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**ANALYSIS OF ANTIOXIDANTS, MINERALS AND VITAMINS
COMPOSITION BETWEEN MALE AND FEMALE INDIAN MACKEREL
- *RASTRELLIGER KANAGURTA***

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ABSTRACT

A majority of consumers do eat fish because of its availability, flavor and palatability, while few do so because of its nutritional value. The muscle tissue was analysed for antioxidant, minerals and vitamin content between both the sexes. Tocopherol and catalase were present in high amount when compared to catalase and SOD. Both male and female counterpart contains high amount of calcium when compared to other minerals analysed. Both the sex contains the presence of riboflavin in huge amount than thiamine. There was no significant deviation in all the parameter analysed between the sexes.

Key Words: *Mackerel, Rastrelliger kanagurta, Antioxidants, Minerals, Vitamins*

INTRODUCTION

Fish is a major source of food to man kind. It provides a significant amount of poly unsaturated fatty acids intake in the diet of a large proportion of people in the developing countries. Along the east coast of India *Rastrelliger kanagurta* is the most popular fish species used as the food (Lakshman *et al.*, 1999). The nutritional value of fish meat comprises the contents of moisture, dry matter, protein, lipids, vitamins and minerals plus the caloric value of the fish. The human body usually contains small amount of these minerals and the deficiency in these principal nutritional elements induces a lot of malfunctioning; as it reduces productivity and causes diseases (Mills, 1980). Besides being used as food, fish is also increasingly demanded for use as feed. It is also useful to the ecologists and environmentalists who are interested in determining the effects of changing biological/environmental conditions on the composition, survival, and population changes within fish species. The nutritional component of the fresh water fish was found to differ between species, sexes, sizes, seasons and geographical localities. Fish consumption has several nutritional and health benefits which are mainly due to the presence of proteins, unsaturated fatty acids, minerals (calcium, iron, selenium zinc etc) and vitamins including A, B3, B6, B12, E, D (Sidhu 2003). The study of mineral elements present in living organisms is of biological importance; since many of such elements take part in some metabolic processes and are known to be indispensable to all living things (Shul'man, 1974). The body usually contains small amount of these minerals, some of which are essential nutrients, been components of many enzymes system and metabolic mechanisms and as such contribute to the growth of the fish. The most important mineral salts are that of calcium, sodium, potassium, phosphorous, iron, chlorine while many others are also needed in trace amounts. The deficiency in these principal nutritional mineral elements induces a lot of malfunctioning; as it reduces productivity and causes diseases, such as inability of blood to clot, osteoporosis, anemia etc. (Shul'man, 1974 and Mills, 1980). The nutritional value of fish meat comprises the contents of moisture, dry matter, protein, lipids, vitamins and minerals plus the caloric value of the fish (Evangelos *et al.*, 1989; Chandrashekar and Deosthale, 1993 and Steffens, 2006).

Mackerel is a very important group of commercial fish tanking second in annual tonnage of marine fishes landed. It is in great demand in fresh condition. 60% consumed fresh, remaining preserved. Large percentage (40%) of the catch is preserved in ice and dispatched to inland areas and 60% catch is salt

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cured or pickled or sun dried or smoked. When the catch is abundant, the surplus is converted into manure which is used for coffee & coconut plantation. The viscera & gills discarded from the curing & canning plants is used in the preparation of fishmeal, Poultry feed, cattle feed & manure. They are of importance not only to India, but also too many countries bordering the Indian ocean & western pacific ocean for which reason several international integrated research projects are sponsored by organization like the indo pacific fisheries council of the FAO.

Therefore, considering these parameters, this study aims in the analysis of difference in the nutritive value of male and female Indian mackerel, *Rastrelliger kanagurta* which is revealed by the measurement of vitamins, some minerals and antioxidants between the two sexes.

MATERIALS AND METHODS

Intermoult *Rastragellier kanagurta* of both sexes, weighing approximately 250g were collected from the local fishermen. The muscle tissues were dissected out for the analysis of various biochemical parameters like antioxidants, minerals and vitamins.

Estimation of Antioxidants

Ascorbic acid was analysed by the spectrophotometric method described by Roe and Keuther (1943). The method proposed by Reddy *et al.*, (1995) was adopted for assaying the activity of Superoxide dismutase. Tocopherol was estimated in the plant samples by the Emmerie-Engel reaction as reported by Rosenberg (1992). Catalase activity was assayed following the method of Luck (1974).

