Research Article

Fatty Acids, Amino Acids and Mineral Composition in *Rohtee ogilbii* from Nira River, Bhor. Maharashtra (India).

Nirbhay Sudhir Pimple[1]*, Sanjay S. Kharat[2]
[1]Assistant Professor, Department of Zoology, Abasaheb Garware College, Karve Road, Pune, India.
[2]Head of Zoology Department, Principal, Modern College of Arts, Commerce and Science, Ganeshkhind, Pune, Inda.
***Corresponding author's E-mail:** nirbhay.pimpale@yahoo.com

Accepted on: 11-06-2016; **Finalized on:** 31-07-2016.

ABSTRACT

The paper presents a comparative analysis of proximate Amino acids, fatty acid and mineral composition of *Rohtee ogilbii* which is an endemic fish species from Western Ghats of Maharashtra. The study depicts the nutritive value of various essential amino acids along with mineral content. Eight essential amino acids with total amount of 2674µg/g were identified. Lysine formed the highest value, followed by leucine. Lipid constituted about 15.67% moisture accounted to 75.27% and Protein content was 24.76%. Environmental factors like age, sex, loss of solids during spawning, richness of fish food in river system could be responsible for variation in protein lipid and mineral contents of fish. Minerals included potassium (39%), phosphorous (24%), calcium (07%), sodium (07%), and magnesium (03 %); while zinc, iron, aluminium and copper were present in trace amounts. The result obtained in the study shows that *Rohtee ogilbii* is a good source of proteins, minerals and essential amino acids and has high nutritional value.

Keywords: *Rohtee ogilbii*, protein, carbohydrates, lipids, minerals, moisture, Essential amino acids, Lysine, Atomic Absorption Spectrophotometer.

INTRODUCTION

Fish is known as a rich source of protein diet. Fish meat contains significantly low lipids and higher water than beef or chicken and is recommended over white or red meats.[1,2] Fish lipids are excellent sources of the essential polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) derive mainly from fish.[3] Fish is rich in active protein which has a unique composition of amino acids, a high omega-3 polyunsaturated fatty acid content such as eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic (22:6 n-3, DHA) and fat-soluble vitamins as well as it represents a good source of micro- and macro-elements.[4,5] The shortage of α-linolenic acid (18:3 n-3, ALA) is responsible for neurological disorders and poor growth.[6] The presence of DHA and EPA have shown significant positive effect in preventing hypertension and cardiovascular diseases[7] and it has beneficial effects by improving defence mechanisms and also show anti-inflammatory activity of long-chain n-3 PUFAs.[8] Essential polyunsaturated fatty acids such as ALA, EPA and DHA are not synthesized in the human body and effectively synthesized only by aquatic organisms; therefore, humans can receive these essential fatty acids by marine and freshwater fishes.[9,10] Fresh water bodies throughout the globe are deteriorating in their physio-chemical status. This is due to the influx of sewage, industrial effluents, and agricultural discharge along with pharmaceutical waste which find their way towards these water bodies. These pollutants find their way in the aquatic fauna and ultimately in tissues of Fish. They interfere with the biochemical process of the organism and disturb the biochemical process.[11] Fish meat contains significantly low lipids and higher water than beef or chicken and is recommended over white or red meats.[12,13] There is increasingly demand of fish. However, information concerning the chemical composition of freshwater fishes in general is valuable to nutritionists concerned with readily available sources of low-fat, high-protein foods such as most freshwater fishes.[14,15]

Moreover, the measurement of some proximate profiles such as protein contents, lipids and moisture contents is often necessary to ensure that they meet the requirements of food regulations and commercial specifications. In recent years, the significance of polyunsaturated fatty acids especially the n-3 and n-6 fatty acids, has gained much attention because of their various biological activities in health and disease management. Recently, several investigations about meat quality and safety of fish from our fishponds were carried out.[16,17] No such studies has been carried out on *Rohtee ogilbii* hence, it was important to assess the lipid content and fatty acid composition. *Rohtee ogilbii* is been consumed by a large population and hence understanding the chemical composition and nutritional quality of this fish would help in formulation of balanced food products, it would provide us with the status of the nutritive value of the raw fish and its benefits to the consumer and would be of great information for commercial fishery Unit. Hence, the study on the total lipid content and fatty acid composition was undertaken.

MATERIALS AND METHODS

Rohtee ogilbii is an endemic species from Western Ghats of Maharashtra. 50 individual of similar body weight and length were collected from Nira River located at Bhor

(Maharashtra) at 18° 10' 0" N Latitude/73° 51' 0" E longitude.

