

INVESTIGATION OF THE CONSTITUENT FATTY ACIDS OF SOME FRESHWATER FISHES COMMON IN NIGERIA

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ABSTRACT

Ugoala, C.; Ndukwe, G. & Audu, T. 2009. Investigation of the constituent fatty acids of some freshwater fishes common in Nigeria. *Braz. J. Aquat. Sci. Technol.* 13(1):65-70. ISSN 1808-7035. The aim of this paper was to investigate the constituent fatty acids of some freshwater fish species as well as compare the nutritional quality of freshwater fish with that of marine fish by comparing the levels of essential fatty acids present. The fatty acids profile of freshwater fish species include minor amounts of odd-number, branched-chain, and even-number fatty acids as well as saturated components, the monounsaturated fatty acids and polyunsaturated fatty acids. The major saturated fatty acids were C14:0 and C16:0. The C18:1 is the prominent monounsaturated fatty acids while the C18:2 is the dominant polyunsaturated fatty acids. The essential fatty acids compositions showed prominence in C18:3n-3 and C18:2n-6. The overall significance of this study had been its revelation that freshwater fish are good source of omega-6 essential fatty acids. The high percent of saturated fatty acids in freshwater fish gives them an advantage in curing processing.

Keywords: Fatty acids composition, Freshwater fish, Marine fish, nutritional quality, Fatty acids methyl ester.

INTRODUCTION

There is an inter and intra species variability in the composition of fatty acids of fish lipids (and of the specific polyunsaturated fatty acids in particular). This could be explained by the existence of a large number of external and internal factors. The external factors are environment, culturing method, and tropic effects. The internal factors include fish species, feeding regime and digestion, life cycle stage, quantitative and qualitative characteristics of lipids- triacylglycerols, phospholipids and their topographical origin- dorsal and ventral part of muscle tissue (Buchtova *et al.*, 2004).

In recent years, there have been a large number of experimental studies into some of the above factors causing changes in the composition of fatty acids in various fish species (Csengeri *et al.*, 1978; Farkas *et al.*, 1978; Vanderwesthuyzen *et al.*, 1984; Suzuki *et al.*, 1986; Viola *et al.*, 1988; Bieniarz *et al.*, 2000). Other authors have studied the impact that various types of heat treatment will have on the fatty acids composition (Gall *et al.*, 1983; Maeda *et al.*, 1985; Tothmarkus and Sasskiss, 1993; Fajmonova *et al.*, 2003).

Detailed information about lipid components and their fatty acids constituents is needed to understand how to diminish oxidative or hydrolytic factors which affect quality of fish. The nature, proportion, and degree of unsaturation of the fatty acids in the lipids are all closely related to the oxidation of the oils. However, the

fatty acids composition of the muscle cell membranes are especially important factors in determining the stability because oxidative changes are initiated from the membrane components of muscle (Buckley *et al.*, 1989). Rancidity development is a vital concern to the food industry because it may result in sensory changes (flavour and aroma), loss of nutritional value (Essential fatty acids, fat-soluble vitamins: A, D, E, K), production of primary and secondary oxidation products (hydro peroxides, free radicals, epoxides, etc). It can also be used for indexing, assisting in technology development.

Fatty acids profile analysis also provide information about the essential fatty acids requirements of fish which would aid the compounding of adequate protein-to-fat ratios feed that would balance energy requirements with caloric intake.

