Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading

Nutritional quality of processed head and bone flours of Tilapia (*Oreochromis mossambicus*, Peters 1852) from Parangipettai estuary, South East Coast of India

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ARTICLE INFO

Article history:
Received 12 February 2012
Received in revised form 22 February 2012
Accepted 23 March 2012
Available online 28 April 2012

Keywords:
Fatty acids
Amino acids
Vitamins and Minerals
Heads
Bones
Predominant
Human diet

ABSTRACT

Objective: To analyze the proximate composition, fatty acids, amino acids and nutritional composition of flour made from Tilapia (*Oreochromis mossambicus*) head and bones. **Methods:** Tilapia fish head and bone are the parts with unknown composition. Fish bones and heads were collected, cleaned and dried. The flours were prepared using 14-mesh stainless steel sieve and analysed further. **Results:** The results in 100 g of Tilapia head flour (THF) were composed of moisture [(5.89±0.01) %] protein [(32.59±0.02) %] total lipids [(0.2014±0.0002)%], and ash [(11.14±0.02) %]. The results in 100 g of Tilapia bone flour (TBF) were: moisture [(4.22±0.02)%], protein [(31.52± 0.02) %], total lipids [(0.8761±0.0002) %], and ash [(0.89±0.01)%]. The fatty acid composition of THF occurring in the highest proportions were alpha linolenic acid [(C18: 3; (2.4390±0.0025)%], stearic acid [(C18:0; (1.9883±0.0025)%], linoleic acid [(C18:2; (1.9860±0.0025)%], palmitic acid [C16:0; (1.1787 ±0.0002) %], oleic acid [(C18:1; (1.5468±0.0002)%] margaric acid acid [(C17:0 (0.4146±0.0002)%] and moroctic acid [(C18:4; (0.1141±0.0002)%]. The fatty acid compositions of TBF occurring in the highest proportions were stearic acid [(C18: 0; (0.7860±0.0025)%], oleic acid [(C18:1; (0.1141±0.0002)%], alpha linolenic acid [(C18: 3; (0.1141±0.0002)%], palmitic acid [(C16: 0; (0.1143±0.0025)%], linoleic acid [(C18:2; (0.0816±0.0002)%], margaric acid acid [(C18:0; (0.0088±0.0003)%] and moroctic acid [(C18:4; (0.0034±0.0003)%]. The major amino acids such as glutamic acid (0.9967%), aspartic acid (1.837%), lysine (1.048%) and leucine (0.807%) were found in THF and in TBF the major amino acids were found as glutamic acid (0.7865%), aspartic acid (0.9686%), lysine (1.342%) and leucine (0.7756%). The THF flour possessed seven essential minerals in milli grams per gram (mg/gm) such as calcium (56.7±0.2 mg), iron (3.098±0.002 mg), potassium (16.78±0.02 mg), magnesium (15.67±0.03 mg), copper (1.414±0.002 mg), sodium (34.67±0.03 mg) and zinc (0.343±0.003 mg). The TBF flour possessed calcium (67.76±0.02 mg), iron (2.41±0.02 mg), potassium (15.67±0.02 mg), magnesium (45.76±0.02mg), copper (0.131±0.001 mg), sodium (29.11±0.01 mg), and zinc (0.2415±0.0002 mg). **Conclusions:** The results indicate that the flours made from the fish head and bone of tilapia may result as a valuable alternative food in the human diet. This is the first study which reports the nutritive value of tilapia (*Oreochromis mossambicus*) byproducts such as heads and bones.

1. Introduction

Fishes received increased attention as a potential source of animal protein and essential nutrients for human diets [1]. Fish and fish products are very important sources of income[4] and high market value[2]. Fish has been shown to be the cheapest source of animal protein in Third World Countries[3]. The consumption of fish is allied to health reimbursement because of a rich content in proteins of high dietary value; minerals, vitamins and distinguishing lipids. It should be considered that fish tissue presents elevated nutritional significance and therefore is a particularly optional dietary module. In addition, fish are a good source of micro and macro-elements such as calcium, phosphorus, selenium and manganese[4]. Fish lipids are well known to be rich in long-chain (LC) n-3 polyunsaturated fatty acids (LC n-3 PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[5].