All fish specimens were washed, and placed in sterile universal bottles and kept at -20 °C. About 30 gms of fish muscle tissue was separated and used for the determination of different tests. Total protein content was estimated by Lowry's method (1951),[18] carbohydrate by Anthrone reagent method),[19] lipid by Folch & Moisture and Ash by AOAC method (2000). The concentration of mineral elements was determined using Atomic Absorption Spectrophotometer (AAS) and calculated in ppm (µg/g dry weight). The muscle tissue was analyzed for its proximate composition.

Water Content (%)

Fish tissue contain maximum amount of water. Fresh fish sample was scarified for the moisture estimation. Automatic moisture analyzer (IR 120, Denver, Moisture analyzer) was used for the estimation. Fish tissue was separated and 1 gm of the tissue was exposed to 100 °C and then gradually the temperature was raised upto to 170 °C until a stable was obtained. The moisture was estimated from the weight loss due to exposure to heating. The moisture was estimated in terms of percentage.

$$Water\ Content\ (\%) = \frac{(Initial\ Weight - Final\ Weight)}{Initial\ Weight} \times 1000$$

Ash

(AOAC-2000)[20] was used to determine the ash content from the fish sample under study. Fish tissues were cleaned washed and 5gms was taken in a previously ignited and weighed silica crucible. Then it was transferred to muffle furnace (Phoenix CEM Corporation, USA) the temperature was maintained at 600 °C for 6 hours which resulted in the fish ash formation. The ash was allowed to cool and the weight difference was calculated.

Carbohydrate by Anthrone Reagent Method (Hedge & Hofreiter 1962)

100 mg of fish tissue was hydrolyse by keeping in boiling water bath for three hours with 5ml of 2.5N Hydrochloric acid and then cool to room temperature. It was then neutralise with solid sodium carbonate until the effervescence ceases. The volume was graduated to 100ml and then centrifuged. 0.5 and 1ml aliquots were used for analysis. The standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. The test tube was graduated to 1ml by adding distilled water which was followed by adding 4ml anthrone reagent. The test tubes was heated in boiling water bath for eight minutes after cooling the reading was obtained for green to dark green coloured absorption maximum at 630 nm.

Carbohydrate present in 100 mg of the sample = (mg of glucose/volume of test sample) X 100

Total Lipid was Estimated by Folch Method

1gm of tissue was homogenised with chloroform/menthol (2/1) and the volume was made 20 times the volume of the tissue sample (1 g in 20 ml of solvent mixture). The whole mixture was then agitated for 15-20min in an orbital shaker at room temperature. The homogenate was filtered. The solvent was then washed with 0.2 volume (4 ml for 20 ml) of water or better 0.9% NaCl solution. After vortexing some seconds, the mixture was centrifuged at low speed (2000 rpm) to separate the two phases. The upper phase was removed by siphoning and kept it to analyze small organic polar molecules. The interface was rinsed one or two times with methanol/water (1/1) without mixing the whole preparation. After centrifugation and siphoning of the upper phase, the lower chloroform phase containing lipids was evaporated under vacuum in a rotary evaporator or under a nitrogen stream if the volume is under 2-3 ml.

Total Protein Estimation

Total Protein Estimation was carried out by standard Lowry's Method. 50 well plates were used to prepare the assay using Bovine Serum Albumin (BSA) as standard protein. The absorbance was measured at 595 nm.

Table 1: Proximate composition (g/100g, dry weight) and caloric values of *Rohtee ogilbii*

Nutrients	*Rohtee ogilbii*
Ash	0.57
Carbohydrates	4.16
Lipids	15.67
Moisture	75.27
Protein	24.76

Values are expressed as ± Standard deviation

Table 2: The mineral constituents (ppm, µg/g dry weight) and ratios (%) of *Rohtee ogilbii*

Minerals	ppm, µg/g dry weight	%
Aluminum	11	1
Calcium	2195	07
Copper	0.8	1
Iron	24	1
Magnesium	723	03
Phosphorous	6845	24
Potassium	11367	39
Sodium	2215	07
Zinc	268	1
Total minerals	**23648.8**	

Table 3: Different groups of amino acids and total ammonia of *Rohtee ogilbii.* The values are expressed as concentrations (µg/g dry weight) and ratios (%)

