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Determination of Fatty Acids from Freshwater Fish Oils Using GC-MS Method

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Abstract: Thirteen species of fish (*Labeo coubie, Citharinus citharus, Hyperopisus bebe, Mormyrops anguilloides, Mormyrus rume, Orechromis niloticus, Sarotherodon galilaeus, Clarias gariepinus, Clarias anguillaris, Heterobranchus bidorsalis, Clariheterobranchus, Lates niloticus* and *Hydrocynus forskalii*) were studied for their oil fatty acids composition. Identification and quantitative measurement of fish oils fatty acids were carried out by gas chromatography coupled with mass spectrometry. GC-MS was applied on fatty acids methyl esters. Fatty acids were identified in order of their retention time. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) formed a large proportion of total lipids. Multi-methyl branched, methyl branched fatty acids contributed a smaller proportion. It can be concluded that these fish oils are excellent sources of essential fatty acids omega-6 and omega-9.

Keywords: Fatty acids; Gas chromatography; Retention time; Freshwater fish

I. Introduction

Fish is increasingly being consumed in the developing world especially as it is the most affordable source of cheap protein. However due to the ever increasing population, demand far exceeds supply since there is not an endless amount of fish. This therefore calls for improved management and utilization of fish stocks. Fish resources are limited. Any collapse of the major stocks would be economically disastrous. Aquaculture is therefore designed at increasing the production of fisheries for human consumption. Different species of plants and animals being cultured continues to increase every year with the advanced culturing/rearing techniques. However, fish culture is being hampered by the high cost and scarcity of inputs like fast growing fish seeds.

Although fatty acid compositions of organisms have been investigated for decades, however, much of the early lipid research was directed at determining the commercial value of fish oils and understanding how fat content relates to various life history functions. Because the composition of certain lipids can be closely related to the types of food recently ingested, recent investigations have been directed at diet analysis and foraging distribution [1, 2]. A low fatty acids diet is generally healthier, but for growing and proper development and function, the human body needs a certain amount of fats. Consumption of foodstuff that contains a large amount of saturated fatty acids is associated with heart disease, diabetes, cancer; therefore, the diet must contain unsaturated fatty

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acids. Polyunsaturated fatty acids (PUFA), especially ω-3 fatty acids docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) are essential dietary nutrients for human health; they are defined "essential" fatty acids since they cannot be synthesized by the human body and consequently they must be provided from the diet [3]. PUFAs play important roles in the human body, such as in the synthesis of specific active compounds, in the brain and eye development of infants or in reducing the bad" cholesterol and thus in the prevention of the coronary heart disease [4, 5 and 6]. Marine organisms (fish, seafood, algae) are the main natural sources of essential fatty acids in human diet (mainly EPA and DHA). Fish oil is considered to have the highest amounts of ω-3 PUFA [7, 8].

Techniques using fatty acids have been used to obtain information on trophic relationships, diet, foraging locations, and stock structure. More recent research suggests that the composition of phospholipid fatty acids prominent in some body tissues (heart tissue, brain, eggs) have a genetic basis that makes analysis of these tissues appropriate for stock identification studies [9, 10]. Fatty acid analyses have been used to monitor changes in aquatic biofilms [11, 12 and 13] and to characterize ground water communities [14]. The approach has now been used to track the fate of sediment transported to the aquatic environment and consequently, the impact of aquatic microflora on soil fatty acid methyl ester (FAME) profiles after transport to the aquatic environment is now known. The results obtained from fatty acids analyses can be included in a database of fatty acid profiles, leading to a more accurate automatic identification.

In this study, we report the use of gas-chromatography coupled with mass spectrometry (GC-MS) for the determination of the fatty acids composition in some freshwater fish oils. Although there are several studies on the fatty acid composition of different species of fish, no information about the content of fatty acids composition of these freshwater fish species that are available.