Estimation of Minerals

The iron was estimated by Ramsay's method (1952). Phosphorus was estimated by the method of Fiske and Subbarow (1925). The determination of copper was carried out using diethyl dithio carbamate following the method of Jeffrey *et al.*, (1989). Calcium was estimated titrimetrically using potassium permanganate.

Extraction of Sample and Estimation of Thiamine

Following the method of Bradbury and Singh (1986b), 5g of finely grind tissue samples (from both male and female) is taken in a pestle and mortar in the presence of 20ml of 0.1N sulphuric acid and centrifuge this suspension at 4000 rpm for 20 minutes. Decant the supernatant and extract residue again with the 10ml of the acid. Pool the collected supernatant fractions into 50ml volumetric flask and make the final volume to the mark with 0.1N sulphuric acid.

Added 3ml of potassium ferric cyanide reagent to the suitable portion of thiamine extract followed by 3ml of 15% sodium hydroxide ensure that the solution was alkaline shake the contents for 30 seconds. Add 10ml of isobutanol shake the mixture thoroughly allowed it to stand till the two phases separate out completely. Discard the lower aqueous layer by adding a small amount of solid anhydrous sodium sulphate. Pipette out volume of standard solution containing 0.5 mg of thiamine hydroxide and measure the fluorescence intensity of the standard and a secondary filtrate at 435nm. From the reference curve determine the result in terms of μg of thiamine for 1g of sample using the following formula: Concentration of unknown = $\frac{U-B}{s-b} \times \text{Concentration of standard}$.

Estimation of Riboflavin

Following the method of Bradbury and Singh (1986b), 2ml of each of the homogenized tissue samples were taken in a test tube and add 0.5ml of glacial acetic acid and 0.5ml of 15% potassium permanganate shake well and stand for 1 minute. Add 0.5ml of 3% hydrogen peroxide till the color disappear completely. Now added 10ml of isobutanol pyridine mixture shake for 30 seconds. The tubes were stand undisturbed for 10 minutes and discard aqueous layer. Add a pinch of sodium sulphate with small dropping funnel to remove trace of moisture shake it and keep it in dark.

RESULTS AND DISCUSSION

The levels of antioxidants among male and female *Rastragellier kanagurta* was shown in Table-1, Figure-1. Free radicals are continuously formed in the body but the aerobic organisms employ a battery

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of defense mechanisms such as antioxidant enzymes glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) to prevent or mitigate oxidative tissue damage (Halliwell *et al.*, 1989). When the liver cell plasma membrane is damaged, many of the enzymes normally located in the liver cell cytosol are released into the blood stream. Their estimation in the blood is a useful quantitative marker of the extent and type of hepatocellular damage (Mitra *et al.*, 1998). In addition, perturbation of the GSH status of a biological system has been reported to increase the lipid peroxidation (Uday *et al.*, 1999). Thiobarbituric acid reactive substances, TBARS, are produced as by-products of lipid peroxidation that occurs in the hydrophobic core of biomembranes (Fraga *et al.*, 1987). At other sites, intake of compounds that induce antioxidant enzyme activity or scavenging of free radicals prevents oxidative damage (Hochstein *et al.*, 1988). Antioxidants are the substances that when present at a low concentration compared with those of the oxidizable substances considerably delay or inhibit the oxidation of the substrate. Antioxidants act as a major defense against radical mediated toxicity by protecting the damage caused by free radicals (Nayan and Janardhanan, 2000).

The variation in the levels of various antioxidants like tocopherol, ascorbic acid, and superoxide dismutase, catalase in male and female *Rastrelliger kanagurta* fish were determined and compared. Of these antioxidants, Catalase shows highest free radical scavenging property when compared to SOD. Similarly, tocopherol was found to present in large amount when compared to the level of Ascorbic acid in both the sex. A similar trend was noticed in the female counter part also. Since, the levels of all the antioxidants and scavenging enzymes were slightly high in female in comparison to male, it indicates that the female *Rastrelliger kanagurta* was under more stress and possesses higher free radical scavenging potential. This present study shows significant variation in the non enzymatic antioxidants such as ascorbic acid and tocopherols. But there is no variation in the superoxide dismutase and catalase.

