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FATTY ACID COMPOSITION AND AMINO ACID PROFILE OF TWO FRESHWATER SPECIES, AFRICAN CATFISH (*CLARIAS GARIEPINUS)* AND TILAPIA (*TILAPIA ZILLII)*

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ABSTRACT

The proximate, fatty and amino acids composition of two commercially important freshwater fish species *Clarias gariepinus* and *Tilapia zillii* purchased from local fishermen in two landing sites in Lagos State, Nigeria were determined. Live specimens of *C.gariepinus* were purchased while samples of *T.zillii* were stored in an ice chest at 4^0C and conveyed to the laboratory for analyses. The chemical composition of the samples was determined using AOAC methods. The protein content of *C. gariepinus* and *T. zillii* was 18.8% and 19.0% (% wet weight), respectively. The total lipid content was high for *C. gariepinus* (9.3%) but low for *T. zillii* (1.1) %. Lipid composition varied in the two species but differences were not statistically significant (P>0.05). The ash was 1.2% for both species. The moisture content was higher in *T. zillii* (80.4%) than in *C. gariepinus* (74.3%). The percentage composition of individual fatty acids as a ratio of total muscle lipids of *C. gariepinus* ranged from 0.1% to 26.0% and that of *T. zillii* from 0.1% to 32.2%. Among them, those occurring in the highest proportions were myristic acid (C14:0, 4.2-5.2%), palmitic acid (C16: 0, 22.0-32.2%), palmitoleic acid (C16:1, 3.6-13.2%), heptadecanoic acid (C17:0, 0.7-3.0%), stearic acid (C18:0, 8.1-9.5%), linoleic acid (C18:2, 1.4-12.3%) and oleic acid (C18:1). Amino acids from sample protein in *C. gariepinus* ranged from 0.30% to 17.81% and that of *T.zillii f*rom 0.27% to 18.16%. The most abundant amino acids in the two fish species were glutamic acid, aspartic acid, leucine and lysine ranging from 9.49% to 18.16%. The ratio of ω3/ω6 fatty acids was less than 1 in *C. gariepinus* while in *T. zillii* it was 2.70. The two freshwater fish species contain essential fatty acids particularly eicosapentaenoic acids and docosahexaenoic acids and essential amino acids for promoting good health, prevention and healing of diseases in humans. The two freshwater fish were therefore found to be important sources of nutrients.

Key words: Proximate, Freshwater, fish, fattyacids, aminoacids.

INTRODUCTION

Fish is highly nutritious, tasty and easily digested. It is much sought after by a broad cross-section of the world's population, particularly in developing countries. It is estimated that around 60 percent of people in many developing countries depend on fish for over 30 percent of their animal protein supplies, while almost 80 percent in most developed countries obtain less than 20 percent of their animal protein from fish. However, with the increased awareness of the health benefits of eating fish and the ensuing rise in fish prices, these figures are rapidly changing. Fish also contains significant amounts of all essential amino acids, particularly lysine in which cereals are relatively poor. Fish protein can be used therefore to complement the amino acid pattern and improve the overall protein quality of a mixed diet [1].

In human nutrition, fatty acids such as linoleic and linolenic acids - important for preventing skin diseases - are considered essential as they cannot be synthesized by the organism. In marine fish, these fatty acids constitute only about 2% of the total lipids, which is a small percentage when compared to many vegetable oils. However, fish oils contain other "essential" polyunsaturated fatty acids which act in the same way as linoleic and arachidonic acids. As members of the linolenic acid family (first double bond in the third position, ω-3 counted from the terminal methyl group), they also have neurological benefits in growing children. One of these fatty acids, eicosapentaenoic (C20: 5 ω 3), has attracted considerable attention since Danish scientists found a significant amount in the diet of a group of Greenland Eskimos who proved virtually free from arteriosclerosis. Convincing evidence now exists for the significant role fish and fish oils play in decreasing the risk of developing cardiovascular diseases and in improving foetal brain development [2].

Muscle is the main part of fish used for human consumption [3] and when fish is suggested as a means for improving health, fatty acid and amino acid composition should be considered. Many heart specialists are recommending that their patients use generous quantities of fish in the diet to avoid excessive consumption of saturated fatty acids and as a means for the patient to obtain adequate protein in the diet without taking in excessive fat, which might result in their becoming overweight. Therefore, fish lipids and proteins have been recognized as being beneficial for human health.

