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VARIATIONS IN THE NUTRITIONAL COMPOSITION OF THE HEAD AND BONE FLOURS OF TILAPIA (*OREOCHROMIS MOSSAMBICUS)* ADAPTED TO ESTUARINE AND FRESHWATER ENVIRONMENTS

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ABSTRACT

Consumption of fish and fish by products assures various health benefits, but on the other hand the fish processing wastes if not discarded properly pose a serious environment threat. Tilapias are commonly available cichlid fishes which are considered to possess various biological importance. The objective of the work is to analyze and compare the similarities and differences in the nutritional quality of the exotic fish *Oreochromis mossambicus* found in brackish water and fresh water environments. The estuary adapted tilapia and freshwater tilapia was collected and processed as head and bone flours. The samples were further analyzed and the results in 100 g of Estuarine Tilapia Head Flour (ETHF) was composed of moisture (5.87 ± 0.003%), protein (32.06 ± 0.02%) total lipids (0.202 ±0.003 %), carbohydrates (1.44 ± 0.005%) and ash (1.15 ± 0.006%). The results in 100 g of Estuarine Tilapia Bone Flour (ETBF) was found as moisture (4.20 ± 0.006%), protein (31.48 ± 0.07%), total lipids (0.217 ± 0.002), carbohydrates (0.13 ± 0.004%) and ash (0.89 ±0.004%). The proximate content in Freshwater Tilapia Head Flour (FTHF) ranged as moisture (5.79 ± 0.01%), protein (32.50 ± 0.02%), total lipids (0.202 ± 0.009%), carbohydrates (1.54 ± 0.02%) and ash (1.16 ± 0.003). The proximate content in Freshwater Tilapia Bone Flour (FTBF) ranged as moisture (5.77 ± 0.01%), protein (32.58 ± 0.03%), total lipids (0.200 ± 0.005%), carbohydrates (1.48 ± 0.02%) and ash (1.23 ± 0.01%). The fatty acid occurring in the highest proportions was alpha linolenic acid both ETHF (2.492±0.003mg) and ETBF (2.374±0.002mg). The fatty acid composition in FTHF occurring in the highest proportion was palmitic acid (0.983±0.002mg) and in FTBF the highest proportion was found in stearic acid (0.785±0.005mg). In the amino acid analysis, the highest values were recorded in phenyl alanine for ETHF (1.986±0.002%) and lysine in ETBF (1.364±0.003%). Phenyl alanine content was found higher in both FTHF (1.889±0.002%) and FTBF (1.981±0.003%). The essential vitamins and minerals were also analyzed and the results are discussed in detail.

Keywords: Fish, nutrition, protein, amino acids, essential, tilapia

INTRODUCTION

In many countries of the world, the huge quantities of fish waste produced are often discarded into the environment and become a source of pollution **(Vignesh and Srinivasan, 2012)**. It has been suggested that about 25% of the total production of marine captured fisheries are discarded worldwide **(AOAC, 2000)**. The discards from the processing plants amount to 20 million tonnes, which is equivalent to 25% of the world's total production from marine capture fisheries **(AMEC, 2003)**. These wastes can be used to produce fish protein concentrate, fish oils and enzymes (such as pepsin and chymotrypsin) as well as other value added products. Solid fish waste consists of head, tails, skin, gut, fins and frames. These byproducts of the fish processing industry can be a great source of value added products such as proteins and amino acids, collagen and gelatin, oil and enzymes **(Esteban *et al*., 2007; Disney *et al*., 1977)**. These wastes contain proteins (58%), ether extract or fat (19%) and minerals. Also, monosaturated acids, palmitic acid and oleic acid are abundant in fish waste (22%) **(Ghaly *et al*., 2013)**. Traditionally, fish waste material has been converted to powdered flour, used as animal feed **(Strom and Eggum, 1981)**. However, the processing of fish produces parts that are not used mainly the head, skin, viscera, and bones. Fishbone is constituted by the remaining meat after the removal of the fillet, bones, and cartilages **(Petenuci *et al*., 2008)**. Fish protein concentrate is produced from edible parts of fish, which is properly dehydrated and milled into flour. It is otherwise referred to as fish flour. It has attractive colour, pleasant flour, and of reduced moisture content which makes it to have relatively longer shelf life. Nutritional studies have shown that fish protein concentrate can be added to weaning food growing infants and nursing mothers **(Adeleke and Odedeji, 2010)**. However, processing methods such as salting, boiling, frying, sun drying, roasting and smoking have been used to preserve and increase its availability to consumers **(Olayemi *et al*., 2011)**. Most processing methods often times involve removal of the head, viscera and other parts of the fish which may have either negative or positive effect on the total nutritive values of the fish **(Saliu, 2008)**.
Tilapias are the most important fish cultured both in tropical and subtropical countries. Tilapias are very sturdy in nature and does adapt in many environmental factors in which other fish cannot survive. Commercially, tilapia are the second most important group of wild-captured fish, after carps, with a global capture 769,936 tonnes (metric tons) in 2007 **(FAO, 2009)**. Tilapia culture surged in the 1990s and currently ranks as the world's eighth most common group of farmed fish species, with a commercial production of 2.5 million tonnes in 2007, corresponding to an estimated value of $3.3 billion **(FAO, 2009)**. Tilapia raised in inland tanks or channels are considered safe for the environment, since the wastes and diseases are contained and does not spread to the wild. However, these fishes have caused attention as being among the most serious invasive species in many subtropical and tropical parts of the world. Tilapia has become a popular seafood to American consumers, mainly because of its high nutritional value, mild taste, and low expense relative to other fin fishes. In Indian scenario the tilapias are commonly found in estuaries, freshwater streams, wells, sewage canals and ponds. This adaptability and availability of this species enabled scientists to choose this fish as a model organism for various research purposes. They are also the most farmed fish in the tropical and sub-tropical regions of and have been playing an increasingly key role in the nation's nutrition as a source of relatively cheap animal protein. In recent times, fish has been reported as the cheapest source of protein used to correct protein deficiency in human diets in the tropic region **(Akinwumi, 2011)**. In India, the Mossambicus variety of Tilapia was being widely cultured in states including Kerala, Andhra Pradesh and Odisha for the last 30 years. It also contains some bioactive compounds with therapeutic properties that are beneficial to human health **(Nnaji *et al*., 2010; Lordan *et al*., 2011)**.