Amino acids	µg/g dry weight	%
Acidic amino acids	426	15.6
Amino acids with aromatic ring	251	11
Amino acids with OH-group	321	15.1
Amino acids with SH-group	132	04.3
Basic amino acids	553	17
Non-polar amino acids	991	34
Total amino acids	**2674**	**2674**
Total ammonia (µg/g)	38	38

Table 4: Essential amino acids (EAA) profile of *Rohtee ogilbii* values are expressed as (µg/g dry weight) in muscle tissues

Amino acids	µg/g dry weight
Leucine	223.3
Isoleucine	128.1
Valine	157.6
Threonine	150.1
Lysine	273.7
Histidine	096.3
Methionine	112.1
Phenylalanine	128.2
Total EAA	1269

RESULTS AND DISCUSSION

Proteins, lipids Carbohydrates, moisture contents and Ash were considered in evaluating the nutritional value of the species studied. *Rohtee ogilbii* is consumed by large population in rural as well as urban region from the state of Maharashtra and Karnataka (India). The result obtained showed the nutritional elements of crude protein recording the highest values and lipid recording the lowest. This makes the fishes important living resources of dietary protein as other sea and freshwater fish.[21,22] Protein estimation is an important tool in assessing various climatic and diseased conditions as it is highly useful in exploiting health status of animals.[23] High lipid fishes had less water and more protein than low-lipid fishes. This is in-line with the report of Steffens[24] that protein forms the largest quantity of dry matter in fish. The mineral constituent of Rohtee ogilbii was studied. The result obtained showed considerable concentrations of potassium, phosphorus, sodium and calcium, which makes the fish as good source of minerals. The concentration of Potassium in the present study was high in comparison with other minerals. Few heavy metals analyzed were present within tolerable limits.

They are in accordance with the study carried out by. The variation in different minerals recorded in *Rohtee ogilbii* could be due to the concentration of these minerals in the water body[25] and the physiologically capacity of the fish to absorb and convert the essential nutrients from the diet or the water bodies where they live. This is supported by the findings of[26,27]. The concentration of Zinc in the present study was 258 ppm, µg/g which is higher this may be due to zinc pollutants which enter the water body and find its way in fish tissue.

Zinc is used as a major component in artificial paints which are used to colour the Ganesh Idol, which are later immersed in water after the festival, there by adding to high level of Zinc in water body.

Microelements are required for the biochemical process in trace amount, but they tend to become harmful when their concentrations in the tissues exceed the metabolic demands.[28]

CONCLUSION

The result obtained in the study shows that *Rohtee ogilbii* is a good source of proteins, minerals and essential amino acids and has high nutritional value.

Basic amino acids forms 17%, Acidic amino acids forms 15.6%,non-polar amino acids with aliphatic side chains constituted 15.1%, amino acids with a sulphur group forms 04.3%, and amino acids with aromatic ring on the side chains forms 11%.

The present work will help to understand the composition of fish which is good sources of protein and minerals. It would be a very informative for commercial fishery science.

- Author (last name) AB (initials), Author BB, Title of Article, Journal name, **volume, year, page numbers.** Doi: xxxx; PMID: xxxxx.

REFERENCES

1. Neil J S, Fish consumption, fish oil, lipids, and coronary heart disease, Circulation, 94, 1996, 2337-2340.
2. Nestel PJN, Fish oil and cardiovascular disease: lipids and arterial function. Am. J. Clin. Nutr., 71, 2000, 228-231.
3. Vignesh S. R., & Srinivasan M. Nutritional quality of processed head and bone flours of Tilapia (*Oreochromis mossambicus*, Peters 1852) from Parangipettai estuary, South East Coast of India. *Asian Pacific Journal of Tropical Biomedicine,* 2012, 368-72.
4. Luczynska J., Markiewicz K. and J Jaworski, Interspecific differences in the contents of macro- and microelements in the muscle of six fish species from lakes of the Olsztyn lake district (North-East of Poland). *Polish Journal of Food and Nutrition Sciences,* 15(1), 2006, 29–35.
5. Beaven R. (Captain) FRGS, Handbook of the fresh water fishes of India. (1990). Narendra Publishing house, Delhi-110006.
6. Cundiff D. K., Lanou A. J. and C. R. Nigg, Relation of omega-3 fatty acid intake to other dietary factors known to reduce

coronary heart disease risk. *American Journal of Cardiology,* 99, 2007, 1230–1233.