Earlier research on fish proteins[6-8], has shown that they have bioactive properties and beneficial health effects

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Foundation Project: Supported by UGC Majar Research Project [Grant No. 32-642/2006(SR)].

which make them a very interesting alternative for the food industry. Several research groups around the world have been working on converting these wastes into useful food and bioactive ingredients[9-11]. Fish mince using by products can also be successfully used directly in various food systems and in physically or chemically altered form to produce an array of nutritional and functional products[12]. Some studies have shown that FPH prepared from fish byproducts such as bones and heads can contribute to increased water holding capacity in food formulations[13,14]. Several studies have indicated that peptides derived from fish by product proteins have antioxidative properties in different oxidative systems[15,16]. Fish protein hydrolysates prepared from fish byproducts can also function as immuno-stimulants, can have anti-carcinogenic effects and anti-anaemia activity[17]. Researchers have identified and reported specific peptides from animal and fish proteins responsible for ACE inhibition[11,18,19]. The other reported bioactivities include antihypertensive, immunomodulatory, neuroactive, antimicrobial, mineral and hormonal regulating properties[20-22]. A review by Underland *et al*[17] presented the health effects of different seafood products including fish proteins and FPH from fish byproducts and a review from Kim & Mendis[6] discussed the bioactive effects of marine rest raw materials.

Tilapias are the most important fishes cultured both in tropical and subtropical countries. According to the FAO[23], the Global tilapia aquaculture production in 2008 stood at nearly 2.8 million tons. Tilapia fillets have high protein, low fat, low calorie, and low carbohydrate. Tilapia fillets are also an excellent source of Phosphorus, Niacin, Selenium, Vitamin B12 and Potassium[24]. 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. With these facts in mind, the aim of the present work is to evaluate the fatty acid composition and nutrient potential of tilapia heads and bones after processing in the form of flour, with the ultimate goal of its consumption by humans. Tilapias were chosen for this work based on the fact that they have good consumer acceptance, economically viable and are in low fat content[23]. 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 source of relatively cheap animal protein. 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 many countries the huge quantities of fish waste produced are often discarded into the environment and thus become a source of pollution. There are no intensive studies on fish wastes and their importance is uncared. Thus this study focuses on the evaluation of all possible nutrients from the head and bone flours made from Oreochromis mossambicus. We point out that there are no reports on the nutritive value (fatty acid, amino acid, vitamin and mineral composition) of tilapia fish head and bone flour in either the national or international literature, which prompted us to evaluate its nutritional value. Thus this is the first study which reports the nutritive value of tilapia (*Oreochromis mossambicus*) byproducts.

2. Materials and methods

2.1. Samples

The fish Tilapia *Oreochromis mossambicus* (Peters, 1852) used for this study were captured from the Parangipettai estuary (South East Coast of India). Their length ranged as 8.9± 11.2 cm to17.3 ±18.4 cm and body weight was 12.3± 14.8 g to 15.3± 21.6 g. The samples *i.e.* the heads and bones were collected fresh and the flour samples were prepared as follows.

2.2. 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 4 hours at 180 ℃. Next, the flour was sieved using a 14-mesh stainless steel sieve. The product obtained, referred to as tilapia fish head and bone flour (THF & TBF), was packed in polyethylene bags, wrapped in aluminum foil after removal of air, and stored in refrigerator at 4℃ for later analysis.

2.3. Analytical methods

2.3.1. 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[25]. Moisture was determined by oven drying at 105 ℃ to constant weight[26]. Total lipid was extracted from the muscle tissues using Bligh and Dyer [27] method. The lipid content was gravimetrically determined. Ash was determined gravimetrically in a muffle furnace by heating at 550 ℃ constant weight[26].

2.3.2. Fatty acids analysis

Fatty acid methyl esters (FAME) were prepared by methylation of the total lipids (TL), as described by Joseph and Ackman[28].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 and 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 ℃, 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.

Table 1
Proximate composition of tilapia head and tilapia bone flour (TBH &THF).

Constituents	Moisture (%)	Crude protein (%)	Carbohydrates (%)	Ash (%)	Total lipids (%)
THF	5.89±0.01	32.59±0.02	1.45±0.02	1.14±0.02	0.2014±0.0002
TBF	4.22±0.02	31.52±0.02	0.14±0.0002	0.89±0.01	0.8761±0.0002

2.3.3. 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[7]. 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 0.84 g of sodium metabisulphite, 30 mL 6N HCl and anti bumping granules were added. The mixture was hydrolysed for 24 h in PEG bath set at 130 ℃ 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.

2.3.4. 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[30].

2.3.5. Statistical analysis

Analysis of variance was used to evaluate the analysis data and data were expressed as Mean±SD. Statistical calculation was performed with SPSS 15.0 for windows.

3. Results

3.1. Proximate composition

The protein content in the THF was found to be 32.59% where as in TBF the protein content was 31.50%. This result emphasizes that both the head and bone flours have more or less equal proportion of protein content. But the carbohydrate content of TBF was 1.45 % low when compared with that of THF 0.143%. The results also showed that the lipid content was high in TBF (0.8761%) and low in THF (0.2014%). The TBF also had low values of moisture and ash when compared with that of THF. The results are summarized in Table 1. Thus the tilapia head and bone flours have an important role as nutritional alternative due its high level of protein content.

