycine	2.96±0.25	7.97±0.03
Alanine	1.92±0.03	7.4±0.31
Cysteine	0.25±0.01	0.56±0.02
Valine	2.7±0.36	3.81±0.24
Methionine	1.05±0.038	1.48±0.35
Isoleucine	2.26±0.17	5.29±0.17
Leucine	5.21±0.22	9.37±0.38
Tyrosine	0.95±0.06	2.63±0.05
Phenyl alanine	2.05±0.06	3.68±0.18
Histidine	0.96±0.03	3.43±0.20
Lysine	3.28±0.14	6.44±0.49
Arginine	0.65±0.017	3.17±0.57
Tryptophan	2.49±0.30	2.79±0.20

Values are expressed as mean±SD for three replicates.

chloroform/methanol (2:1). Aliquots of the chloroform layer extract were evaporated to dryness under nitrogen and the lipids were quantified gravimetrically.

Amino acids analysis

Total amino acid composition was determined following the method of Ishida *et al* (1981) using a Shimadzu chromatograph LC-10AS amino acid analyzer equipped with an ion exchange column, quaternary pump, a 20 µl injection valve and fluorescence detector. Mobile phase (Buffer A) contained trisodium citrate, distilled ethanol and perchloric acid (pH 3.2) and Buffer B had sodium citrate, NaOH and Boric acid (pH 9.8). The flow rate was constant at 0.4 ml/min, and the oven temperature was set at 60°C. The fluorescence excitation and emission wavelengths were 340 nm and 450 nm respectively. 100 mg of samples were hydrolyzed in 6 N HCl in evacuated sealed tubes at 110°C for 24 h. After derivatization by o-phthalaldehyde, amino acids were identified and quantified by comparison of their retention times with those of standards (Sigma). The results were expressed in percentage.

Table 3 : Comparison of Fatty acid composition (% of fatty acids in terms of total fatty acids) of Commerson's anchovy and Indian mackerel.

Fatty acids	Mackerel	Anchovy
C 12:0	0.131±0.13	0.736±0.075
C 13:0	0.0223±0.02	1.153±0.06
C 14:0	6.497±0.25	4.26±0.06
C 15:0	0.729±0.73	1.36±0.08
C 16:0	26.705±0.82	21.1±0.19
C 17:0	1.296±0.03	1.39±0.08
C18:0	8.585±0.07	10.29±0.40
Others	2.191±0.18	2.62±0.20
Total Saturated	46.157±1	42.91±1
MUFA		
C 16:1, n7	6.599±0.17	6.207±0.08
C 17:1,n7	0.041±0.01	ND
C18:1, n9	5.381±0.12	18.12±0.29
C20:1, n9	0.327±0.05	0.186±0.07
C22:1, n9	0.064±0.01	ND
Others	0.647±0.05	0.14±0.02
Total MUFA	13.059±0.25	24.65±0.39
PUFA		
C 18:2, n6	1.684±.005	1.68±0.59
C 18:3, n3	0.376±0.02	1.30±0.26
C 20:2, n6	0.459±0.15	0.56±0.07
C 20:3, n6	0.158±0.001	0.17±0.06
C 20:4, n6	1.520±0.22	2.77±0.62
C 20:5, n3	9.142±0.13	4.99±0.31
C 22:6, n3	25.447±0.51	16.97±0.48
Others	1.338±0.18	3.53±18
Total PUFA	**40.125±0.72**	**31.96±1.7**
Grand Total	**99.34±0.59**	**99.52±0.72**

Values are expressed as mean±SD for three replicates

Table 4 : Comparison of Macro Minerals profile (g %).

Minerals	Sodium	Potassium	Calcium
Mackerel	0.107±0.06	2.397±0.08	0.07±0.05
Anchovy	0.362±0.04	2.347±0.05	1.4±0.01

Values are expressed as mean±SD for three replicates

Fatty acid analysis

Fatty acids methyl esters (FAMEs) were analyzed by the method of Metcalfe *et al* (1966) with slight modifications (Sankar *et al,* 2010). A fraction of the lipid extract was saponified with 0.5N NaOH in methanol followed by methylation in 14% boron trifluoride in methanol (BF_3/MeOH). Methyl esters of the fatty acids thus obtained were separated by gas chromatography [Thermo Trace GC Ultra] equipped with a capillary column [30m long and 0.25mm diameter] and a flame ionization detector. The carrier gas was nitrogen and the flow rate was 0.8ml/min initial temperature was set as 110°C and was increased 2.7°C/min until a temperature of 250°C was obtained. Injector and Detector temperature was kept at 260°C and 275°C respectively. Fatty acids separated were identified by the comparison of retention times those obtained by the separation of a mixture of standard fatty acids. Measurement of peak areas and data processing were carried out by Thermochrom card software. Individual fatty acids were expressed as a percentage of total fatty acids.

