Determination of fatty Acid Composition in Hemichromis Bimaculatus (gill, 1862) and Eutropius Niloticus (rüppell, 1829) From Makwaye Lake Zaria, Kaduna State

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ABSTRACT

Background: Fatty acid analysis was conducted to determine the qualitative fatty acid in *Hemichromis bimaculatus* and *Eutropius niloticus* fish species. The two species of fish were obtained from Makwaye Lake, Bomo, Zari, Kaduna state. Fish oil was extracted by soxhlet extraction method and the presence of fatty acid was determined by Gas Chromatography Mass Spectrometry (GCMS). Identification of fatty acid was achieved by comparing with the retention time and molecular mass of mass spectra standard obtained from the library of GCMS machine to identify the fatty acid composition. A total of sixteen fatty acids were detected in the two fish species studied, of which six were present in both species; Cyclopropaneoctanoic acid (C16:0), palmitic acid (16:0) and Strearic acid (18:0) from saturated fatty acid (SFAs) group while Palmitoleic acid (C16:1), Oleic acid (18:1) from monounsaturated fatty acid (MFAs) group and Linoleic acid (C18:2) from polyunsaturated fatty acid (PFAs) group were present in both species. Others are (SFA): Myristic acid (C14:0), (MFA): Pentacyclic acid (C15:1), cis-Hypogeic acid (C16:1) and Gaidic acid (16:1) followed by (PFA) which is Dihomo-γ-linolenic acid (C20:2). *H. bimaculatus* had a total fatty acid concentration of about 11.62% (2.28% SFA, 3.35% MFA and 5.99% PFA) whereas *E. niloticus* had a total fatty acid concentration of about 58.99% (3.02% SFA, 1.89% MFA and 54.08% PFA). Among the two species the most dominant fatty acid was Linoleic acid in *H. bimaculatus* with an area percentage of 10.56% and in *E. niloticus* with 54.08% respectively. The relative abundance of fatty acid can be attributed to temperature and dietary intake of the fish; however, these fish species are good sources of highly nutritional fatty acids beneficial to man and are recommended for consumption.

Keywords: Fish oil, Fatty acids, *H. bimaculatus*, *E. niloticus*.

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INTRODUCTION

Fish is a favorite foodstuff for the majority of societies (FAO/WorldFish)). It contains important nutritional components and serves as a source of energy for human beings (1). Regular consumption of fish can promote the defense mechanism for protection against invasion of human pathogens because fish food has antimicrobial peptide (2). An excessive amount of lipid derived from guts, head, fins and scales can be used for development of nutraceutical and

food industries which are the rich sources of important nutrients including the long-chain polyunsaturated fatty acids (PUFAs) eicosapentaenoic (EPA) and docosahexaenoic (DHA) which is associated with reduced heart disease risk and other health conditions (3).

Fish and other types of seafoods are an important source of protein worldwide. Globally, they comprise about 6 percent of dietary protein, but for 3 billion people, fish account for up to 20 percent of the average per-capita intake of animal protein (4). Because of the potential health benefits of fish, the Dietary Guidelines for Americans, (5) (DGA) recommend that people consume 8 ounces of seafood per week especially marine-derived "oily" fish such as salmon, mackerel, sardines, pompano, anchovies, swordfish, trout, and tuna to provide an average daily consumption of 250 mg of EPA/DHA per day (6). Other fresh water fish which are readily available in Nigerian water bodies provide these fatty acids, but levels are low that very large amounts of fish would have to be consumed each day to meet the recommendation. Although another omega-3 fatty acid, alpha linolenic acid (ALA), can be converted into EPA and DHA, the conversion is fairly limited in humans. Five of the top 10 consumed seafood that are rich in omega-3 fatty acid are shrimp, light tuna, *salmon*, pollock, and catfish (7). Generally, these fatty acids reduces the risk of Cardiovascular disease, inflammation, Atherosclerosis, Arrhythmias, developmental disabilities, mental health, (8) Cognitive aging, Brain and visual functions, Atopic diseases and Cancer among others (9) (10). This study seeks to find other sources of nutritionally beneficial fatty acid from fish species present in freshwater environment. *Hemichromis bimaculatus* and *Eutropius niloticus* are fish species with commercial importance in many parts of Africa as important food fish (12) which feeds from mid-water and surface waters on fish, insects, crustaceans, ostracods, snails, seeds, leaves, roots, diatoms, algae, and fruit. They are also readily available fish species within the study area with high consumption rates in the community.

MATERIALS AND METHODS

Study Area

The fish samples from the two species were collected from Makwaye Lake, Ahmadu Bello University farm. The farm is about 17km from Zaria on the Zaria-Funtua Road. The embankment is 3352.8 meters long of 2926.08 meters in width. The area of the lake at full capacity is 18.18 hectares while the volume at full capacity is 82.6 hectares. The lake is located on the latitude 11°12'19.10"N and longitude 7° 357.3" E in Samaru Zaria, Kaduna State Nigeria (11).