Water bodies in Nigeria harbour a variety of fish species that serve as food and an economic resource to the country. Some of the most important species accounting for about 90% of Nigeria's fishery include croakers, catfishes, tilapias, threadfins, soles and the clupeids [4]. Fresh water fish constitute 69.6% of the total fish supply available to Nigeria [5]. This represents a major source of animal protein supply to Nigeria, which has a low *per capita* protein consumption [6]. Presently, there has been expansion of aquaculture in Nigeria, especially the culture of African catfish (*Clarias gariepinus*) and tilapia (*Tilapia zillii*) due to their tolerance to a wide range of temperatures, fast growth, adaptation to diverse environments, as well as to low oxygen and high salinity levels [7]. Knowledge of the fatty acid composition is desirable for important fish species such as the catfish and tilapia, not only because of the value of the fish as produce, but also because of possible new industrial

applications of the fish oil. In light of recent dietary and medical emphasis on the role of fatty acids in human physiology, it is important to know the fatty acid composition of this group of fish.

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). Long chain, n-3 PUFA cannot be synthesized by humans and must be obtained from the diet [8]. It is known that polyunsaturated fatty acids can regulate prostaglandin synthesis and hence induce wound healing [9, 10]. The ω-3 and ω-6 polyunsaturated fatty acids (PUFA) have been shown to have positive effects on cardiovascular diseases and cancers [11]. It is thus important, for human health, to increase the consumption of fish and fish products which are rich in polyunsaturated fatty acids [12, 13]. Polyunsaturated fatty acids composition may vary among species of fish, even among fresh water and marine fish [14]. Certain amino acids like aspartic acid, glycine and glutamic acid are also known to play a key role in the process of wound healing [15].

Lipids, protein, fatty and amino acids present in some fish species are well documented in temperate regions of the world. Two of the most important species, catfish *Clarias gariepinus* and tilapia *Tilapia zillii* were chosen for this work based on the fact that they have good consumer acceptance, are economically viable and are in low fat content. They are also the most farmed fish in the tropical and sub-tropical regions of Africa and have been playing an increasingly key role in the nation's nutrition as source of relatively cheap animal protein [4]. Yet, their nutrition profile is lacking in literature. Aspects of the biology of Tilapia species occurring in the Lagos lagoon [16, 17], their abundance in Lake Kianji [18] and abundance of *Clarias gariepinus* in Anambra River Basin 2 [19] as well as fatty acid of smoked *C. gariepinus* in Northern Nigeria [20] have been extensively studied. Despite high demand, commercial value and distribution of these fish, there is the need for precise data on their nutrient composition in South-west Nigeria where they are highly consumed.

Thus, this study was carried out to determine the proximate, fatty acid and amino acid composition of the two common freshwater fish species *Clarias gariepinus* and *Tilapia zillii*.

MATERIALS & METHODS

Collection of samples

African catfish (*Clarias gariepinus*) and Tilapia (*Tilapia zillii*) fish samples for this study were obtained from Makoko and Epe Landing sites in Lagos, Nigeria. Live specimens of *C. gariepinus* were purchased while samples of *T. zillii* were stored in an ice chest at 4^0C and conveyed to the laboratory. The specimens were packaged in separate labeled polythene bags and eventually stored in a cold store (-22 °C) at the Nigerian Institute of Oceanography and Marine Research (NIOMR), Victoria Island, Lagos.

Biometric measurements

Fish samples were thawed in the open air in the laboratory and individual data for length, weight and sex taken and recorded. The standard length was measured using a measuring board. The weight was measured with a Satorious top loading electronic weighing balance, Satorious- Werke GMBH model (Type 1106/ Fabr. Nr. 2608053).

Sample preparation

Each fish was dissected, gutted and the gonad removed to determine the sex by visual examination. The fish sample was then cleaned, filleted and placed in a Waring Blender and homogenized for 15 min. Samples for the different chemical analyses were then taken from the homogenous material. Triplicate determinations were carried out on each sample.