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 our previous study, we have reported the nutritional quality of the head and bone flours of *O. mossambicus* and in the present study we compare the head and bone flour nutritional value of *O. mossambicus* collected from an estuary and freshwater. The mineral and proximate composition of fish varies greatly from one species and one individual to another depending on age, sex, environment, season, area of catch and processing method **(Nurnadia *et al.*, 2013; Adeniyi *et al.*, 2012)**. Thus, in the present study, we point out that there are limited reports in national and international literature on the proximate composition, amino acids, vitamins, fatty acids and mineral composition in the wastes and discards of a fish species that survives in two different environments such as brackish water and freshwater.

MATERIAL AND METHODS

Samples

The availability of the study fish are abundant both in fresh and estuarine water. The fresh water Tilapia was collected from ponds and wells in Cuddalore district. Their length was measured and it ranged as 7.7± 10.2cm to14.3 ±16.4 cm and body weight ranged from 10.3± 15.8g to 16.3± 22.6g. The estuarine tilapia was collected from Vellar estuary in Portonovo, South east coast of India. Their length ranged as 9.7± 12.2cm to13.5 ±18.4 cm and body weight ranged from 12.3± 17.8g to 17.9± 24.6g. The samples i.e. the heads and bones were collected fresh and the flour samples were prepared in laboratory as there are no fish waste processing units nearer to the sampling area.

Sampling

The tilapia heads and bones were separated and washed with filtered water, cleaned with paper towels and dried for 25 minutes. After drying the bones were ground in an endless-screw grinder, placed on trays and dried in an oven for four (4) hours at 180ºC. Next, the flour was sieved using a 14-mesh stainless steel sieve. The product obtained, referred to as Estuarine tilapia fish head (ETHF), Freshwater tilapia head flour(FTHF), Estuarine tilapia bone flour (ETBF) and Freshwater tilapia bone flour (FTBF), was packed in polyethylene bags, wrapped in aluminum foil after removal of air, and stored in refrigerator at 4 ºC for later analysis. The complete processing duration of the sampling was around 20 days. The processed samples were analyzed in Instrumentation facility, CAS in Marine Biology, Annamalai University.

Analytical methods

Moisture and Ash content analysis

Proximate composition analyses of the samples were done in triplicate for protein, moisture, lipid and ash contents. The crude protein was determined by the Kjeldahl procedure **(AOAC, 1984)**. Moisture was determined by oven drying at 105ºC to constant weight **(AOAC, 1990)**. Total lipid was extracted from the muscle tissues using **Bligh and Dyer (1959)** method. The lipid content was gravimetrically determined. Ash was determined gravimetrically in a muffle furnace by heating at 550 ºC constant weight **(AOAC 1990)**.

Fatty acids analysis

The fats were converted to free fatty acids by saponification. The fatty acids were converted to their methyl esters and into heptane. Internal standards were used for estimation of actual fatty acids present in the fat. Identification/quantification of fatty acids was achieved by gas chromatography, the former being resolved by elution times. Internal standards were used for estimation of actual fatty acids present in the fat. Identification/quantification of fatty acids was achieved by gas chromatography. The lipids were esterified according to **Metcalfe *et al*, (1966)**. The fatty acid methyl esters were analyzed on a Thermo quest trace gas chromatograph equipped with SP-2330 fused silica capillary column, 30x0.25 mm ID 0.20 µm film thickness. Column injector and detector temperatures were 240 and 250°C, respectively. Carrier gas, helium; split ratio 1/150; column flow 75 ml/min; make-up 30 ml/min (He) range 1; sample injection 0.5 µl. The fatty acid methyl mixture No. 189-19 was used for standards (Sigma). The fatty acids were calculated by percentage of total lipid.

Estimation of Amino acids

The experimental lyophilized samples were finely ground for estimating the amino acids in the HPLC (Merck Hitachi L-7400) following the method of **Baker and Han (1994)**. About 0.5 g sample was weighed into a 100 ml flat bottomed flask, 1 ml of Norleucine standard solution, 5 ml of performic acid in ice bath. The oxidation procedure was carried out in a fridge for 16 h after which 0.84g of sodium metabisulphite, 30 ml 6N HCl and anti-bumping granules were added. The mixture was hydrolyzed for 24 h in PEG bath set at 1300C after which it was allowed to cool and 30 ml of 4 M lithium hydroxide added. The pH was adjusted to 2.1 and the mixture made up to 100 ml final volume. About 5 ml of the sample was filtered through 2 µm filter and this was run through a Biochrom 20 Amino Acid analyzer.