The aim of the study reported here was to investigate the fatty acids constituents of some freshwater fish species present in a Nigerian Lake by determining the amounts of saturated, monounsaturated, and polyunsaturated n-3 and n-6 fatty acids.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh captured fishes were sorted and identified. They include *Mormyridae* (*Hyperopisus bebe*, *Mormyrops anguilloides* and *Mormyrus rume*), *Cichlidae*

(*Oreochromis niloticus* and *Sarotherodon galilaeus*), *Clariidae* (*Clarias gariepinus* and *Clarias anguillaris*), and *Heterobranchus bidorsalis*, *Centropomidae* (*Lates niloticus*), *Clariheterobranchus* (a hybrid of *Clarias* spp and *Heterobranchus* spp) and *Characidae* (*Hydrocynus forskalii*) were obtained from Fishermen at the Kainji Lake Dam site. The fishes were weighed, beheaded, eviscerated and cleaned prior to freezing. In an attempt to obtain a homogeneous sample from each species, their flesh was removed from their backbones, minced, blended and immediately extracted using chloroform-methanol mixture in the ratio of 2:1.

Lipid Extraction

Lipid extractions were carried out on minced fish samples (10 g each) using the extraction methods of Folch *et al* (1957). In this method, chloroform-methanol was used in the ratio of 2:1. A total of three repetitions were performed per sample. The solvent mixtures were concentrated in vacuo using a rotary evaporator maintained at 35°C and the extracts were stored in sample vial respectively.

Fatty Acids Analysis

The method of Park & Goins (1994) was used in the methylation of the extracted fish lipids. Methylene chloride (100 μ L) and 1 mL 0.5M NaOH in methanol were added to oil extract in a test-tube and heated in a water bath at 90°C for 10 min. The test tube was removed from the water bath and allowed to cool before the addition of 1 mL 14% BF₃ in methanol. The test tube was heated again in a water bath for 90°C for 10 min, and cooled to room temperature. One mL of distilled water and 200 μ L hexane was added to the test tube respectively and then fatty acid methyl ester was extracted by vigorous shaking for one minute. After centrifugation, the top layer which contains the fatty acids methyl ester was collected and transferred into a sample vial for analysis. The fatty acids profiles were determined using an Agilent Gas Chromatograph, Model 6890N fitted with an Agilent Mass Selective Detector, 5973 series. Separation was carried out in a capillary column (30 x 0.25 mm id x 0.25 μ m DB wax). The starting temperature was 150°C maintained for 2 minutes at a heating rate of 10 °C/minute. The total running time was 22 minutes. Helium was the carrier gas while the injection volume was 1 μ L.

RESULTS AND DISCUSSION

Hyperopisus bebe, *Mormyrops anguilloides*, *Mormyrus rume*, *Oreochromis niloticus*, *Sarotherodon galilaeus*, *Clarias gariepinus*, *Clarias anguillaris*, *Heterobranchus bidorsalis*, *Lates niloticus*,

Clariheterobranchus, and *Hydrocynus forskalii* are commercially important to freshwater fisheries in Nigeria, and they constitute a major part of landing, aquaculture and sources of animal protein. The fatty acids compositions of these fishes vary greatly. Therefore, a substantial normal variation is observed (Table 1) for the fatty acids constituent of these fishes as well as those of marine origin (Table 2) and their degree of unsaturation (Table 3). The fatty acids profiles include minor amounts of odd-number, branched-chain, and even-number fatty acids. These varieties as well as the quantity and quality of fatty acids noticed may be due to differences in subspecies, diet, spawning cycle, season and environment. The degree of unsaturation of fish oils vary with seasons. It rises as the water temperature falls and vice versa (IFFO BULLETIN No.18).

The knowledge of fatty acids composition of fishery species has fundamental importance in the application of different technological processes in fish preservation, processing and product development.

Clarias anguillaris and the tilapia species seem to be the most saturated fishes among the freshwater species (Table 3). The major saturated fatty acids identified in these species were C14:0 and C16:0. This high proportion of saturated fatty acids in these species could account for low iodine value in their oils. However, substantial amount of C18:2n-6 and C18:1 (Table 3) could also necessitate high iodine value. High iodine value shows the level of unsaturation in the fish oil. C18:1 and C18:2n-6 presence may encourage lower melting point. C18:2n-6 and C18:3n-3 content among other factors (viscosity, colour, iodine value and peroxide value) determine oil quality for industrial purposes. Saturated fatty acids and cholesterol are major dietary contributors to coronary heart disease, due to their oxidation in the presence of light and molecular oxygen through a free radical reaction (Diplock, 1993). The branched chain fatty acids contents in the freshwater species studied were generally low especially in *Hyperopisus bebe*. Branched chain fatty acid has an important advantage. Branched chain fatty acids influence lower melting point, lower cholesterol levels, provide energy, influence some ribosomal functions which are necessary for peptide elongation and form an integral part of biomembranes while those esterified with cholesterol stimulate protein synthesis. (Hradec *et al*, 1974).