Table 1: Changes in the level of antioxidants in male and female *Rastrelliger kanagurta* (Values of Mean \pm SD, N=5, mg/g wet tissue weight)

| S. No. | Antioxidants | Male fish | Female fish |
|--------|----------------------|----------------|----------------|
| 1. | Ascorbic acid | 1.3 \pm 0.04 | 1.6 \pm 0.04 |
| 2. | Tocopherols | 12.6 \pm 0.2 | 13.6 \pm 0.1 |
| 3. | Superoxide dismutase | 1.03 \pm 0.9 | 1.2 \pm 0.1 |
| 4. | Catalase | 40.9 \pm 0.8 | 40.6 \pm 0.5 |

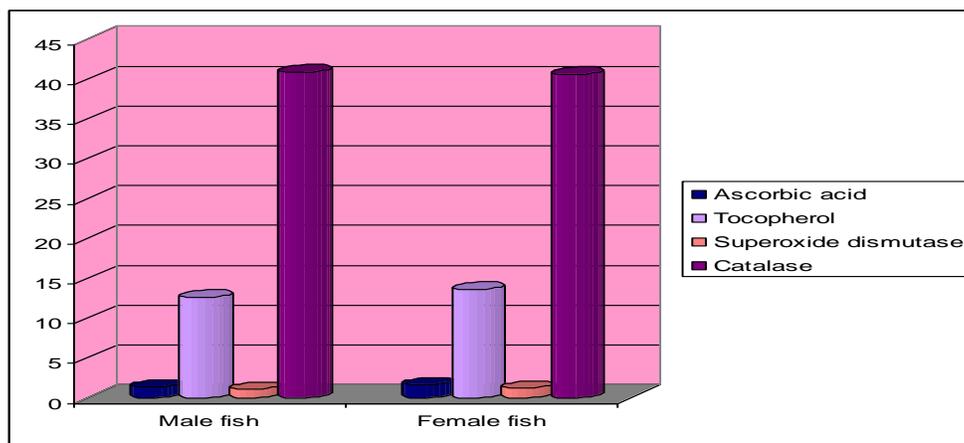


Figure 1: Changes in the level of antioxidants in male and female *Rastrelliger kanagurta*

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Table 2: Changes in the level of minerals in male and female *Rastrelliger kanagurta* (Values of Mean \pm SD, N=5 mg/g wet tissue weight)

| S. No. | Mineral constituents | Male fish | Female fish |
|--------|----------------------|--------------------|--------------------|
| 1. | Copper | 0.01 \pm 0.001 | 0.02 \pm 0.001 |
| 2. | phosphorus | 0.36 \pm 0.05 | 0.46 \pm 0.05 |
| 3. | Iron | 0.001 \pm 0.0005 | 0.002 \pm 0.0005 |
| 4. | calcium | 1.6 \pm 0.04 | 1.8 \pm 0.04 |

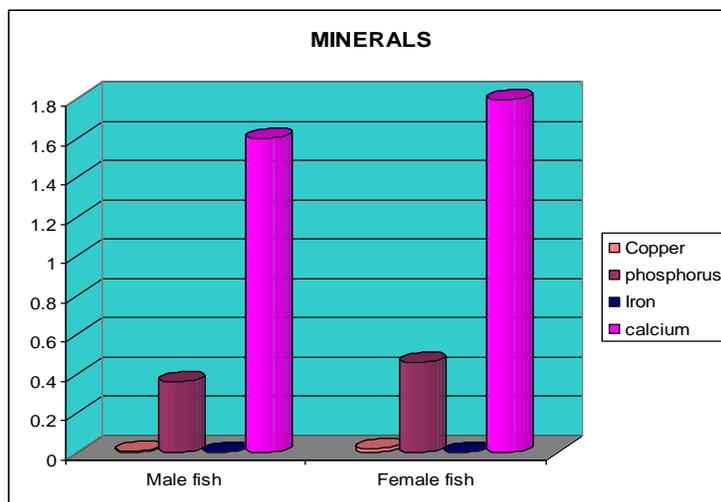


Figure 2: Changes in the level of minerals in male and female *Rastrelliger kanagurta*.