7. Kris-Etherton P. M., Harris W. S. and L. J. Appel, for the nutrition committee. AHA scientific statement. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation,* 106, 2002, 2747–2757.
8. Dorea J. G., Persistent, bioaccumulative and toxic substances in fish: human health considerations. *Science of the Total Environment,* 400, 2008, 93–114.
9. Sushchik N. N., Gladyshev M. I. and G. S. Kalachova, Seasonal dynamic of fatty acid content of a common food fish from the Yenisei River, Siberian grayling, *Thymallus arcticus*. *Food Chemistry,* 104(4), 2007, 1353–1358.
10. Nirbhay S. Pimple and Sanjay S. Kharat Concentration of Heavy Metals in Few Tissues of, *Rohtee Ogilbii* From Nira River Bhor District (Maharashtra). India. International Journal of Scientific research. (2013), 517-519.
11. Neil JS Fish consumption, fish oil, lipids, and coronary heart disease, Circulation, 94, 1996, 2337-2340.
12. Nestel PJN Fish oil and cardiovascular disease: lipids and arterial function. Am. J. Clin. Nutr., 71, 2000, 228-231.
13. Mozaffarian MD, Rozenn NL, Lewis HK, Gregory LB, Russell PT, Davis SS Cardiac benefits of fish consumption may depend on type of fish meal consumed. Circulation. 107, 2003, 1372-1382.
14. Foran JA, Carpenter DO, Hamilton MC, Knuth BA, Schwager SJ, Risk- based consumption advice for farmed Atlantic and wild pacific salmon contaminated with dioxins and dioxin-like compounds. Environ. Health. Persp., 33, 2005, 552-556.
15. AOAC Association of Official Analytical Chemists Official Methods of Analysis. (17th ed.). W. Hortuntzed (Ed), Washington. (2000).
16. Spirić A., Trbović D., Vranić D., Đinović J., Petronijević R., Milijašević M., Janković S. and T. Radičević, Uticaj masnih kiselina u hrani na sastav masnih kiselina i količinu holesterola kod kalifornijske pastrmke (*Oncorhynchus mykiss*). *Tehnologija mesa,* 50(3–4), 2009, 179–188(Sr).
17. Ćirković M., Trbović D., Ljubojević D. and V. Djordjević, Meat quality of fish farmed in polyculture in carp ponds in Republic of Serbia. *Meat technology,* 52, 2011, 106–121.
18. Lowry O. H., Rosenbrough A. L., Farr & Randall R. J. Protein measurement with Folin phenol reagent. J. Biol. Chem., 193, 1951, 265.
19. Folch J., Lees M and G.H. Sloane Stanley. *J. Biol. Chem*., 226, 1957, 497-509.
20. AOAC Association of Official Analytical Chemists Official Methods of Analysis. (17th ed.). W. Hortuntzed (Ed), Washington. (2000).
21. Vlieg P, Murray T Proximate composition of albacore tuna, Thune's alalunga, from the temperate South Pacific and Tasman Sea. N. Zealand J. Marine Freshwater Res., 22, 1988, 491-496.
22. Zuraini A, Somchit MN, Solihah MH, Fatty acid and amino acid composition of three local Malaysian Channa spp. Fish Food Chem., 97, 2006, 674-678.
23. Guha P., Kaptan E., Bandyopadhyaya G., Kaczanowska S., Davila E., Thompson K., Martin S. S., Kalvakolanu D. V., Vasta G. R., and Ahmed H. Cod glycopeptide with picomolar affinity to galectin-3 suppresses T-cell apoptosis and prostate cancer metastasis". Proceedings of the National Academy of Sciences. DOI: 10.1073/pnas. (2013), 1202653110.
24. Steffens W. and M. Wirth, Influence of nutrition on the lipid quality of pond fish: common carp (*Cyprinus carpio*) and tench (*Tinca tinca*). *Aquaculture International*, 15, 2007, 313–319.
25. Yeannes IM, Almandos ME, Estimation of fish proximate composition starting from water content. J. Food Comp. Anal., 16, 2003, 81-92.
26. Ricardo CM, Cyrino JEP, Portz L, Trugo LC, Effect of dietary lipid level on nutritional performance of the surubim, Pseudoplatystoma coruscans. Aqua. 209, 2002, 209-218.
27. Adewoye SO, Fawole OO, Omotosho JS, Concentrations of selected elements in some freshwater fishes in Nigeria. Sci. Focus, 4, 2002, 106-108.
28. Hogstrand C, Wood CM. The physiology and toxicology of zinc in fish, In E. W. Taylor (Ed.), Toxicology of Aquatic Pollution, Cambridge: University Press, 1996, 61-84.

Source of Support: Nil, **Conflict of Interest:** None.