II. Experimental Section

II.1. Sample Collection and Preparation

Freshly captured *Labeo coubie, Citharinus citharus, Hyperopisus bebe, Mormyrops anguilloides, Mormyrus rume, Orechromis niloticus, Sarotherodon galilaeus, Clarias gariepinus, Clarias anguillaris, Heterobranchus bidorsalis, Clariheterobranchus, Lates niloticus* and *Hydrocynus forskalii* fishes were sorted and identified. They 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 fleshes were removed from their backbones, minced, blended and immediately extracted using chloroform-methanol mixture in the ratio of 2:1.

II.2. Extraction of lipids

From the whole fillet, lipids were extracted from 5 to 6 g of fish fillet through the use of the Folch extraction technique. This method involves mechanical homogenisation of the fatty tissue with 2:1 chloroform: methanol mixture to a final volume 20 times the volume of the tissue. To prevent autoxidation, 10 mg/l of butylated hydroxytoluene was added to all samples. For complete recovery of fatty acid and isolation of non-fatty acid compounds, the extracts were repeatedly washed three times with 4 ml of 20 mg/l sodium sulphate salt solution for each 20 ml of chloroform: methanol. The extracts were allowed to separate into layers, and the lower chloroform phase containing lipids was collected and evaporated under a nitrogen stream to pre-concentrate the extracts before derivatisation. Preparation of methyl esters before any gas chromatographic analysis, triacylglycerols (fatty acid) were converted into low-molecular weight non-polar derivatives by modifying their functional groups. This was done to improve volatility of the fatty acid compounds. Derivatisation was done according to the method explained by [14] acid-catalysed methanolysis, which involves hydrolysis of the lipid extract in 5 ml of 36% HCl in 100 ml of methanol, sealed in test tubes and derivatised at 80°C for 5 to 6 h. After cooling of the mixture, obtained fatty acid methyl esters (FAMEs) were finally extracted with 4 ml of petroleum ether and washed with 10 ml of de-ionised water.

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II.3. Gas-liquid chromatography

Gas chromatography analysis was performed using an Agilent Gas Chromatograph, Model 6890N fitted with an Agilent Mass Selective Detector, 5973 series. 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 1µL. The injection port was maintained at 250°C, and the split ratio was 20:1. Oven temperature programming was done from 70 to 280°C at 10°C/min, and it was kept at 280°C for 5 min. Interference temperature was kept at 250°C. Ionization mode was electron impact ionization and the scanning range was from 40 amu to 400 amu. Mass spectra were obtained at 0.5 sec. interval. The spectra of the compounds were matched with NIST and Wiley library. The structures were defined by the % similarity values and confirmed by the study of classical fragmentation pattern, base peak and molecular ion peaks of the compounds.

III. Results and Discussion

Gas chromatography mass spectroscopy analysis was carried out in *Labeo coubie, Citharinus citharus, Hyperopisus bebe, Mormyrops anguilloides, Mormyrus rume, Orechromis niloticus, Sarotherodon galilaeus, Clarias gariepinus, Clarias anguillaris, Heterobranchus bidorsalis, Clariheterobranchus, Lates niloticus* and *Hydrocynus forskalii*. An extraction procedure which involves extraction and methylation in a single step was selected because of its reported advantages, rapidity, simplicity, and low cost. The fatty acid methyl esters (FAMEs) profile was determined. It is known that individual fatty acids can be identified by GC because of their different retention times. The spectra of the compounds were matched with NIST and Willey library. Their structures were identified by the % similarity values. They were confirmed by the study of classical fragmentation pattern, base peak and molecular ion peaks of the compounds. The detailed tabulations of GC-MS analysis of the extracts are given in Tables 1-12 below respectively.

