

Biochemical Composition, Mineral Content and FTIR analysis on five different tissues of fresh water fish *Heteropneustes fossilis* in Tenkasi district

M.Umamageshwari*, S. Uma Rajeswari **, R. Sivasakthi **

PG and Research Department of Zoology, Pasumpon Muthuramalinga Thevar College, Melaneelithanallur, Affiliated to Manonmaniam sundarnar university, Tirunelveli, Tamilnadu, India.

Abstract

The nutritional qualities of Carbohydrate, Protein, Lipid, Moisture and Minerals of *Heteropneustes fossilis* were investigated. The investigation showed that the chemical composition is not the same in all fishes. It was also observed that the biochemical composition of fish depends mostly on size, age, sex, season, as well as breeding season. In this study the protein content was high in liver was found to be 111.317 ± 0.37 and ovary in 106.033 ± 0.33 *Heteropneustes fossilis*. The carbohydrate content was high in muscle and liver were 4.473 ± 0.03 , 4.417 ± 0.19 respectively among the species. Lipids content was more in intestine 4.35 ± 0.03 . Comparatively less quantity of moisture are estimated in the ovary and liver. A mineral content like Calcium and Sodium content was high in liver was found to be 155.31, 95.370 ppm. Iron and potassium was high in ovary in 10.473, 42.504 ppm. All biochemical parameters differ significantly ($P < 0.001$) among the species under investigation. Research exploration has been conducted in order to find the better way for exercising the wastes product generated from food processing diligence. The present work is a compendium of information on biomedical and pharmaceutical uses of fish processing wastes.

Keywords: *Heteropneustes fossilis*, protein, lipid, liver, ovary, and minerals etc.

INTRODUCTION

Fresh water fish constitutes an essential source of protein in our food [1]. The depletion of fishery resources from the sparkling water throughout the world, as properly as developing dietary deficiency in growing country, underscore the requirement for greater utilization of handy fishery assets as human meals [2]. The biochemical and mineral composition shows the fish quality. Therefore, proximate biochemical and mineral composition of a fish helps to examine its dietary and suitable for eating price in phrases of strength in contrast to exclusive fishes. The predominant parts of fish are additionally divided into four categories, namely; protein, carbohydrate, lipid, and moisture. The chemical composition is historically used as an indicator of the dietary value, in addition, due to the fact of the physiological circumstance of fish and its habitat [3, 4]. The biochemical evaluation of these ingredients may range considerably from species to species, having a bet on age, sex, surroundings and season [5, 6]. Fish obtained high-quality molecule and vital vitamins for human diets [7, 8]. Fishes appearing lengthy migrations earlier than they attain the particular floor would possibly make use of protein moreover to lipids for energy, so depleting every the lipid and protein reserves, main to an everyday discount of the organic compound of the fish [9]. The biochemical composition of fish is intently regarding feed intake, migratory and sexual adjustments in reference to spawning. The fish will have hunger intervals for herbal or physiological motives such as at some point of migration, spawning or attributable to exterior elements like as scarcity of food. Fishes are the expensive components of excessive grade protein and one of kind natural products. They are critical supply of animal protein and have been extensive ordinary as a supply of protein, carbohydrate and lipid for the renovation of wholesome physique [10]. Protein contents of fish give rich source of some important amino acids [11]. These vital amino acids and protein are ultimately providing health benefits for human beings [12]. Most of the undeveloped countries of are facing lot of nutritional deficiency due to low levels of proteins, vitamins and minerals in their diet [13]. Lipids are molecules composed of fatty acid and glycerol that makes up the fats and oils. Water in the form of moisture, is necessary for all organisms to maintain homeostasis in the body. Fish meat is usually preferred in respect of other white and red meat as it has low levels of lipid and high levels of water when compared with chicken, mutton, and beef [14]. There are inverse relationships between water and lipid contents while investigation fish muscle. If in case moisture levels are low in percentage, than fat and