Estimation of Vitamins and minerals

The samples were finely ground for estimating the vitamins and minerals. The samples were analyzed by Liquid chromatography by following the protocol British Pharmacopoeia 01/2005:0218.

Statistical analysis

Analysis of variance was used to evaluate the analysis data and significant differences among means were determined by Independent Samples-T Test (P<0.05). Statistical calculation was performed with SPSS 15.0 for windows.

RESULTS AND DISCUSSION

Proximate composition

The analysis to know the proximate composition of the head and bone flours showed promising results. The protein content of ETHF was found to be 32.06 ± 0.02% whereas in FTHF the protein content was 32.50 ± 0.02%. The protein content of ETBF was found to be 31.48 ± 0.075 whereas in FTBF the protein content was 32.58 ± 0.03 %. This result emphasizes that both the head and bone flours have an insignificant difference of their of protein content. Likewise the lipid content was estimated as 0.202 ±0.003% for ETHF and 0.202 ± 0.009 for FTHF. The ETBF's lipid content was estimated as 0.217 ± 0.002% and FTBF's content was 0.200 ± 0.005%. No major contrasts or variations in the lipid content was noticed. The amount of carbohydrate estimated in ETHF was 1.44 ± 0.005% and in FTHF it was 1.54 ± 0.02%. In ETBF and FTBF the carbohydrate percentage ranged low as 0.13 ± 0.004% and 1.48 ± 0.02% respectively. The carbohydrate content in ETBF was found low in comparison to that of FTBF. The moisture content in FTHF was 5.79 ± 0.01% where as in ETHF it was 5.87 ± 0.003%. Minute differences were noticed between the FTHF and ETHF moisture content. In FTBF and ETBF there was a considerable variation in the moisture content. The values ranges from 5.77 ± 0.01% (FTBF) to 4.20 ± 0.006% (ETBF). The ash content was found as FTHF (1.16 ± 0.003%), ETHF (1.15 ± 0.006%), FTBF (1.23± 0.01%) and low in ETBF (0.89 ±0.004%). The composition of the fish waste varies according to the type of species, sex, age, nutritional status, time of year and health. Most of the fish contains 15-30% protein, 0-25% fat and 50-80% moisture **(Murray and Burt., 2001; Ghaedian *et al.*, 1998)**. **Suvanich *et al*, (2006)** reported that the composition of catfish, cod, flounder, mackerel and salmon varied according to the species. Mackerel had the highest fat content (11.7%) and cod had the lowest (0.1%). Salmon had the highest protein content (23.5%) and flounder had the lowest (14%). The moisture content of the five fishes varied between 69 and 84.6% but the ash content of all species was similar **(Ghaly *et al.*, 2013)**. The variation in the four samples is shown graphically (Fig1 and 2).

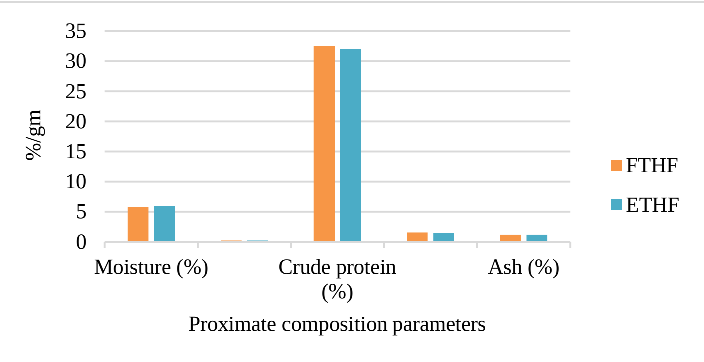

Figure 1 Variation in the proximate composition of Freshwater (FTHF) and Estuarine (ETHF) adapted tilapia head flours

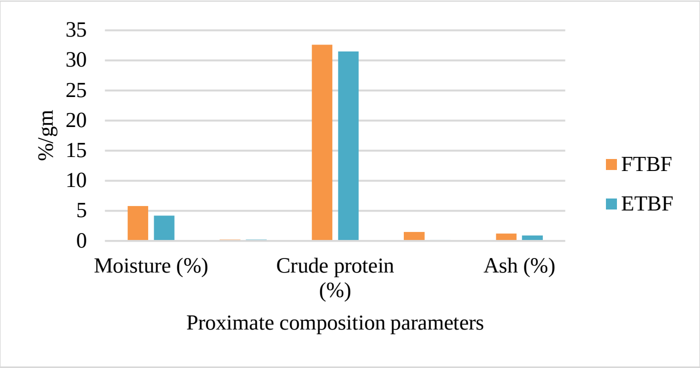

Figure 2 Variation in the proximate composition of Freshwater (FTBH) and Estuarine (ETBF) adapted tilapia bone flours