Labeo coubie and *Citharinus citharus* are freshwater teleost fish species that utilise any conceivable food resource. They feed on living plant matter and detritus. Polyunsaturated fatty acids (38%) and monounsaturated fatty acids (25%) were found in *Labeo coubie*. The percentage of saturated fatty acids was the lowest representing 25% of the total fatty acid content (Table 2) in *Citharinus citharus*. Oleic acid is the common fatty acid in *Labeo coubie* and *Citharinus citharus.*

Table 1: *GC-MS Data for Labeo coubie compounds*

Peak	Name of Compound	Retention Time (Min)	Molecular Ion Peak (m/z)	Base Peak (m/z)
1	**5:0** Pentanoic acid	14.28	102	73
2	**7:0** Heptanoic acid	15.65	130	74
3	**8:3** 2,4,6-Octatrienoic acid	16.28	138	73
4	**17:3** 7,9,11-Heptadecatrienoic acid	16.66	264	55
5	**18:2** 9,12-Octadecadienoic acid	17.25	280	98
6	**18:1** 9-Octadecanoic acid	17.35	282	55
7	**22:1** 11-Docosanoic acid	17.55	338	74
8	**22:0** Docosanoic acid	17.95	340	55

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Table 2: *GC-MS Data for Citharinus citharus compounds*

Peak	Name of Compound	Retention Time (Min)	Molecular Ion Peak (m/z)	Base Peak (m/z)
1	**11:1** 5-Undecaenoic acid	10.88	184	55
2	**14:1** 7-Tetradecaenoic acid	14.28	226	73
3	**14:0** Tetradecanoic acid	15.66	228	74
4	**16:2** 4,6-Hexadecadienoic acid	16.30	252	73
5	**18:1** 9-Octadecaenoic acid	16.66	282	55
6	**19:1** 9-Nonadecaenoic acid	17.36	296	55
7	**19:0** 9-methyloctadecanoic acid	17.98	298	55
8	**22:5n-3** 3,5,7,9,11-docosapentaenoic acid	19.74	330	55

MORMYRIDAE: This class is well represented in local waters with 26 different species belonging to 6 genera. They are bottom-dwellers around deep, rocky pool or deep water around fallen trees. The fleshes of most species contain excess of fatty oil. The high oil content makes them difficult to cure. These species feed on molluscs, larvae of *chironomid* and *chaoborid* flies.

Table 3: *GC-MS Data for Hyperopisus bebe compounds*

Peak	Name of Compound	Retention Time (Min)	Molecular Ion Peak (m/z)	Base Peak (m/z)
1	**12:0** Duodecanoic acid	4.82	200	55
2	**13:0** Tridecanoic acid	9.31	214	55
4	**15:1** 5-methyltetradecaenoic acid	11.00	240	73
5	**18:3n-3** 3,5,7-Octadecatrienoic acid	11.18	278	55
6	**18:2** 7,9-dimethylhexadecadienoic acid	14.78	280	55
7	**22:1n-11** 11-docosaenoic acid	15.36	338	55

Table 4: *GC-MS Data for Mormyrops anguilloides compounds*

Peak	Name of Compound	Retention Time (Min)	Molecular Ion Peak (m/z)	Base Peak (m/z)
1	**12:0** Duodecanoic acid	4.82	200	73
2	**18:3n-3** 3,5,7-Octadecatrienoic acid	9.29	278	74
3	**18:2** 7,9-dimethylhexadecadienoic acid	15.24	280	73
4	**18:3n-6** 6,8,10-Octadecatrienoic acid	15.41	278	99
5	**20:0** 7,9-dimethyloctadecanoic acid	15.50	312	55
6	**20:1n-9** 9-eicosanoic acid	15.57	310	74
7	**20:2** 9,11-eicosadienoic acid	15.64	308	55
8	**20:3n-6** 6,8,10-eicosatrienoic acid	15.67	306	73
9	**20:3n-3** 3,5,7-eicosatrienoic acid	15.69	306	74
10	**20:4n-6** 6,8,10,12-eicosatetraenoic acid	15.72	304	73
11	**20:5n-3** 3,5,7,9,11-eicosapentaenoic acid	15.78	302	55
12	**21:0** Uncosanoic acid	15.81	326	55
13	**21:1n-11** 11-uncosaenoic acid	16.47	324	55
14	**22:2** 9,11-docosadienoic acid	16.54	336	74
15	**23:0** Tricosanoic acid	16.57	354	55