Fatty acids composition is also important as an aspect of quality of raw material, sensory attributes and storage stability. *Hyperopisus bebe* and *Mormyrus rume* content of the monoene fatty acids is about ninety percent with C18:1 the prominent monounsaturated fatty acids. Polyunsaturated fatty acids attained the highest value (90%) in *Hydrocynus forskalii*. High contents of polyunsaturated fatty acids in *Lates niloticus*, *Mormyrops anguilloides*, *Hydrocynus forskalii* and

Table 1. Fatty acids profile of freshwater fish.

FATTY ACIDS	<i>Clarias anguillaris</i>	<i>Lates niloticus</i>	<i>Mormyrops anguilloides</i>	<i>Mormyrus rume</i>	<i>Hydrocynus forskali</i>	<i>Heterobranchus bidorsalis</i>	<i>Hyperopisus bebe</i>	<i>Clarias gariepinus</i>	<i>Clariheterobranchus</i>	<i>Sarotherodon galilaeus</i>	<i>Oreochromis niloticus</i>
12:0	11.20		5.23	4.58		19.89	3.58		9.79		
13:0	3.43			0.98		4.30	0.65				
14:0	7.99					14.52		31.61		3.81	17.82
14:1	19.63							1.23			
15:0	4.79			4.58		9.14	3.58	5.43		5.08	4.53
15:1	2.74			1.96		8.60	0.65	27.90	2.25		
16:0		2.15								36.38	34.14
16:1										10.15	12.39
17:0										4.65	1.81
17:1		0.92									
18:0	2.05	3.38						3.46		9.31	5.74
18:1										12.27	9.37
18:1cis		4.92						2.22			
18:1trans	4.11	4.31						15.56			
18:2n-6										4.65	3.02
18:3n-3		6.15	1.31			6.18	0.98	2.22		2.12	3.63
18:2cis		22.15	14.05			4.57					
18:2trans		3.38	1.31				0.33	1.98	19.26		
18:3n-6		5.85	12.09					4.94			
18:4										2.12	1.51
20:0		3.08	6.54						13.16		
20:1n-9		4.31	13.73						7.38		
20:2		3.69	5.23						12.68		
20:3n-6		7.07	4.25					1.48	4.17		
20:3n-3		2.78	4.25			4.30					
20:4n-6		3.69	9.48					1.98	3.85	2.96	1.51
20:5n-3		3.69	3.27							0.17	0.60
21:0		10.46	7.52						3.85		
21:1n-11			2.61			13.98					
22:0	36.53										
22:1n-11				86.27			89.25				
22:2		6.46	0.65		89.57	12.09					
22:5n-3										2.54	1.51
22:6n-3										3.81	2.42
23:0	2.51		1.31		10.43						
24:0		1.54									

2-piperidinone also found in *Mormyrops anguilloides*

Clariheterobranchus (Table 3) and in the marine species, explains why they have very short keeping quality. They are very easily deteriorated if not well preserved. These fishes may not therefore be suitable for preservation through smoking, frying and sun drying except if antioxidants are used. Large or large oily fish require longer periods of processing, therefore cold smoking technique (45-60°C) could be recommended for such fish species.

The presence of high levels of polyunsaturated fatty acids also means that when oils from these species are heated above 150°C, formation of dimers and trimers as well as ring compounds could happen, while high amount of hydrogen are required for hydrogenation.