protein contents would be seen high in percentage with more energy stored in fat form in fish muscle [15]. Fish meat is also rich source of minerals and most abundant micro elements such as calcium, sodium, magnesium, phosphorus, iron and potassium etc. are also present. *Heteropneustes fossilis* is famous for being cheap and best source of essential protein amongst medium sized fish species for their biochemical composition in order to make it good choice for the people of locality. The current examination on the nutritional compounds of edible and economical fishes provides a substantial range of nutritional information, bringing to our attention the richness of healthy nutrients present in the eatable portion of fish. The study concludes that locally obtainable fish food can be a substantial aid in redressing the problems of malnutrition and can help in pharmaceutical industries to formulate drugs and medicines obtained from biochemical assessment of fresh water fishes.

MATERIALS AND METHODS

Collection of samples

Heteropneustes fossilis were collected from Tenkasi rearing pond. They were treated with 5% KmnO₄ for five minutes for dormant disinfection. The fish weighing 100-150 gms of *Heteropneustes fossilis* was maintained at constant temperature of $28 \pm 1^{\circ}\text{C}$ under laboratory conditions for acclimation. Fish sample in fresh organ like liver, intestine, gills, muscle and ovary were washed, filtered, finely minced and homogenized for chemical analysis

Estimation of Biochemical Parameters

The Folin- Ciocalteu phenol method of Lowry [16] was adopted for the estimation of protein. The carbohydrate was estimated by Anthrone method [17]. Estimation of lipid [18] respectively. Moisture content of the samples was determined according the method of [19]. Pre weighed dry samples were dried the moisture content in an oven at 105°C until a constant mass was obtained.

Aim and Objective of Present Study

The aim of the Present study was to determine the relative composition of Carbohydrate, Protein, Lipids, moisture and minerals in *Heteropneustes fossilis*.

To detect the proximate composition of Carbohydrate, protein, lipids, moisture and minerals in the liver, gills, intestine, muscle and ovary.

To establish the biochemical relationship among the FTIR Spectroscopy.

RESULTS

Proximate analysis

The current investigation offers with proximate composition in carbohydrate, protein lipid moisture and mineral values in liver, intestine, gills, muscle, and ovary of *Heteropneustes fossilis*. Both, seasonal and size wise variation in biochemical, mineral and FTIR analysis are tabulated in Table 1-7., Fig. 1 and 3 respectively. This end result interpreted that proximate composition of protein, carbohydrate, lipid, moisture and minerals in fish relies upon on season, intercourse and reproductive cycle. The biochemical parts of the physique components of *H. fossilis* have been proven in Table 1 and figure 1 of the bio molecules analyzed, protein, carbohydrate, lipid and moisture had been observed to be most in liver, muscle and ovary. The liver of *H.fossilis* consists of the excessive quantity of protein (111.317 ± 0.37 mg/g) whereas the gills comprise low quantity of protein (21.470 ± 0.18 mg/g). The carbohydrate content used to be excessive in muscle (4.473 ± 0.03 mg/g) and low in gills (2.92 ± 0.36 mg/g). The whole lipid values excessive of gut (3.15 ± 0.03 mg/g) and low quantity of lipid in gills (1.202 ± 0.05 mg/g). The moisture content excessive of intestine (77.46%), and low of liver (67.76%) respectively (Fig 2). Minerals elements of tissues of *H.fossilis* were presented in the table 2 and figure 3. Among the estimated minerals, calcium was high in liver and muscle (155.31, 103.622 ppm) respectively. The high sodium content recorded in liver was 95.370 ppm. The both iron and potassium was high in ovary (10.473, 42.504 ppm).