Table 2
Fatty acids (mg/100 g) in tilapia head and in bone flour (THF &TBF)(n=3).

Fatty acids	THF	TBF
Palmitic acid (C16:0)	1.1787±0.0002	0.1143±0.0025
Stearic acid (C18:0)	1.9883±0.0025	0.7860±0.0025
Oleic acid (C18:1)	1.5468±0.0002	0.1141±0.0002
Linolenic acid (C18:2)	1.9860±0.0025	0.0816±0.0002
Alpha linolenic acid (C18:3)	2.4390±0.0025	0.1141±0.0002
Moroctic acid (C18:4)	0.1141±0.0002	0.0034±0.0003
Margaric acid (C18:0)	0.4146±0.0002	0.0088±0.0003

Table 3
Essential amino acids (%/g dry weight) in tilapia head and tilapia bone flour (TBH &THF).

Amino acids (in %/gm)	THF (%/g)	TBF (%/g)
Aspartic acid	1.837	0.9686
Glutamic acid	0.9967	0.7865
Asparagine	1.454	1.3165
Serine	0.343	0.6054
Gultamine	0.393	0.3981
Glycine	1.414	1.4141
Threonine	0.847	0.8564
Arginine	0.4411	0.4454
Alanine	0.1181	0.1076
Cystine	0.3430	0.3354
Tyrosine	0.1417	0.1577
Histidine	0.4343	0.3043
Valine	0.8881	0.8971
Methionine	0.4343	0.3982
Iso-leucine	1.414	1.2181
Phenyl alanine	1.978	0.9937
Leucine	0.807	0.7756
Lysine	1.048	1.342
Proline	1.414	0.5545
Tryptophan	0.887	0.7866

3.2. Fatty acid content

Fatty acids in fishes are derived from two main sources namely biosynthesis and diet[31].The fatty acid composition as a percentage of eluted methyl esters of the two flours is summarized in Table 2. The sequence of the fatty acids is ordered according to their chromatographic retention times. The saturated fatty acids were found to 3.16% in THF and 0.89% in TBF. The unsaturated fatty acids were found to be 1.96%/100 g in THF and 0.12% in TBF. The PUFA content was 2.55%/100 g in THF and 0.11% in TBF. The fatty acid composition of THF occurring in the highest proportions were alpha linolenic acid (C18: 3; 2.43%), stearic acid (C18: 0; 1.983%), linoleic acid (C18:2; 1.9860%), palmitic acid (C16: 0; 1.17%), oleic acid (C18:1; 1.54%) margaric acid acid (C17:0; 0.41%) and moroctic acid (C18:4; 0.11%). The fatty acid compositions of TBF occurring in the highest proportions were stearic acid (C18: 0; 0.7860%), oleic acid (C18:1; 0.114%), alpha linolenic acid (C18: 3; 0.1141%), palmitic acid (C16: 0; 0.1143%), linoleic acid (C18:2; 0.0816%), margaric acid acid (C17:0; 0.0088%) and moroctic acid (C18:4; 0.0034%). EPA, DHA and other fatty acids were was detected in very low amounts. The results were not significant in the statistical analysis (P< 0.05).

Table 4

Essential Vitamins in tilapia head and tilapia bone flour (TBH &THF)

Vitamins	THF	TBF
Vitamin A	145.6000±0.2500	12.5600±0.0300
Vitamin D	4.4500±0.0250	5.8000±0.0300
Vitamin E	1.9898±0.0002	4.8800±0.0250
Vitamin K	0.8981±0.0002	ND
Vitamin B6	0.4343±0.0002	0.2424±0.0002
Vitamin B12	0.1414±0.0003	0.1416±0.0002
Vitamin C	25.6400±0.0250	45.6500±0.0200

*Not detected.

Table 5

The mineral constituents in % of tilapia head and tilapia bone flour (TBH &THF) (n=3).

Minerals (%)	THF	TBF
Calcium	56.700±0.200	67.7600±0.0200
Magnesium	15.670±0.030	45.7600±0.0200
Zinc	0.343±0.003	0.2415±0.0002
Iron	3.098±0.002	2.4100±0.0200
Copper	1.414±0.002	0.1310±0.0010
Sodium	34.670±0.030	29.1100±0.0100
Potassium	16.780±0.020	15.6700±0.0200

3.3. Amino acid content

Twenty different amino acids were obtained in the head and bone flours of tilapia (*Oreochromis mossambicus*). Nine essential amino acids that are very important for the human body are all present in the THF and TBF flours. These essential amino acids are lysine, leucine, valine, isoleucine, threonine, phenylalanine, methionine, histidine and tryptophan. The major amino acids such as lutamic acid (0.9967%), aspartic acid (1.837%), lysine (1.048%) and leucine (0.807%) were found in THF and in TBF the major amino acids were found as glutamic acid (0.7865%), aspartic acid (0.9686%), lysine (1.342%) and leucine (0.7756%). The amino acid composition of THF and TBF is listed in Table 3 and the retention peaks for the essential amino acids in THF and TBF is shown in Figure 1 and Figure 2.