Amino acid content

Amino acids are the building blocks of proteins. They have wide nutritional value, taste, medicinal action and chemical properties. They are used as food additives, in pharmaceutical applications, feed and food supplements. The amino acids such as arginine, glycine, glutamate and histidine are used in protein pharmaceuticals as an excipient for drug development **(Ghaly *et al.*, 2013)**. The largest consumer of amino acids is the food flavoring industry which uses monosodium glutamate, alanine, aspartate and arginine to improve the flavor of food. The second largest consumer of amino acids is the animal feed industry which uses lysine, methionine, threonine, tryptophan and others to improve the nutritional quality of animal feed. The amino acids can also be used in various pharmaceutical applications such as protein purification and formulations and production of antibiotics such as Jadomycin **(Arakawa *et al.*, 2007)**. The analysis for identifying the presence of essential 20 amino acids in the flour samples was done. The amino acid Phenyl alanine was found to be high in ETHF (1.986±0.002%) followed by aspartic acid (1.841±0.003%) and asparagine (1.452±0.0045). In FTHF, phenylalanine (1.889±0.002%) was found high followed by aspartic acid (1.435±0.002%) and isoleucine (1.396±0.002%). The amino acid Glycine (1.443±0.002) was recorded high in ETBF followed by lysine (1.364±0.003) and asparagine (1.326±0.002). In FTBF, phenylalanine (1.981±0.003%) was found higher than aspartic acid (1.414±0.002%) and isoleucine (1.412±0.004%). While comparing the other amino acids content among the ETHF, FTHF, ETBF and FTBF no noticeable variations found. Alanine was the least found amino acid in all four samples i.e ETHF (0.119±0.002%), FTHF (0.109±0.002%), ETBF (0.109±0.003 %) and FTBF (0.107±0.002%). But these minute variations has to be taken into consideration on the fact that they can be either risk or benefit factors. The graphical illustration (Fig 3 and 4) shows the variation between the samples amino acid content. The values recorded for all the amino acids is shown in Table 1.

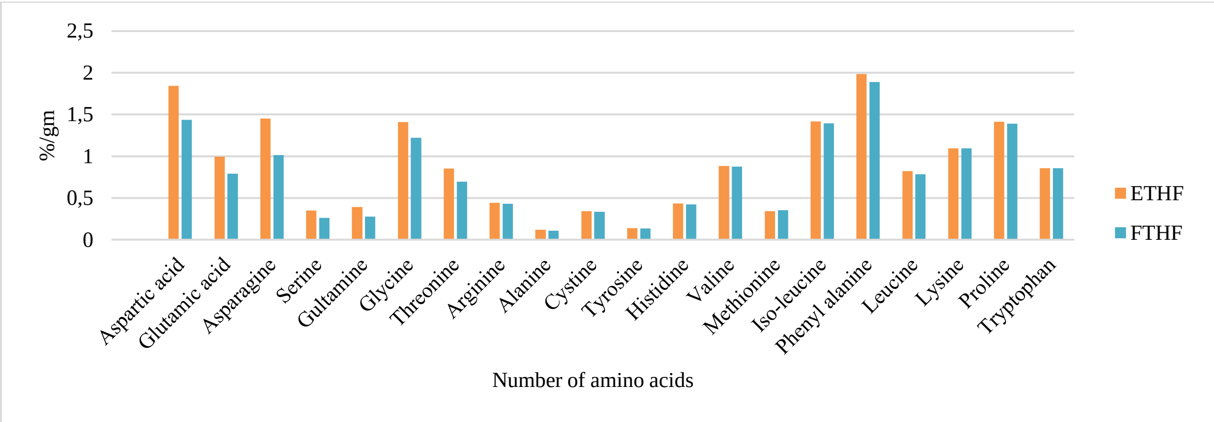

Figure 3 Amino acid composition of Freshwater (FTHF) and Estuarine (ETHF) tilapia head flours

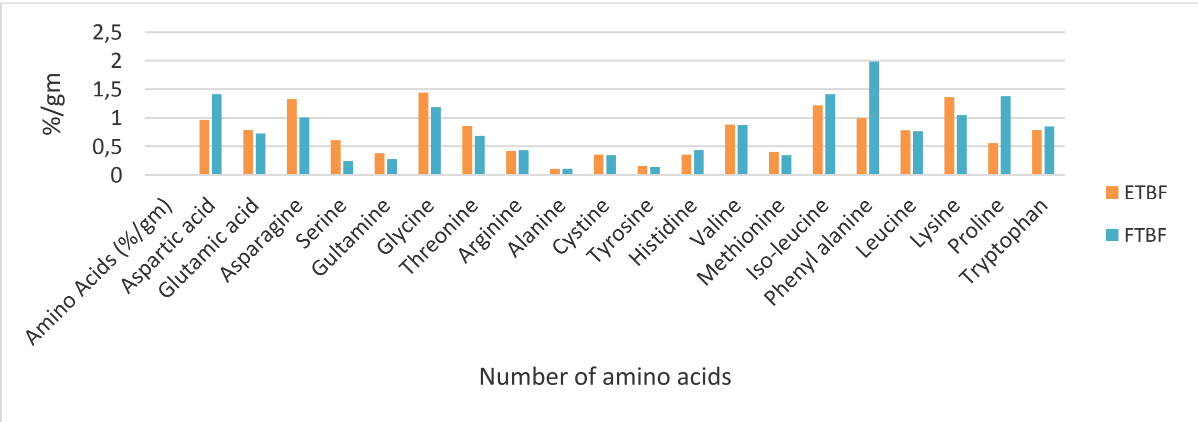

Figure 4 Amino acid composition of Freshwater (FTBF) and Estuarine (ETBF) tilapia bone flours

Table 1 Essential amino acids (%/gm dry weight) in estuarine tilapia head (ETHF), freshwater tilapia bone flour (FTHF), estuarine tilapia bone flour (ETBF) and freshwater tilapia head flour (FTBF)