Table 5 : Comparison of Micro Mineralsprofile (g%).

Minerals	Copper	Iron	Zinc
Mackerel	0.001±0.04	0.005±0.01	0.013±0.03
Anchovy	0.001±0.08	0.004±0.01	0.021±0.03

Values are expressed as mean±SD for three replicates

RESULTS AND DISCUSSION

Crude protein content in the muscle was usually in the broad range of 11–24% (wet weight), depending on the species and variety, the state of nutrition, and the reproductive cycle of the animals, as well as the parts of the organisms (Sikorski, 1994; Spinelli and Dassow, 1982). Protein content of mackerel is 19.2 g% that of anchovy is 17.68g%. Protein content of anchovy is less than that of mackerel. According to Suzuki (1981) the white meat contains less lipids than the dark meat and is usually composed of about 18% to 23% of protein, depending on the species and time of harvesting). However, significant variations in protein content are observed between fish species, and a relation seems to exist between chemical composition and depth of occurrence (Childress and Nygaard, 1973; Yancey *et al,* 1992).

In mackerel lipid content is higher as compared to anchovy.Moisture content of anchovy is higher than mackerel. The lipid and moisture shows an inverse relationship between moisture and fat. This is in concordance with studies by Devi (2006), Ninan (2003) and Mohan, *et al* (2008). Ash content of mackerel is lower than that of anchovy.

Essential amino acids are essential for the growth and maintenance of the body, and they want to supply through diet. These fishes contain all the essential amino acids, so consumption of both fishes is good for health. The level of essential amino acid is higher in anchovy than that of mackerel, Therefore, It is advisable that to consume anchovy, especially in growing age and pregnancy.

Generally, the fatty acid compositions of fish oil vary with their feeding habits, environmental conditions, age, maturity and type of species (Çelik *et al,* 2005; Haliloglu *et al,* 2004; Ratkowsky *et al,* 1996; Saito *et al,* 1999). For example, a marine planktonic diet results in low levels of n-6 PUFA, of which EPA and DHA are the predominant fatty acids (Justi *et al,* 2003). Essential fatty acids like eicosapentaenoic acid (EPA-C20:5 n-3) and docosahexaenoic acid (DHA-C22:6 n - 3) content is very high in mackerel when compared to anchovy. DHA is essential for the normal functional development of the retina and brain, particularly in premature infants (Conner, 2000). Both mackerel and anchovy contain high levels of DHA. So consumption of these fishes in the time of pregnancy is recommended DHA content is high in mackerel in compared to anchovy. According to Osman *et al* (2001) total PUFA content in Indian mackerel body was 53.4%.

Fish oil supplementation decreases the psoriatic lesions (Ziboh *et al,* 1986; Maurice *et al,* 1987; Bittener *et al,* 1988; Dewsbury *et al,* 1989; Mayser *et al,* 1998). This effect might be attributed to the presence of ω-3 PUFA present in it (Turini *et al,* 1994; Lee *et al,* 1984; Lee *et al,* 1985; Nathaniel *et al,* 1985; Letkowith*et al,* 1990). ù-3 PUFA's have lipid lowering property, so it decreases they risk of cardio vascular diseases (Von *et al,* 1985; Dyerberg *et al,* 1978; Harris *et al,* 1983; Phillipson*et al,*1985; Flaten*et al,*1990).

Studies by Zuta *et al* (2003) have shown that Indian mackerel processing wastes comprising skin, viscera and muscle tissue were subjected to urea complexation and the concentrated PUFA content was found to be 22.6%, 15.2%, and 21.2%, respectively.

Minerals have an important role in human metabolism (Belitz *et al,* 2001). Increased calcium level is seen in anchovy compared to that of mackerel. It is because of the bone present in the mince of the anchovy. In the case of anchovy bones are also edible. So anchovy is the source of calcium it is beneficial for growing children to meet their calcium needs. It is observed that the mineral content is significantly lower as compared to that of anchovies. The minerals accumulate in fish body through the food chain and water. However, it has been demonstrated that the concentration of trace minerals in fish tissues depends on various factors such as nourishment sources, biological differences, seasonal factors and environmental conditions (Lal, 1995; Zeynali *et al,* 2009). In conclusion, Commerson's anchovy is rich in essential amino acids and fatty acids required for the human healthcare. Inexpensive anchovies also contain significant amount of nutritionally important elements and minerals. Hence, Commerson's anchovy may be considered as a healthy food item containing essential nutrients required for the growing children and pregnant women.

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