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Table 5: *GC-MS Data for Mormyrus rume compounds*

Peak	Name of Compound	Retention Time (Min)	Molecular Ion Peak (m/z)	Base Peak (m/z)
1	**12:0** Duodecanoic acid	4.82	200	73
2	**13:0** Tridecanoic acid	7.88	214	74
3	**15:0** 5-methyltetradecanoic acid	9.32	242	73
4	**15:1** 5-methyltetradecaenoic acid	9.90	240	55
5	**21:1n-11** 11-uncosaenoic acid	11.02	324	55

From Tables 3-5, the results seem to suggest that *Mormyrops anguilloides* is the best in terms of fatty acids compositions. It has about 60% of PUFA including the eicosapentaenoic acid (EPA). *Mormyrus rume* is composed more with saturated fatty acids. Branched chain fatty acids (15:0, 18:2 and 20:0) and 12:0 and 21:1n-11 is common in the oils of the *Mormyridae.* Branched chain fatty acids could be responsible for the lower melting point of these oils. However, *Mormyrops anguilloides* oils seems to be better in quality due to the presence of ω-3 (18:3n-3, 20:3n-3 and 20:5n-3) and ω-6 (18:3n-6, 20:3n-6 and 20:4n-6).

CICHLIDAE: These are important figure in many fisheries because of their great adaptability, high fecundity, and rapid growth rate. They feed on insects' larvae and plant materials. The *Cichlidae* from the analysis has 35% saturated fatty acids in its composition while about 50% of the fatty acids are the PUFA. The ratio of ω-3 to ω-6 is about 2:1. Branched chain fatty acids (15:0, 16:0, 17:0 and 18:2), ω-3 fatty acids (18:3n-3, 18:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3) and ω-6 fatty acids (18:2n-6 and 20:4n-6). The fatty acid profile of the *Cichlidae* was dominated by polyunsaturated fatty acids, especially ω-3, which includes eicosatetraenoic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

Table 6: *GC-MS Data for Orechromis niloticus and Sarotherodon galilaeus compounds*

Peak	Name of Compound	Retention Time (Min)	Molecular Ion Peak (m/z)	Base Peak (m/z)
1	**14:0** Tetradecanoic acid	2.17	228	73
2	**15:0** 5-methyltetradecanoic acid	3.21	242	74
3	**16:0** 5,7-dimethyltetradecanoic acid	4.30	256	73
4	**16:1** 8-hexadecaenoic acid	7.95	254	55
5	**17:0** 7-methylhexadecanoic acid	8.89	270	55
6	**18:0** Octadecanoic acid	11.68	284	55
7	**18:1** 9-Octadecaenoic acid	11.71	282	74
8	**18:2n-6** 7,9-octadecadienoic acid	14.51	280	55
9	**18:3n-3** 3,5,7-Octadecatrienoic acid	16.53	278	73
10	**18:4n-3** 3,5,7,9-octadecatetraenoic acid	16.63	276	74
11	**20:4n-6** 6,8,10,12-eicosatetraenoic acid	16.66	304	73
12	**20:5n-3** 3,5,7,9,11-eicosapentaenoic acid	16.68	302	55
13	**22:5n-3** 3,5,7,9,11-docosapentaenoic acid	16.71	330	55
14	**22:6n-3** 3,5,7,9,11,13-docosapentaenoic acid	16.73	328	55

CLARIIDAE: These are divided into two genera-Clarias and *Heterobranchus*, each having three species. Clarias live mostly in swamps, where they feed on weeds, insects' larvae, snails, crustacean, worms and small fishes. They have oily flesh which can be rendered into oil. *Heterobranchus* flesh is less oily than that of *Clarias*.