Proximate composition analysis

The moisture content of the fish species was determined using the air oven drying method using a known weight of the fillet at 105°C until a constant weight was obtained [21]. Ash content was determined by incineration of the dried sample obtained from moisture determination in a muffle furnace at 525°C for 24h. Crude protein content was calculated by converting the nitrogen content, determined by Kjeldahl's method (6.25 X N) [21]. The lipid extractions were performed by modification of the method of Bligh and Dyer as described by AOAC [21,22].

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. The analysis was carried out by a commercial analysis laboratory (Eclipse Scientific Group Laboratory, Chatteris- Cambridge, Uk).

Amino acids analysis

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 hydrolysed for 24 h in PEG bath set at 130^0C 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 analyser. The data was collected in the form of chromatograms. The analyser was ion exchange with several buffers at varying pH running through the column. Each sample takes four hours to run through the system. The analysis was carried out by a commercial analysis laboratory (Institute of Grassland and Environmental Research Laboratory, Aberystwyth, UK).

Statistical analysis
The descriptive statistics (mean, standard deviation, range) were conducted while statistical significance of differences ($P \leq 0.05$) was determined by analysis of variance (ANOVA) with SPSS version 10.0 [23].

RESULTS

The standard length ranged from 16.3cm - 35.8cm; 9.3 cm -20.1cm and the weight from 106.2g-274.6g; 51.3g-289.6g for *C.gariepinus* and *T. zillii,* respectively.

Proximate composition
The proximate composition of the two freshwater species *C. gariepinus* and *T. zillii* is shown in Table1.

Moisture content
The moisture content ranged from 65.64 to 80.17% with a mean of 74.3% for *C. gariepinus* and 71.77 to 80.47% and mean of 80.4% for *T. zillii*. No significant difference was noted ($p > 0.05$) between the species.

Protein content
The protein content of *C. gariepinus* ranged from 18.34 to 20.32% while that of *T. zillii* ranged from 17.75% to 20.84%. The mean protein content ranged from 18.8% in *C. gariepinus* to 19.0% in *T. zillii*. No significant difference was noted ($p > 0.05$) between the species. The highest protein content was recorded on *T. zillii.*

Lipid content
The lipid value of *C. gariepinus* ranged from 0.71 % to 9.84% and that of *T. zillii* from 0.28 to 1.37%.The mean total lipid content ranged from 1.1% to 9.3%. No significant difference was noted ($p>0.05$) between the species. The highest lipid content was obtained on the samples from *C. gariepinus.*

Ash content
The ash content of *C. gariepinus* ranged from 1.00% to 2.92% and 0.97% to 1.38% in *T. zillii*. The mean crude ash in both species was 1.2% (% wet weight). No significant difference was noted ($p > 0.05$) between the species.

Fatty acid content
The fatty acid composition as a percentage of eluted methyl esters of the two freshwater species is summarized in Table 2. The sequence of the fatty acids is ordered according to their chromatographic retention times. The fatty acid composition of *C. gariepinus* ranged from 0.1% to 26% and that of *T. zillii* from 0.1% to 32.2%. Among them those occurring in the highest proportions were myristic acid (C14: 0; 4.2-5.2%), palmitic acid (C16: 0; 22.0-32.2%), palmitoleic acid (C16:1; 3.6-13.2%), heptadecanoic acid (C17:0; 0.7-3.0%), stearic acid (C18: 0; 8.1-9.5%), oleic acid (C18:1 16.2-26.0%) and linoleic acid(C18:2; 1.4-12.3%).

The species *C. gariepinus* has the ω-3: ω-6 ratio of less than 1 (0.39), whereas *T. zillii* has 2.70. The PUFA/Saturated (P/S) ratio obtained was 0.60 and 0.22 (Table 3).

Amino acid content

Twenty different amino acids were obtained in the two freshwater fish species. The total amino acid for *C. gariepinus* was 168.84 mg g^{-1} and *T.zillii* was 170.26 mg g^{-1}. Nine essential amino acids that are very important for the human body are all present in the two fish species. These essential amino acids are lysine, leucine, valine, isoleucine, threonine, phenylalanine, methionine, histidine and tryptophan.