Amino Acids (%/gm)	ETHF (%/gm)	FTHF (%/gm)	ETBF (%/gm)	FTBF (%/gm)
Aspartic acid	1.841±0.003	1.435±0.002	0.967±0.003	1.414±0.002
Glutamic acid	0.997±0.002	0.791±0.002	0.787±0.001	0.723±0.002
Asparagine	1.452±0.004	1.016±0.002	1.326±0.002	1.006±0.002
Serine	0.349±0.005	0.261±0.002	0.608±0.001	0.245±0.002
Glutamine	0.393±0.001	0.278±0.002	0.375±0.003	0.276±0.002
Glycine	1.410±0.001	1.221±0.002	1.443±0.002	1.191±0.002
Threonine	0.853±0.002	0.697±0.002	0.861±0.002	0.686±0.002
Arginine	0.443±0.002	0.431±0.001	0.421±0.003	0.432±0.001
Alanine	0.119±0.002	0.109±0.002	0.109±0.003	0.107±0.002
Cysteine	0.344±0.002	0.337±0.002	0.356±0.002	0.343±0.002
Tyrosine	0.141±0.003	0.138±0.001	0.161±0.002	0.141±0.003
Histidine	0.435±0.003	0.423±0.002	0.356±0.002	0.434±0.003
Valine	0.884±0.002	0.878±0.003	0.881±0.002	0.877±0.002
Methionine	0.345±0.003	0.353±0.002	0.406±0.003	0.343±0.003
Iso-leucine	1.416±0.003	1.396±0.002	1.216±0.003	1.412±0.004
Phenyl alanine	1.986±0.002	1.889±0.002	0.994±0.003	1.981±0.003
Leucine	0.821±0.002	0.784±0.002	0.780±0.002	0.763±0.002
Lysine	1.094±0.002	1.095±0.002	1.364±0.003	1.047±0.002
Proline	1.414±0.002	1.391±0.002	0.556±0.003	1.379±0.003
Tryptophan	0.857±0.004	0.859±0.002	0.787±0.004	0.849±0.002

Legend: Values are expressed as mean ± standard deviation, n=3

Fatty acid content

In the present study, seven essential fatty acids was identified in the head and bone flour samples. The fatty acid analysis highlights the content of alpha linolenic acid as high in ETHF (2.492±0.003) where Moroctic acid was recorded low (0.117±0.002). In FTHF sample palmitic acid was highly observed and moroctic acid was found low (0.003±0.006). In the bone flour analysis, Alpha linolenic acid (2.374±0.002) was recorded high in ETBF and moroctic acid (0.105±0.002) was recorded low. In the FTBF sample, stearic acid (0.785±0.005) had the maximum range and low range in moroctic acid (0.003±0.004). While comparing the results in head flours, the ETHF samples exhibited higher ratios than that of FTHF samples. Likewise similar conclusion was derived for bone flour samples (ETBF and FTBF). The range variation in fatty acid content between the samples is shown (Fig 5 and 6). The values recorded were tabulated (Table 2). Nutraceutics are natural food products that provide health and medical benefits, including the prevention and treatment of disease. Among these food products essential fatty acids stand out, particularly the n-3 long-chain polyunsaturated fatty acids (LC-PUFA), such as eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6n-3) **(Navarro Garcia *et al*., 2014)**.The fatty acids C16:0 and C18:0 can be used as energy sources. Additionally, they may serve as structural components of phospholipids **(Perez *et al*., 1999)**.

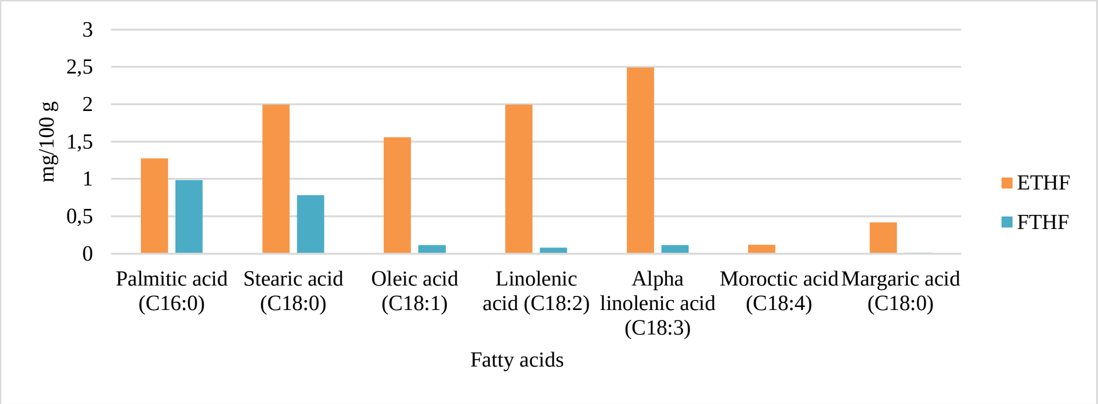

Figure 5 Fatty acid composition of Freshwater (FTBH) and Estuarine (ETBF) tilapia head flours