Characterization of the tissue of *H.fossilis* (FT-IR) Spectral Analysis:

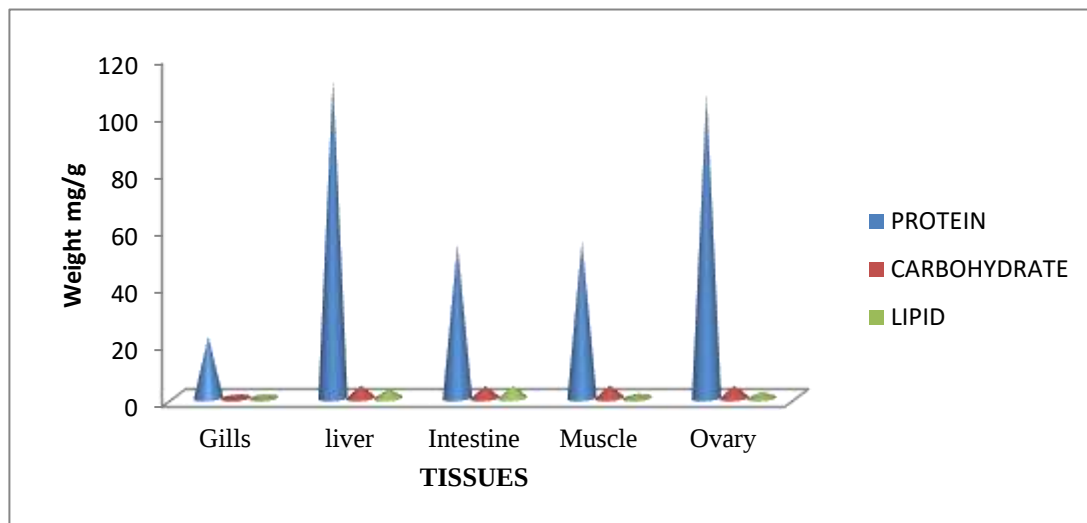
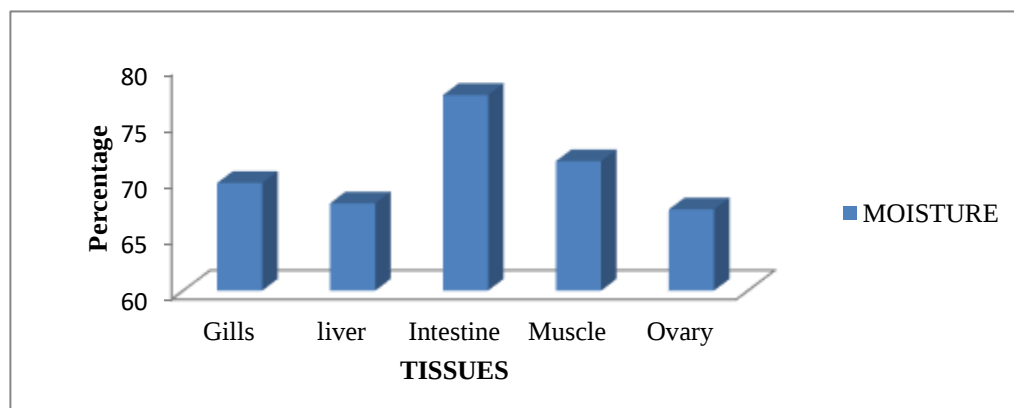
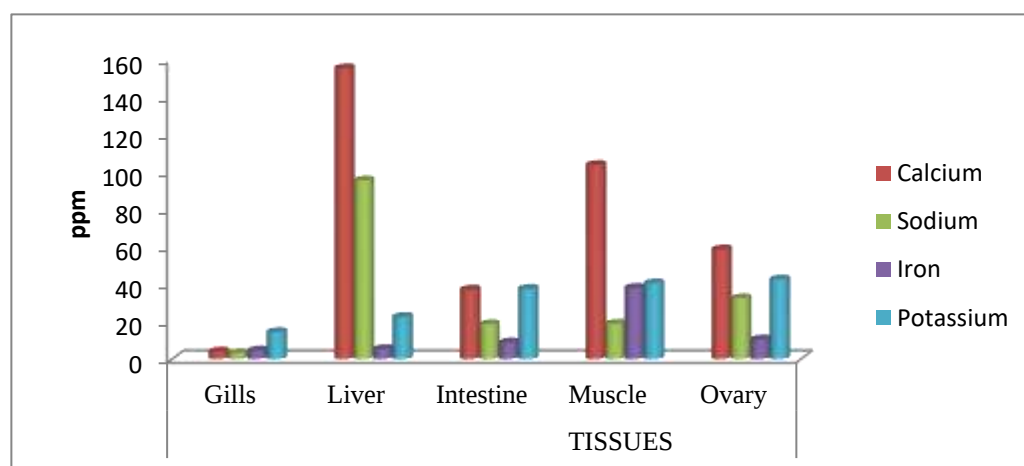
The current find out about used to be carried out to analyze the composition of the tissues of the freshwater fish *H. fossilis* the important of FTIR spectroscopy. The depth and the absorption bands in FTIR spectrum are at once associated to the molecules (Damian *et.al.*, 2007). The FTIR spectra of the fish tissue of *H. Fossilis* in the $4000-400$ cm^{-1} range. The spectrum incorporates various bands springing up from the contribution of one-of-a-kind practical companies belonging to protein, carbohydrates, and lipids. The absorption band and undertaking had been proven in Table 3, 4, 5, 6 and 7. The principal peaks depicted in the figures indicate the robust band at frequencies displaying their corresponding practical team in *H.fossilis*. From the frequency table, the pattern confirmed the presence of important practical companies for the presence of protein and lipid.