The amino acid composition as a percentage of total protein is shown in Table 4. The major amino acids are glutamic acid, aspartic acid, lysine and leucine and ranged from 9.49% to 18.16% of total protein in the fish flesh. Levels of other amino acids ranged from 0.30% to 17.81% in *C. gariepinus* and 0.27% to 18.16% in *T. zillii*.

DISCUSSION

Fish, like other animals, have the ability to accumulate lipids in their body. There are a number of classifications available by which fish is divided into groups, for example according to their lipid content. The species *C. gariepinus* belong to the group of high protein-medium fat while *T. zillii* is of high protein- low fat category [24]. The total fat content in *C. gariepinus* was about 9% by weight, while *T. zillii* was 1%. It was reported that low –fat fish have higher water content, as observed in this study (Table 1) [25]. The species *T .zillii* had the highest moisture content. Ash in muscle declined steadily during the starvation of non-fatty fish once the water content has increased above a critical value. Fat content is influenced by species, geographical region, age, and diet [26].

In general, the fatty acid composition of the fish analyzed is in agreement with the data available on the fatty acid composition of similar fish species as reported in previous work [27]. Of the saturated fatty acids, palmitic acid (C16:0) was found to have the greatest proportion in the two fish species analyzed.

The two fish species contained arachidonic acid (C20:4), which is a precursor for prostaglandin and thromboxane biosynthesis [28]. Arachidonic acid can interfere with the blood clotting process and attach to endothelial cells during wound healing [14] although the level of arachidonic acid was very low in the two species. This, however, was contrary to the result obtained in three local Malaysian Channa spp fish [29]. The levels of DHA in the current work suggest that the two fish species can have a healing effect for muscle pain and inflammation [30]. Docosahexaenoic acids (DHA) and EPA had been reported to have preventive effects on human coronary artery disease [31]. Therefore, fish have been suggested as a key component for a healthy diet in humans [14].

The ω-3:ω-6 fatty acids ratio has been suggested to be a useful indicator for comparing relative nutritional values of fish oils. The ratio of ω-3: ω-6 PUFA ranged from 0.39 (*C. gariepinus*) to 2.70 (*T .zillii*) indicating much species variation as was reported in previous work [32]. The ratio of ω-3:ω-6 fatty acids in total lipid of

freshwater fish was typically in the range 0.5-3.8 compared to 4.7-14.4 in marine fish. It was suggested that a ratio of 1:1 or 1:5 would constitute a healthy human diet [24]. The two freshwater fish had the ω-3:ω-6 ratio within the recommended ratio.

It is generally recognized that PUFA composition may vary among species of fish but little attention has been paid to the PUFA composition of different species when selecting fish for diets. All fish were considered of similar nutritional value and selection was made primarily on the basis of availability, freshness, flavour and similar factors [32]. Results of clinical and epidemiological research suggest that EPA and DHA, found only in fish and sea foods, possess extremely beneficial properties for the prevention of human coronary artery disease [33]. Fish oils have been proposed as antithrombotic dietary supplements [33]. Therefore, when fish are suggested for health promotion, both the fat content and the PUFA distribution must be considered. The present study indicated that the two freshwater fish species had significant quantities of EPA and DHA and were therefore an average source of polyunsaturated fatty acids (PUFA).

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 [34]. 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 regrowth and tissue healing [35,36]. Other reports of similar nature provided valuable information on selecting fish and fish oils for nutritional purposes [32, 37, 38].

CONCLUSION

In this study we confirm that the two freshwater fish species examined had high protein -medium oil (*C. gariepinus*) while *T. zillii* had high protein - low oil. They are a good source of fatty acids and amino acids. The two fish species can be used generously in the diets to avoid excessive consumption of saturated fat which if consumed without a sufficient supply of polyunsaturated omega-3s will be used to construct cell membranes. The resulting cell membranes are, however, less elastic, a situation that can have a negative effect on the heart because it makes it harder to return to a resting state. As a result, the two freshwater fish species contain essential fatty acids particularly eicosapentaenoic acids and docosahexaenoic acids and essential amino acids for promoting good health, prevention and healing of diseases in human beings. The two freshwater fish were, therefore, found to be important sources of nutrients.