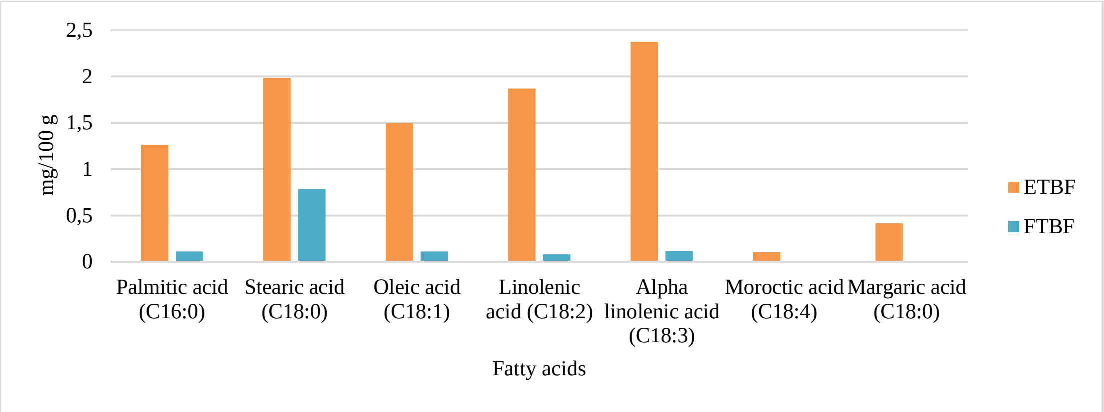

Figure 6 Fatty acid composition of Freshwater (FTBH) and Estuarine (ETBF) tilapia bone flours

Table 2 Fatty acids (mg/100 g) in estuarine tilapia head (ETHF), freshwater tilapia bone flour (FTHF), estuarine tilapia bone flour (ETBF) and freshwater tilapia head flour (FTBF)

Fatty acids	ETHF	FTHF	ETBF	FTBF
Palmitic acid (C16:0)	1.274±0.001	0.983±0.002	1.261±0.002	0.112±0.003
Stearic acid (C18:0)	1.995±0.002	0.783±0.002	1.984±0.002	0.785±0.005
Oleic acid (C18:1)	1.557±0.002	0.114±0.003	1.498±0.002	0.113±0.005
Linolenic acid (C18:2)	1.996±0.002	0.081±0.005	1.869±0.004	0.081±0.005
Alpha linolenic acid (C18:3)	2.492±0.003	0.113±0.007	2.374±0.002	0.114±0.002
Moroctic acid (C18:4)	0.117±0.002	0.003±0.006	0.105±0.002	0.003±0.004
Margaric acid (C18:0)	0.420±0.002	0.009±0.002	0.416±0.002	0.008±0.004

Legend: Values are expressed as mean ± standard deviation, n=3

Vitamins and Minerals

It is essential to know the vitamin and mineral content of any product that is desirable to human consumption. The amount of vitamins and minerals is species-specific and can also vary seasonally. In general, fish meat is a good source of the B vitamins and also of the A and D vitamins in the case of fatty species. Some freshwater species such as carp have high thiaminase activity so the thiamine content in these species is usually low. As for minerals, fish meat is regarded as a valuable source of calcium and phosphorus in particular but also of iron, copper and selenium. Saltwater fish have a high content of iodine **(FAO, 2005)**. The analysis of vitamin content in the head flours showed that Vitamin A (144.5±0.02) was found abundant in ETHF and FTHF (145.3±0.06) followed by Vitamin C (ETBF- 25.63±0.08; FTFH-25.62±0.1). The low ranged vitamin in ETHF was Vitamin B12 (0.14±0.05) and in FTFH (0.11±0.04) it ranged very low. Comparing the results for bone flours Vitamin A was abundant in ETBF and in FTBF. Lower values were recorded for vitamin B12 for ETBF and FTBF (Table 3). The comparative results are shown in the graphs (Fig 7 and 8). The results for mineral analysis in head flours showed that calcium was abundant in both ETHF (56.83±0.1) and FTHF (56.72±0.1) samples. The sodium content was moderate in ETHF (34.70±0.06) and FTHF (34.67±0.052). The lower ranged mineral was Zinc in both ETHF (0.352±0.005) and FTHF (0.339±0.007). The calcium content in bone flour was high and it ranged as ETBF (67.84±0.04) and FTBF (56.71±0.01). In ETBF, Copper (0.124±0.002) was found low whereas in FTBF Zinc (0.354±0.04) was low. In bone flour samples, the ETBF sample had noticeable Magnesium (45.63±0.06) content which was comparatively low in FTBF (15.64±0.09) sample. When comparing the samples, the bone flour samples exhibited higher mineral content. The values recorded are tabulated (Table 4) and the variation in the mineral content between the samples is shown (Fig 9and 10). Fish bones contain 60-70% minerals including calcium, phosphorous and hydroxyapatite **(Kim and Mendis, 2005)**. Generally, calcium is deficient in most of the regular diets and to improve calcium intake, consumption of small whole fish can be nutritionally valuable. The fish bones obtained from the fish processing waste can be used to provide calcium **(Ghaly *et al*., 2013)**. In order for bones being a fortified food, they should be converted into edible form of softening their structure with hot water treatment, hot acetic acid solutions or by super-heated steam cooking **(Ishikawa *et al*., 1990)**. Fish bones are a very good source of hydroxyapatite which can be used as a bone graft material in medical and dental applications **(Ghaly *et al*., 2013)**.