In all the fish species analysed, the dominant polyunsaturated fatty acids are of the Omega-6 series and are found chiefly in C18:2 fatty acids. The essential

fatty acids compositions showed prominence in C18:3n-3 and C18:2n-6. C22:6n-3 was noted in the tilapia species.

Clarias anguillaris, *Mormyrus rume*, *Hydrocynus forskali* and *Clariheterobranchus* have undetectable levels of Omega-3 fatty acids. Omega-6 fatty acids were not detected in *Clarias anguillaris*, *Mormyrus rume*, *Hydrocynus forskali*, *Heterobranchus bidorsalis*, and *Hyperopisus bebe*. The essential fatty acids were lacking in *Clarias anguillaris*, *Mormyrus rume*, and *Hydrocynus forskali*. C22:6n-3 were absent in all the species except the tilapias. The tilapia species contain all the essential fatty acids although they vary in composition. C18:2n-6 contents in *Lates niloticus* and *Clariheterobranchus* as well as *Mormyrops anguilloides* are high in that order. The oils were characterized by low levels of Omega-3 polyunsaturated fatty acids. Fish oils rich in C18:2n-6 is essential in human nutrition

Table 2: Fatty acids compositions of some marine fish species. Values courtesy of IFFO Fish oil bulletin no. 18.

FATTY ACIDS	Anchovy	Horse mackerel	Menhaden	Sardine	Capelin	Herring	Mackerel	Norway pout	Sand eel	Sprat
14:0	8.48	7.95	8.95	5.68	6.53	8.50	7.80	5.57		
14:1		0.55	0.30							
15:0	0.73	16.70	0.65							
16:0	19.43	7.35	19.10	17.80	10.23	14.27	16.10	12.30	14.63	16.50
16:1	8.85		11.50	6.80	9.30	6.13	9.00	4.70	7.03	6.80
16:2			1.65							
16:3		1.40	1.90							
16:4		1.65	1.10							
17:0	0.93	0.60	0.90							
18:0	4.45	3.05	3.45	3.09	1.30	1.03	1.80	2.70	1.80	2.17
18:1	14.20	9.30	10.00	7.85	14.03	9.67	12.90	13.90	9.05	15.67
18:2	1.78	1.15	1.25	2.58	1.23	1.37	1.30	1.23	7.23	1.97
18:3	0.68	0.60	1.35	1.87	0.60	1.50	1.10	1.17	5.68	1.73
18:4		1.90	2.70		5.33	3.00	2.50	4.30		
20:0		0.35		0.63						
20:1	3.70	5.55	0.90		12.67	12.60	12.10	10.23	15.08	10.27
20:2					0.40	0.57	0.20	0.60		
20:3		0.30				0.40				
20:4	0.70	0.85	2.25		1.67	5.10		2.57		
20:5	16.23	13.65	14.10	8.43	9.97		7.60	8.23	9.05	6.33
21:5		0.35	0.65							
22:0		0.20								
22:1	1.85	9.55	0.30	5.07	10.63	21.83	13.90	11.70	15.53	14.10
22:2					0.60	0.37		0.60		
22:4										0.67
22:5	0.98	2.00	2.45		0.63	0.80	0.60	10.67	0.73	0.65
22:6	9.55	9.35	8.55	15.53	9.57	6.47	7.70	14.33	8.90	8.73

because of the presence of the fatty acids. These fatty acids are not synthesized by the body but are required for tissue development. Our result however, generally suggests that Wild freshwater fish has low Omega-3: Omega-6 ratios (Table 3).