TABLE 1. Biochemical composition of *H. fossilis* Tissues

S.NO	Biochemical constituents (mg/g)	TISSUES				
		Gills	Liver	Intestine	Muscle	Ovary
1	Protein	21.470±0.18	111.317±0.37	53.563±0.08	54.861±0.28	106.033±0.33
2	Carbohydrate	1.202±0.05	4.417±0.19	4.198±0.35	4.473±0.03	4.334±0.14
3	Lipid	1.16±0.01	3.15±0.01	4.35±0.03	1.57±0.05	2.15±0.01
4	Moisture (%)	69.61	67.79	77.46	71.56	67.25

TABLE 2. Minerals composition of *H. fossilis* Tissues

S.NO	Mineral elements (ppm)	TISSUES				
		Gills	Liver	Intestine	Muscle	Ovary
1	Calcium	4.035	155.31	37.126	103.622	58.548
2	Sodium	3.148	95.370	18.591	18.856	32.500
3	Iron	4.582	5.334	8.861	38.027	10.473
4	Potassium	14.421	22.516	37.541	40.463	42.504

Figure 1. Biochemical constituents in *H. fossilis* tissue sample**Figure 2. Moisture content in *H. fossilis* tissue sample****Figure 3****Minerals content in *H. fossilis* tissue sample**

Characterization of the tissue of *H.fossilis* (FT-IR) Spectral Analysis

Table 3.FTIR frequency of *H.fossilis* Gills

S.NO	IR frequency Number(cm^{-1}) Intestine	Definition of the spectral assignments
1	1637.66	C=O amide I band, protein
2	1560.41	C-N Amide II band, mainly protein
3	1459.08	Stretching-C=O inorganic carbonate
4	1384.36	CH and CH ₂ aliphatic bending group, Protein and Lipid
5	1113.41	Amide III band region, mainly protein

Table 4.FTIR frequency of *H.fossilis* Liver

S.NO	IR frequency Number(cm^{-1}) Liver	Definition of the spectral assignments
1	1637.30	C=O amide I band, mainly protein
2	1540.07	C-N Amide II band, mainly protein
3	1458.87	Stretching-C=O inorganic carbonate
4	1384.30	CH and CH ₂ aliphatic bending group, Protein and Lipid
5	1110.95	Amide III band region, mainly protein