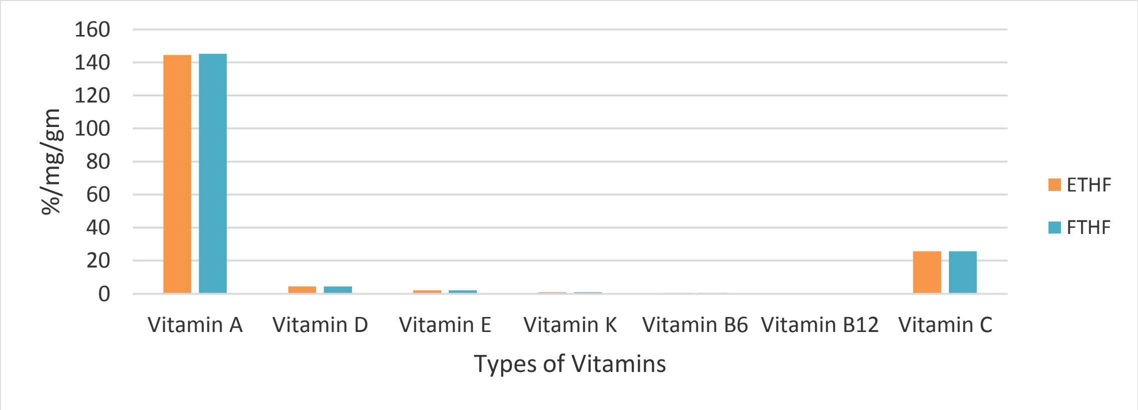

Figure 7 Vitamin content in the Freshwater (FTHF) and Estuarine (ETHF) tilapia head flours

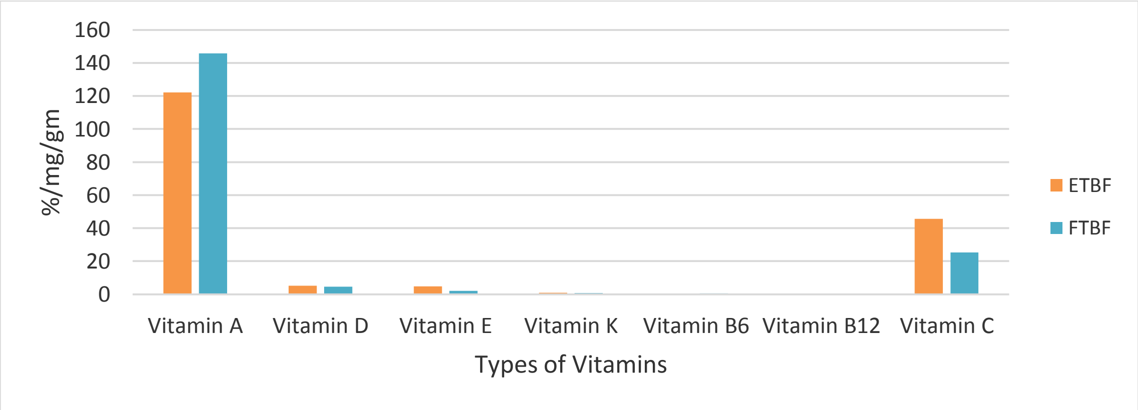

Figure 8 Vitamin content in the Freshwater (FTBH) and Estuarine (ETBF) tilapia bone flours

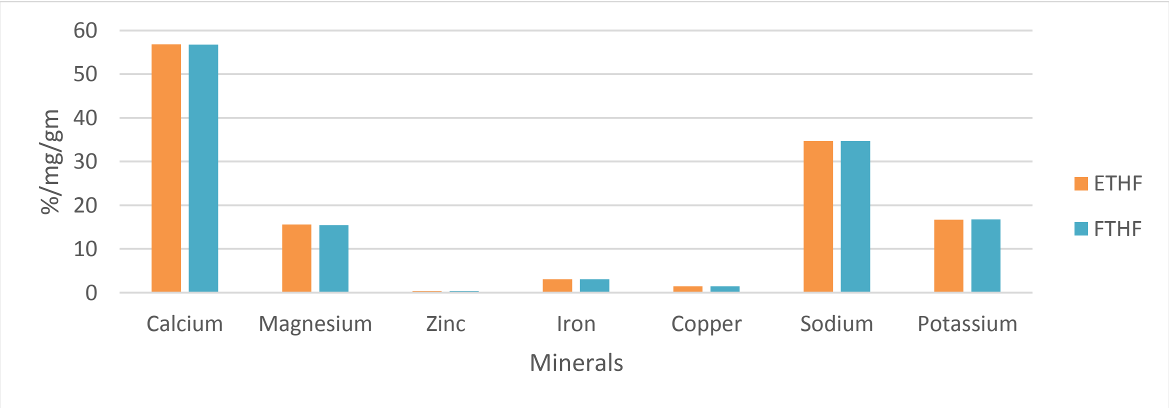

Figure 9 Minerals in the Freshwater (FTHF) and Estuarine (ETHF) tilapia head flours

Table 3 Essential Vitamins in estuarine tilapia head (ETHF), freshwater tilapia bone flour (FTHF), estuarine tilapia bone flour (ETBF) and freshwater tilapia head flour (FTBF)

Vitamins	ETHF	FTHF	ETBF	FTBF
Vitamin A	144.5±0.02	145.3±0.06	122.2±0.2	145.7±0.3
Vitamin D	4.443±0.07	4.446±0.03	5.23±0.4	4.466±0.04
Vitamin E	1.989±0.06	1.985±0.6	4.68±0.1	1.982±0.08
Vitamin K	0.89±0.06	0.81±0.02	0.78±0.02	0.73±0.001
Vitamin B6	0.43±0.04	0.39±0.01	0.24±0.02	0.41±0.006
Vitamin B12	0.14±0.05	0.11±0.04	0.14±0.02	0.13±0.002
Vitamin C	25.63±0.08	25.62±0.1	45.61±0.05	25.32±0.5