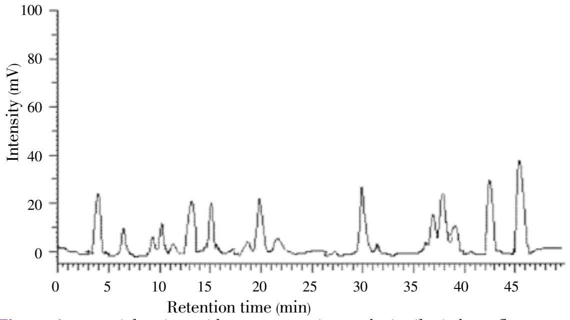

Figure 1. Essential amino acids (EAA) retention peaks in tilapia bone flour (TBF)

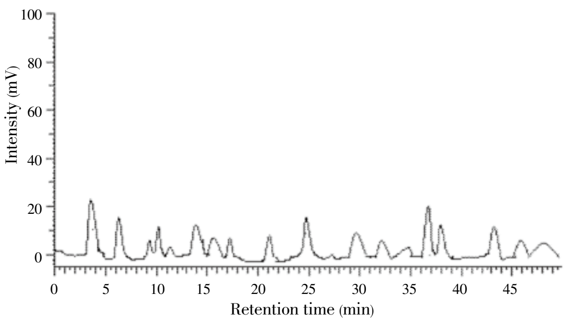

Figure 2. Essential amino acids (EAA) retention peaks in tilapia head flour (THF).

3.4. Vitamins and minerals

The TBF and THF flours possessed seven essential minerals such as calcium, magnesium, zinc, iron, copper, sodium and potassium. Among the seven vitamins reported Vitamin A was predominant in THF which ranged as 145.6 mg/g and vitamin C was found higher in TBF which ranged as 45.65 mg/g. The TBF lacked vitamin K where as THF contained 0.8981mg/gm of vitamin K. The list of vitamins detected in the THF and TBF is listed in Table 4. Calcium was reported as the major mineral in both the head and bone flours. The minerals estimated are listed in table 5. The results indicate that vitamin A and C are dominant in both the flours.

4. Discussion

Amino acids are also important in healing processes and the composition of amino acids in fish is similar to that in man, people can acquire essential amino acids in abundance and proper balance by eating fish. The essential amino acids cannot be manufactured in human bodies, but can be obtained from food. The present study indicated that the two species had all the essential amino acids. Deficiency in the essential amino acids may hinder healing recovery process. Leucine promotes the healing of bones, skin and muscle tissue. Isoleucine is necessary for haemoglobin formation, stabilizing and regulating blood sugar and energy. Glycine, which is one of the major components of human skin collagen, together with other essential amino acids such as alanine form a polypeptide that will promote re growth and tissue healing. Other reports of similar nature provided valuable information on selecting fish and fish oils for nutritional purposes.

Amino acids are also important in healing processes and the composition of amino acids in fish is similar to that in man, people can acquire essential amino acids in abundance and proper balance by eating fish. The essential amino acids cannot be manufactured in human bodies, but can be obtained from food. Stevanato[32] concluded that tilapia heads (*Oreochromis niloticus*) can be used for human consumption, as a nutritive and low-cost food, adding value to a waste product that would otherwise contribute to environmental pollution. The present study indicated that the two samples had all the essential amino acids. The results illustrate that flours made from head and bones of tilapia (*Oreochromis mossambicus*) present a good nutritive value in relation to their protein, total lipids and ash content. These processed foods will serve as a nutritive low cost food which would increase the importance of wastes which if left uncared may cause pollution to the environment. Fish waste is rich in potentially valuable oils, minerals, enzymes, pigments and flavors etc. Knowledge about quality and composition is a necessity. Currently, the major wastes (heads, viscera, skin, and skeleton) are underutilized and often create disposal problems and environmental concerns. These may also have many alternative uses in pharmaceutical, agricultural, aquaculture and industrial applications. In the light of these findings, it may be concluded that these by products are suitable items in the human diet if the nutritive value is further characterized in prfundity.

Conflict of interest statement

We declare that we have no conflict of interest.

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