Table 5.FTIR frequency of *H.fossilis* Intestine

S.NO	IR frequency Number(cm^{-1}) Intestine	Definition of the spectral assignments
1	3450.05	OH of carbohydrate, Protein, and polyphenol
2	1637.60	C=O amide I band, protein
3	1458.81	Stretching-C=O inorganic carbonate
4	1384.32	CH and CH ₂ aliphatic bending group, lipid
5	1111.47	Amide III band region, mainly protein

Table 6.FTIR frequency of *H.fossilis* Muscle

S.NO	IR frequency Number(cm^{-1}) Muscle	Definition of the spectral assignments
1	2341.38	C=C conjugated C=C, lipid
2	1637.68	C=O Amide I band, mainly protein
3	1543.81	C-N Amide II band, mainly protein
4	1459.15	Stretching-C=O inorganic carbonate
5	1384.31	CH and CH ₂ aliphatic bending group, lipid

Table 7.FTIR frequency of *H.fossilis* ovary

S.NO	IR frequency Number(cm^{-1}) Ovary	Definition of the spectral assignments
1	2922.90	CH ₂ asymmetric stretching in saturated fatty acid chain
2	2363.45	C=C conjugated C=C, lipid
3	1735.52	C=O Stretching, Ester phospholipid
4	1543.51	C=N Amide II band, mainly protein
5	1384.25	CH and CH ₂ aliphatic bending group, lipid

DISCUSSION

Fish has the important source of easily digestible protein and constitutes a major share in the world food market and can meet the protein hunger, nutritional security in the upcoming years. The proximate composition of fish species greatly varies during the spawning season due to physiological reason and changes of climate conditions [20, 21]. More studies have been carried out on the proximate composition of fishes [22, 23]. The proximate composition of fish species often different from one to another area [24, 25]. The essential cause of change in proximate composition and minerals may be due intake of food, size, habitat and season of fish [26, 27, 28]. Among all the major constituents studies the protein is an important component in the fish body, which is essential for all living organisms. Protein is not only for enzyme development and hormonal activity [29], but is also an essential source of energy in all living [30]. According to Tripathy *et al.*, the increase in protein content of liver, intestine, gills muscles and ovary in normal condition to exposure of some chemical Impact [31]. In conclusion our data suggested that protein content ranged from 21.470 ± 0.18 to 111.317 ± 0.37 . Protein content in liver and ovary (111.317 ± 0.37 , 106.033 ± 0.33 mg/g) which was observed in the study in accordance with the authors [32]. A numerous research discovered increase in protein concentration in the liver and ovary with increase in size of the fish. Reason for increasing protein in ovary because of the spawning of fish during breeding. Liver is an important organ involved in metabolic processes and in the detoxification and xenobiotics, which was analyzed [33]. The decrease in the content of protein observed in the present study in most of the fish tissues may be due to directing the free amino acids for the synthesis of protein or due to metabolic moderate of the keto acids to gluconeogenesis pathway for the synthesis of protein [34]. Carbohydrates are main source of energy in the living organism and play an important role in the cellular metabolism by providing energy to the all body cells. The carbohydrate content ranged from 1.202 ± 0.05 to 4.473 ± 0.03 mg/g. In the present study, maximum carbohydrate was observed in muscle. However, in muscle of *Heteropneustes fossilis*, the carbohydrate content (4.35 ± 0.03 mg/g). Glycogen is the major form of storage in liver and muscle. Liver is the site of metabolism. Second large quantity of carbohydrate present in liver (4.417 ± 0.19 mg/g). Among all tissues, liver showed higher carbohydrate content because of synthesis in enzyme. Fish liver is a powerful organ for the study of environmental quality biomarkers, due to its role in the animal metabolism by [35]. Lipid from fish known to contain polyunsaturated fatty acids, which help to rectifying coronary