Legend:Values are expressed as mean ± standard deviation, n=3

Table 4 The mineral constituents in % of estuarine tilapia head (ETHF), freshwater tilapia bone flour (FTHF), estuarine tilapia bone flour (ETBF) and freshwater tilapia head flour (FTBF)

Minerals (%)	ETHF	FTHF	ETBF	FTBF
Calcium	56.83±0.1	56.72±0.1	67.84±0.04	56.71±0.01
Magnesium	15.62±0.09	15.46±0.09	45.63±0.06	15.64±0.09
Zinc	0.352±0.005	0.339±0.007	0.240±0.008	0.354±0.04
Iron	3.098±0.001	3.096±0.008	2.390±0.01	3.086±0.01
Copper	1.424±0.007	1.430±0.003	0.124±0.002	1.420±0.002
Sodium	34.70±0.06	34.67±0.052	29.09±0.009	34.63±0.006
Potassium	16.66±0.04	16.75±0.040	15.66±0.009	16.76±0.004

Legend: Values are expressed as mean ± standard deviation, n=3

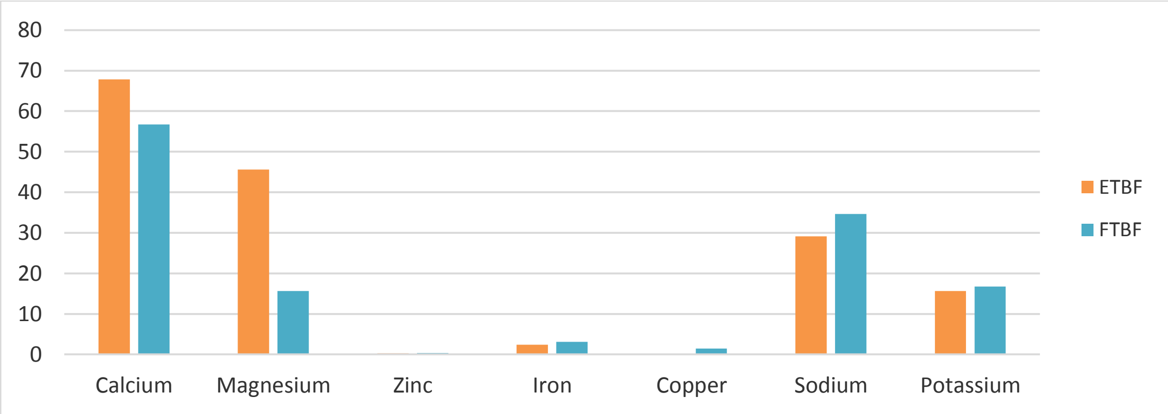

Figure 10 Minerals in the Freshwater (FTBH) and Estuarine (ETBF) tilapia bone flours

CONCLUSION

Fish waste can also be used for production of various value added products such as proteins, oil, amino acids, minerals, enzymes, bioactive peptides, collagen and gelatin. The fish proteins are found in all parts of the fish. The amino acids present in the fish can be utilized in animal feed in the form of fishmeal and sauce or can be used in the production of various pharmaceuticals **(Ghaly *et al.*, 2013)**. Fish protein and mineral contents are recognized for their nutritional and functional properties in the human diet. Fish is one of the most important sources of animal protein available, and has been widely accepted as a good source of protein and other elements for the maintenance of a healthy body **(Arannilewa, 2005)**. Moreover, the consumption of fish has been linked to health benefits such as reduced risk of coronary heart disease. A preventive and/or curative effect has also been reported for arterial hypertension **(Millar and Waal-Manning, 1992)**, human breast cancer **(Rose and Connoll, 1993)**, inflammatory diseases **(Belluzi *et al.*, 1993; James and Cleland, 1996)**, asthma **(Dry and Vincent, 1991; Hodge *et al.*, 1996)** and disorders of the immune system **(Kenneth, 1986; Levine and Labuza, 1990)**. In addition, fish oil helps to prevent brain aging and Alzheimer's disease **(Kyle, 1999)**. There is growing evidence that fish based nutrition are good for the heart and blood vessels and control for cardiovascular diseases. Therefore eating fish or taking fish oil, both freshwater and marine, is being encouraged **(Kumaran *et al.*, 2012)**. The Information about some proximate parameters such as protein, lipids, carbohydrate, moisture and ash is often necessary to ensure that they meet the dietary requirements, food regulations and commercial specifications **(Fawole *et al.*, 2007, Onyia *et al.*, 2010)**. The present study highlights that leftovers or discards of fish products are rich in some nutrients and minerals which can in turn be a beneficial factor for human or for animals. The present study records minor variations in the nutrient composition of fresh water Tilapia nad Estuarine tilapia which is not a major concern until and unless any external factors influences the ratio. The environment in which the fishes survive may be highly polluted, which might have influenced the nutrient content of the fishes. Fishes from polluted waters does not posses rich nutrients generally and there are possibilities of sampling errors which may have reflected in the variation. Thus, studies like the present one has to be often cross checked and validated. Also the rate of pollution in sampling areas and study animals has to be taken into consideration in the future studies. The present study also paves way for further research in fish waste management and also to compare the nutrient composition of fishes from the same and different family based on their adapting environment. The future potential of fish wastes need to be understood by which the research and development can target the most effective and efficient way to manage wastes which can mutually benefit human and the environment.

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