Table 1: Proximate composition of two freshwater species *C. gariepinus* and *T. zillii*

Species	Composition			
	Protein (% w/w)	Lipid (% w/w)	Ash (% w/w)	Moisture (% w/w)
C. gariepinus	18.8±1.9	9.3±0.3	1.2±0.5	74.3±3.7
T.zillii	19.0±1.9	1.1±0.4	1.2±0.2	80.4±3.7

Values are monthly mean ±SD, of three separate determinations.

Table 2: Fatty acid composition of two freshwater species *C. gariepinus* and *T. zillii.*

Fatty acid	*C. gariepinus*	*T. zillii*
C8:0 Caprylic acid	ND	ND
C10:0Capric acid	ND	0.1
C12:0Lauric acid	3.1	0.1
C14:0Myristic acid	4.2	5.2
C14:1Myristoleic acid	0.2	0.5
C15:0Pentadecanoic acid	1.4	0.3
C16:0 Palmitic acid	22.0	32.2
C16:1Palmitoleic acid	3.6	13.2
C17:0Heptadecanoic acid	3.0	0.7
C17:1Heptadecenoic acid	0.2	0.2
C18:0Stearic acid	8.1	9.5
C18:1Oleic acid	26.0	16.2
C18:2Linoleic acid	12.3	1.4
C18:3Linolenic acid (omega3)	0.9	1.0
C18:3Linolenic acid (omega6)	0.6	ND
C18:4Octadecatetraenoic acid	1.6	ND
C20:0Arachidic acid	0.2	0.2
C20:1Gadoleic acid	2.5	0.6
C20:2Eicosadienoic acid	ND	0.6
C20:3Eicosatrienoic acid (omega3)	0.1	ND
C20:3Eicosatrienoic acid (omega6)	0.6	ND
C20:4Arachidonic acid (omega3)	0.3	0.3
C20:4Arachidonic acid (omega6)	0.6	0.6
C20:5Eicosapentaenoic acid	0.7	1.0
C22:0Behenic acid	0.1	0.8
C22:1Cetoleic acid	1.3	ND
C22:4Docosatetraenoic acid	1.3	0.6
C22:5Clupanodonic acid	3.7	1.0
C22:6Docosahexaenoic acid	3.5	3.0
C24:0Lingnoceric acid	ND	ND

Notes: Values are % of eluted methyl esters;
ND = Not Detected

Table 3: Fatty acid ω-3/ ω-6 and polyunsaturated /saturated fatty acid ratio in two freshwater fish species.

Species	ω-3:ω-6	Ratio of P/S
C. gariepinus	0.39	0.60
T.zillii	2.70	0.22

P/S: Polyunsaturated /saturated fatty acid ratio.

Table 4: Amino acid composition of two freshwater fish species as a percentage of total protein.

Amino Acid	*Clarias gariepinus*	*Tilapia zillii*
Cystine	1.16 ± 0.06	1.15 ± 0.07
Taurine	0.53 ± 0.03	1.51 ± 0.09
Aspartic acid	11.35 ±0.61	11.17±0.69
Methionine sulphone	3.17 ±0.17	3.17 ±0.19
Threonine	4.81 ±0.26	4.80 ±0.29
Serine	4.48 ±0.24	4.47 ±0.27
Glutamic acid	17.81 ±0.96	18.16 ±1.11
Glycine	5.07 ±0.27	5.20 ±0.32
Alanine	6.45 ±0.35	6.77 ±0.41
Valine	5.34 ±0.29	5.18 ±0.32
Isoleucine	5.22 ±0.28	5.04 ±0.31
Leucine	9.53 ±0.51	9.49 ±0.58
Tyrosine	1.15 ±0.06	1.47 ±0.09
Phenylalanine	4.19 ±0.23	4.06 ±0.25
g-aminobutyric acid	0.51 ±0.03	0.66 ±0.04
Ornithine	0.65 ±0.04	0.27 ±0.02
Lysine	10.64 ±0.57	10.37 ±0.63
Arginine	6.82 ±0.37	11.66 ±0.72
Hyroxyproline	0.30 ±0.00	0.30 ±0.02
Proline	3.81 ±0.21	3.95 ±0.24
Total amino acids	168.84	170.26

Note: Values are mean of triplicate ± Standard deviation.

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