Comparison of Freshwater Fish and Marine Fish

The fatty acid composition of marine fish species (Table 2) have similar pattern. The freshwater species however, differs in their fatty acids compositions pattern. A study of Table 2 places the marine fish species into two groups. Group one include Anchovy, Horse mackerel, Menhaden and Sardine while Capelin, Herring, Mackerel, Norway pout, Sand eel and Sprat make up group two. Group one are higher in saturated fatty acids, particularly C16:0, higher in polyunsaturated fatty acids (C20:5), lower in monounsaturated fatty acids (C20:1 and C22:1), similar in fatty acids composition, and may be more susceptible to oxidation compared with group two oils because of the higher level of unsaturation of the former.

Our results revealed that freshwater fish has more Omega-6 series of the polyunsaturated fatty acids while the marine has more Omega-3 series (Tables 3). The prominent Omega-3 being C22:6 while the C18:2 are

for the Omega-6 series. This may suggest that the dietary essential fatty acids requirements for marine fish for Omega-3 polyunsaturated fatty acids may be higher than that of freshwater fish. It could also suggest that marine species are suppliers of C18:3n-3; the initial building blocks for all Omega-3 fatty acids in the human body although *Clarias anguillaris*, *Lates niloticus*, *Heterobranchus bidorsalis*, *Clarias gariepinus*, *Clariheterobranchus*, *Oreochromis niloticus* and *Sarotherodon galilaeus* compares favourably with marine in C18:3n-3. However, only *Lates niloticus* seems to compares well with marine species on C20:5n-3 content. The marine species are better in C22:6n-3. The freshwater species are in C18:2n-6 as well as in C20:4n-6 contents. The latter fatty acid is a major constituent of membrane lipids (phospholipids) and is the principal precursor by enzymatic action of hormone-like compounds known as eicosanoids including the prostaglandins (prostanoids, isoprostanes, and isofurans). The eicosanoids produced from C20:4n-6 cause the strongest inflammatory response in humans. Inflammation is one of the body defense mechanisms that reduce the spread of infection.

Table 3. Major fatty acids found in freshwater fish. Nd-not detected, SFA-saturated fatty acid, MUFA-monounsaturated fatty acid, and PUFA-polyunsaturated fatty acid.

Fatty Acids Class	<i>Clarias anguillariass</i>	<i>Lates niloticus</i>	<i>Mormyrops anguilloides</i>	<i>Mormyrus rume</i>	<i>Hydrocynus forskali</i>	<i>Heterobranchius bidorsalis</i>	<i>Hyperopisus bebe</i>	<i>Clarias gariepinu</i>	<i>Clarihetero-branchus</i>	<i>Sarotherodon galilaeus</i>	<i>Oreochromis niloticus</i>
SFA	73.51	20.63	33.00	11.77	10.43	50.28	8.79	40.49	50.41	59.21	64.04
MUFA	26.48	14.46	16.34	88.23	nd	22.58	89.90	46.91	9.63	22.42	21.76
PUFA n-3	nd	12.62	8.83	nd	nd	10.48	0.98	2.22	nd	8.64	8.16
PUFA n-6	nd	16.61	25.82	nd	nd	nd	nd	8.40	8.02	7.61	4.53
PUFA total	nd	64.91	50.66	nd	89.57	27.14	1.31	12.60	39.96	18.37	14.20
n-3:n-6		0.76	0.34	nd	nd	10.48	0.98	0.26	0.00	1.14	1.80
BRANCHED	4.79	34.76	15.36	nd	nd	4.57	0.33	19.76	19.26	nd	nd

CONCLUSION

The paper investigated the constituent fatty acids of some freshwater fish species as well as compares the nutritional quality of freshwater fish with that of marine fish by comparing the levels of essential fatty acids present. The overall significance of this study had therefore been its revelation that marine fishes are better sources of omega-3 essential fatty acids while freshwater fish are good source of omega-6 essential fatty acids. The regular pattern of marine fish fatty acids compositions makes them of advantage to the Hydrogenator. The high percent of saturated fatty acids in freshwater fish gives them an advantage in curing processing. The freshwater fish may have better oil quality than their marine counterparts because of the high content of 18:2n-6 and 20:4n-6 Fatty acids.

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