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Table 7: GC-MS Data for Clarias gariepinus compounds

Peak	Name of Compound	Retention Time (Min)	Molecular Ion Peak (m/z)	Base Peak (m/z)
1	**14:1** 7-Tetradecaenoic acid	11.62	226	73
2	**14:0** Tetradecanoic acid	11.67	228	73
3	**15:0** 5,7-methyltetradecanoic acid	11.71	242	73
4	**15:1** 5-methyltetradecaenoic acid	13.65	240	55
5	**18:0** Octadecanoic acid	14.47	284	55
6	**18:1** 9-Octadecaenoic acid	14.85	282	55
7	**18:3n-3** 3,5,7-Octadecatrienoic acid	14.91	278	74
8	**18:2** 7,9-dimethylhexadecadienoic acid	15.79	280	55
9	**18:3n-6** 6,8,10-Octadecatrienoic acid	15.83	278	73
10	**20:3n-6** 6,8,10-eicosatrienoic acid	18.06	306	74
11	**20:4n-6** 6,8,10,12-eicosatetraenoic acid	18.18	304	73

Table 8: *GC-MS Data for Clarias anguillaris compounds*

Peak	Name of Compound	Retention Time (Min)	Molecular Ion Peak (m/z)	Base Peak (m/z)
1	**12:0** Duodecanoic acid	9.32	200	73
2	**13:0** Tridecanoic acid	9.68	214	74
3	**14:1** 7-Tetradecaenoic acid	9.90	226	73
4	**14:0** Tetradecanoic acid	11.02	228	55
5	**15:0** 5,7-methyltetradecanoic acid	11.20	242	98
6	**15:1** 5-methyltetradecaenoic acid	12.63	240	55
7	**18:0** Octadecanoic acid	14.82	284	74
8	**18:1** 9-Octadecaenoic acid	15.37	282	55
9	**22:0** Docosanoic acid	19.63	340	73
10	**23:0** Tricosanoic acid	21.15	354	73

Table 9: *GC-MS Data for Heterobranchus bidorsalis compounds*

Peak	Name of Compound	Retention Time (Min)	Molecular Ion Peak (m/z)	Base Peak (m/z)
1	**12:0** Duodecanoic acid	8.47	200	73
2	**13:0** Tridecanoic acid	9.32	214	74
3	**14:0** Tetradecanoic acid	9.36	228	73
4	**15:0** 5,7-methyltetradecanoic acid	11.01	242	55
5	**15:1** 5-methyltetradecaenoic acid	11.04	240	73
6	**18:3n-3** 3,5,7-Octadecatrienoic acid	11.20	278	55
7	**18:2** 7,9-dimethylhexadecadienoic acid	14.80	280	74
8	**20:3n-3** 3,5,7-eicosatrienoic acid	18.36	306	55
9	**21:1n-11** 11-uncosaenoic acid	19.51	324	73
10	**22:2** 9,11-docosadienoic acid	19.55	336	74

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Table 10: *GC-MS Data for Clariheterobranchus compounds*

Peak	Name of Compound	Retention Time (Min)	Molecular Ion Peak (m/z)	Base Peak (m/z)
1	**12:0** Duodecanoic acid	15.32	200	73
2	**15:1** 5-methyltetradecaenoic acid	15.52	240	74
3	**18:2** 7,9-dimethylhexadecadienoic acid	15.91	280	73
4	**20:0** 7,9-dimethyloctadecanoic acid	16.19	312	55
5	**20:1n-9** 9-eicosanoic acid	16.56	310	55
6	**20:2** 9,11-eicosadienoic acid	16.64	308	55
7	**20:3n-6** 6,8,10-eicosatrienoic acid	17.07	306	74
8	**20:4n-6** 6,8,10,12-eicosatetraenoic acid	17.67	304	55
9	**21:0** Uncosanoic acid	18.70	326	73

The major SFA which was found in the *Clariidae* were 12:0, 14:0, 15:0, palmitic (C16:0) and stearic (C18:0), 21:0, 22:0, 23:0. According to [15] palmitic acid (C16:0) is the
principal fatty acid at all evolutionary and trophic levels. Fair amount of methyl-branched FAs was found. The major contributors were 5, 7-methyltetradecanoic acid, 5-methyltetradecaenoic acid, 7, 9-dimethylhexadecadienoic acid and 7, 9-dimethyloctadecanoic acid

Genetic selection in fish breeding may allow a desired FA composition, thus enhancing the position of the fish in the market place. In the case of *Clariheterobranchus*, the hybridisation process did not make the breeds differ. Therefore, the FA was not significantly different from the parents.