Minerals are required to regulate osmotic balance. Bone formation and integrity and as components in enzymes and hormones. Minerals were absorbed directly from water through gills and skin. The composition of various mineral constituents like copper, phosphorus, iron, calcium in male and female *Rastrelliger kanagurta* was shown in Table-2, Figure-2. Of these, calcium was present in significantly higher quantity in comparison to all the other constituents followed by phosphorous. The presence of equal amount of copper and iron correlates well with their biological role as copper is essential for the absorption of iron in fish. The high amount of calcium reflects the huge content of calcium present in the natural habitat of this species. Since, the levels of all the minerals were slightly high in female in comparison to male. There is significant variation in minerals like calcium, phosphorous, iron and copper under this study, but relatively almost similar content when the comparison is made sex wise. The variations recorded in 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). Variations recorded in the values obtained for both calcium and phosphorous could be due to an increase in the proportion of bone to flesh as the fishes grow; this is in conformity with the submission of Boyd and Davis (1978).

Thiamine and Riboflavin are vitamins, also called vitamin B1 & B2 respectively. Vitamin B1 is found in many foods including yeast, cereal grains, beans, nuts, and meat. It is often used in combination with other B vitamins and found in many vitamin B complex products. Vitamin B complexes generally include vitamin B1 (thiamine), vitamin B2 (riboflavin) and others. Thiamine is used for AIDS and boosting the immune system, diabetic pain, heart disease, alcoholism, aging, a type of brain damage called cerebellar syndrome, canker sores, vision problems such as cataracts and glaucoma, motion sickness, and improving athletic performance (<http://www.nlm.nih.gov/medlineplus/druginfo/natural/965.html>).

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Table 3: Changes in the level of vitamins in male and female fish of *Rastrelliger kanagurta*

| S. No. | Vitamins constituents | Male fish | Female fish |
|--------|-----------------------|-------------|-------------|
| 1 | Thiamine | 0.01±0.005 | 0.01±0.005 |
| 2. | Riboflavin | 0.003±0.005 | 0.002±0.005 |

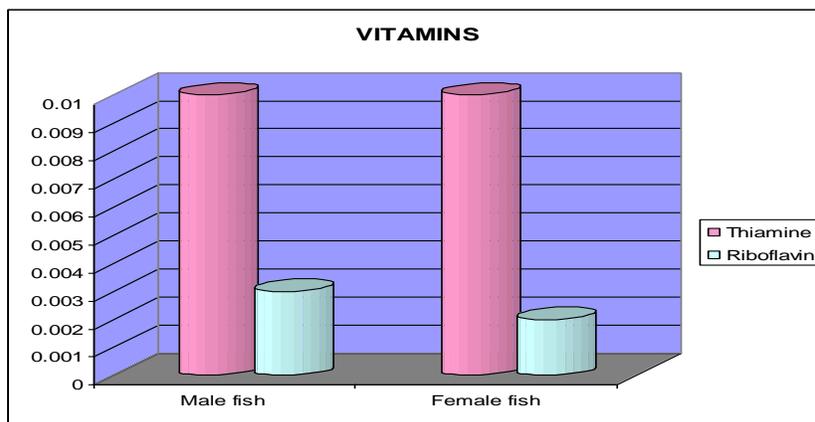


Figure 3: Levels of Vitamins in male and female *Rastrelliger kanagurta*

An assessment of the vitamin content was carried out between both the sexes of *Rastrelliger kanagurta*, thiamine and riboflavin in particular which are important for the normal growth of the fish. The results shown in Table-3, Figure-3 indicates similar trend as witnessed with the other two parameters determined above i.e. the minerals and the anti-oxidants. The results voice that there isn't a substantial difference in the total thiamine content between the two sexes, whereas the male had a meager excess quantity of 0.001mg of riboflavin in comparison to female. This present study shows variation in vitamin riboflavin. But there is no significant variation in thiamine under this study.

The variations recorded in the concentration of the different nutritional components in the fish examined could have been as a result of the rate in which these components are available in the water body (Yeannes and Almandos, 2003).

CONCLUSION

Results from the study have clearly shown that both the sex of *Rastrelliger kanagurta* used in the study contained considerable amount of antioxidants, vitamins and minerals which are vital in supplementing nutrition to mankind. It can therefore be concluded from the study that differences and similarities in nutritive contents with respect to almost all the minerals and vitamins under study between both the male and female *Rastrelliger kanagurta* fish do exist. The variation in nutrient composition in fish can be related to factors such as habitat, feeding habits, human activities and industrial processes. Hence, it was clear that it is not possible to assess the nutritional supremacy between the male and female *Rastrelliger kanagurta*.

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