heart diseases from human beings. In the present study, the lipid content of different tissues varied from 1.16 ± 0.01 to 4.35 ± 0.03 mg/g however in *H.fossilis* lipid content (4.35 ± 0.03 mg/g) in the intestine is high when compared to other tissues. Most of the intestinal lipase activity is located in proximate part of intestine [36]. Maturity and Age variation in the same species and may also contribute to the significant difference in the total lipid content [37]. The decreased level of tissue lipid content may be due to inhibition of oxidative phosphorylation or mobilization of glycerol and agreed with [38]. The differences in these values may be due to many factors such as lipid content in fish different according to species, seasons and geographical variations. Moisture increase might be due to spawning season and availability of more water and more activeness and vigour of fish. Moisture is one of the major components of all species and all type of fish [39]. In the current study, moisture content of fish ranged from 67.25%-77.46%. The moisture content present in intestine (77.46 ± 0.007). Similar to the results obtained by other researchers such as [40] determined moisture ranges from 77.69 to 79.11%. Percentage of moisture is a good indicator of its relative contents of energy, protein, carbohydrate and lipid. Intestine must observe massive quantity of water, so the moisture content increased in intestine of *H.fossilis*.

Mineral elements constitute important components of hormones and enzyme activators in human nutrition. Mineral deficiencies can cause structural, functional pathologies and biochemical analysis. The foods are excellent sources of Ca, P, I, P, Na, Fe and Zn. In the present study, calcium content higher in liver and muscle (155.31, 103.622 ppm). Normally higher calcium intakes may be beneficial in preventing kidney stone formation. On the other hand, helps in kidney function, muscle contraction, normal heart beat and nerve signalling. In the current study, sodium content of fish high in liver 95.370 ppm. In humans, sodium is an essential nutrient that regulates osmotic equilibrium, blood pressure and pH. Iron is used as regulate the all body tissues. In the present study these minerals were found to be maximum in liver and muscle and minimum in gills.

Infrared spectroscopy is important for identification of organic compounds because of its very complex spectrum which consists of many peaks [41]. In this study, samples will be observed using FTIR which the purpose is to identifying the functional groups contained in the sample. The FTIR spectra of the fish all tissue of *H.fossilis* in the 4000–400 cm^{-1} range. The spectrum was normalized with respect to the amide A (3450–1110- cm^{-1}) band as seen table 2. The spectrum contains several bands arising from the contribution lot of functional groups belonging to protein, carbohydrates and lipids. The nutritional value of the different organisms

depends on their biochemical constituents like proteins, carbohydrates, and lipids [42]. In this study the tissue level protein occurrence of such variations were obvious, maybe because of pollution stress in the fish habitat [43, 44].

CONCLUSION

In conclusion our data suggested that FTIR technique is potentially applicable to proximate composition of fish in different tissues of *H.fossilis*. The band areas and intensities of amide bands in tissue indicate the protein quantity of the system in all tissues. The band areas of symmetric and asymmetric CH₂ stretching modes present in the tissues suggested the presence of lipids. The result further suggests that the major biochemical constituents such as proteins, carbohydrate, and lipids can be easily evidenced by FTIR spectroscopy. The filleting wastes are enriched with greater beneficial effects, carried out of enhance fish processing waste into commercial enterprise utility of merchandise. This study concludes that obtainable fish food can be a substantial aid in redressing the problems of malnutrition and can help in pharmaceutical industries to medicines and formulate drugs from biochemical and mineral profile of fresh water fishes.

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AUTHORS

First Author- Ms. M.Umamageshwari, Research Scholar, PG and Research Department of zoology, Pasumpon Muthuramalinga Thevar College, Melaneelithanallur, Tenkasi, Tamilnadu, India.

E.Mail: umamageshwari190@gmail.com

Second Author- Ms. R. Sivasakthi, Research Scholar, PG and Research Department of zoology, Pasumpon Muthuramalinga Thevar College, Melaneelithanallur, Tenkasi, Tamilnadu, India.

Corresponding Author- Dr. S. Uma Rajeswari, Assistant professor, PG and Research Department of zoology, Pasumpon Muthuramalinga Thevar College, Melaneelithanallur, Tenkasi, Tamilnadu, India.