CENTROPMIDAE: *Lates niloticus* is the sole local species of this family. It grows to about two metres long and could weigh of up to 80 kg. *Lates* are carnivores and feed mostly on young fishes. The level of saturation *Lates niloticus* is very minimal.

Table 11: *GC-MS Data for Lates niloticus compounds*

Peak	Name of Compound	Retention Time (Min)	Molecular Ion Peak (m/z)	Base Peak (m/z)
1	**16:0** 5,7-dimethyltetradecanoic acid	11.64	256	73
2	**17:1** 8-heptadecaenoic acid	11.67	268	74
3	**18:0** Octadecanoic acid	11.71	284	73
4	**18:1** 9-Octadecaenoic acid	13.65	282	55
5	**18:3n-3** 3,5,7-Octadecatrienoic acid	14.47	278	98
6	**18:2** 7,9-dimethylhexadecadienoic acid	14.56	280	55
7	**18:3n-6** 6,8,10-Octadecatrienoic acid	15.01	278	55
8	**20:0** 7,9-dimethyloctadecanoic acid	15.21	312	73
9	**20:1n-9** 9-eicosanoic acid	15.32	310	74
10	**20:2** 9,11-eicosadienoic acid	15.52	308	73
11	**20:3n-6** 6,8,10-eicosatrienoic acid	15.91	306	55
12	**20:3n-3** 3,5,7-eicosatrienoic acid	16.19	306	98
13	**20:4n-6** 6,8,10,12-eicosatetraenoic acid	16.56	304	73
14	**20:5n-3** 3,5,7,9,11-eicosapentaenoic acid	16.64	302	74
15	**21:0** Uncosanoic acid	17.07	326	73
16	**22:2** 9,11-docosadienoic acid	17.67	336	55
17	**24:0** Tetracosanoic acid	18.70	368	73

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CHARACIDAE: This class is represented by three genera and fifteen species, all of which are predators. The flesh is white and tasty, dry and not oily, but has excellent keeping qualities after smoking. The young feed on insects and water beetles, while the adults prey on other fishes, especially *Alestes.* The low concentrations of lipid in the muscles of this species could be due to poor storage mechanism and the use of fat reserves during spawning activities. Total body composition reflects the diet or nutrition regimen of the fish.

Table 12: *GC-MS Data for Hydrocynus forskalii compounds*

Peak	Name of Compound	Retention Time (Min)	Molecular Ion Peak (m/z)	Base Peak (m/z)
1	**22:2** 9,11-docosadienoic acid	20.41	336	73
2	**23:0** Tricosanoic acid	20.82	354	55

The differences in individual contents of fatty acids when compared to the bibliographic references may be due to the species involved or environmental factors. Fatty acids composition of any fish depends on the diet, seasonal variation, environment, salinity and temperature [16]. Most FA composition data in the literature originate from species on diverse diets and of varying ages, and involved various tissues.

IV. Conclusion

Labeo coubie, Citharinus citharus, Hyperopisus bebe, Mormyrops anguilloides, Mormyrus rume, Orechromis niloticus, Sarotherodon galilaeus, Clarias gariepinus, Clarias anguillaris, Heterobranchus bidorsalis, Clariheterobranchus, Lates niloticus and *Hydrocynus forskalii* are ideal dietetic food and their consumption would help prevent nutritional deficiencies. This is due to the presence of appreciable amount of mono, di and polyunsaturated fatty acids. Fatty acids help in the transport of cholesterol and thus preventing atherosclerosis, thrombosis and effectively involved in the transport of cholesterol from blood. The results obtained might be considered as important from chemotaxonomic point of view.

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