SOURCE: (Abolude, 2007).

Sample Collection and Processing

Average sized fresh samples of the two fish species *Hemichromis bimaculatus* and *Eutropius niloticus* were obtained from Makwaye Lake, Zaria, Nigeria. The samples obtained were transported to the Department of Zoology, Ahmadu Bello University Zaria, Nigeria in ice, and keys/description for identification according to (12) (13) were utilize. Identification of fish as well as data collection on parameters including body weight and length was achieved using a weighing scale and meter rule for the measurements. The mean weight of the two freshwater fish species is 6.38g for *H. bimaculatus* and 27.28g for *E. niloticus* with total lengths of 7.3cm and 17.53cm respectively. The fish sample were eviscerated, filleted manually and thoroughly washed to remove dirt and enhance purity, then oven dried at 65-75°C for 48hours to reduce moisture. The fish samples were further crushed and ground into powder after undergoing moisture content elimination in an oven. They were then package, labeled and stored in Hydrobiology laboratory, Department of Biology, Ahmadu Bello University Zaria, in preparation for analysis.

FISH OIL EXTRACTION

The fish oil extraction was carried out using soxhlet extractor and methanol as the solvent at the Hydrobiology laboratory, Department of Biology, Ahmadu Bello University Zaria,

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Determination of Fatty Acid Composition

The fatty acid determination involves two consecutive steps; Conversion of lipid into corresponding fatty acid methyl esters, and chromatographic analysis according to AOAC (14).

Conversion of Lipid into Corresponding Fatty Acid Methyl Esters (FAME)

Approximately 20mg of extracted fish oil was weighed and added with 1.5ml of NaOH and 0.50ml methanol in a 15ml capped centrifuge tube. The mixture was heated in a water bath at 100°C for 5minutes and then cooled at room temperature. The mixture was added with 2.0ml of borontrifluoride (BF_3, 12%) in methanol and heated again in a water bath at 100°C for 30minutes. Next, the tube was cooled in running water at room temperature before adding 1ml of isooctane. It was vigorously stirred for 30seconds before adding 5.0ml of saturated sodium chloride solution to facilitate the phase separation. The esterified sample was placed in the refrigerator and left to rest for better phase separation. After collecting the supernatant another 1.0ml of isooctane contains 0.05% butylated hydroxytoluene (BHT) as antioxidant was added into the tube and stirred. The supernatant was collected and added to the previous fraction. The sample was concentrated to a final volume of 1.0ml for later injection into the gas chromatograph.

Chromatographic Analysis of Fame

The fatty acid methyl ester was analyzed using a GCMS-GP2010SE gas chromatograph system, equipped with auto sampler, oven and flame ionization detector.

The separation was carried out with helium as carrier's gas (1.8ml/min). A fused silica capillary column (Omega wax TM-320, 30m x 0.32mm i.d) was used. The column temperature was programmed starting at a constant temperature of 60°C during 2minute, heated to 120°C at 2min, held at 120°C during 2min, heated again to 300°C at 3min and finally held at 230 for 3.50min. A split ratio (50:1) at 250°C was used. The flame ionization detector was also heated to 250°C. Peak identification of fatty acid in the analyzed samples were carried out by comparing with the retention time and molecular mass of mass spectra of standard obtained from library of the GCMS machine and also confirmed from the mass spectrometer fragmentation pattern at Bob Global Resource Limited Abuja (GCMS-QP2010SE SHIMADZU, JAPAN).

RESULTS

The study of fatty acid composition of the two freshwater fish species, *H. bimaculatus* and *E. niloticus* had a total of sixteen fatty acids that were detected, seven were of the saturated fatty acid (SFA) group, six were of the monounsaturated fatty acid (MFA) group whereas three were of the polyunsaturated fatty acid PFA) group.

There are a total of nineteen (19) peaks in figure 1 with an intensity of 46,797,256. Out of which peaks with higher area percentage were selected and the retention time of each peak ranging from 6, 7, 8, 9, 11, 15, 17, and 18 their corresponding retention time and area percentage value are: 17.958 (0.65%), 17.992 (0.98%), 18.136 (0.83%), 18.326 (10.56%), 18.971 (1.41%), 20.416 (4.47%), 21.476 (5.72%) and 21.611 (3.18%). These values were compared with the retention time and molecular mass of mass spectra standard obtained from the library of GCMS machine to identify the fatty acid composition at each peak.

There are a total of nineteen (23) peaks in figure 1 with an intensity of 90,810,208. Out of which peaks with higher area percentage were selected and the retention time of each peak ranging from 1, 4, 6, 7, 9, 11, 17, and 21 their corresponding retention time and area percentage value are: 16.698 (0.06%), 17.221 (3.96%), 17.966 (1.11%), 18.000 (1.37%), 18.480 (54.08%), 19.198 (2.17%), 20.440 (5.13%), and 21.640 (2.81%).

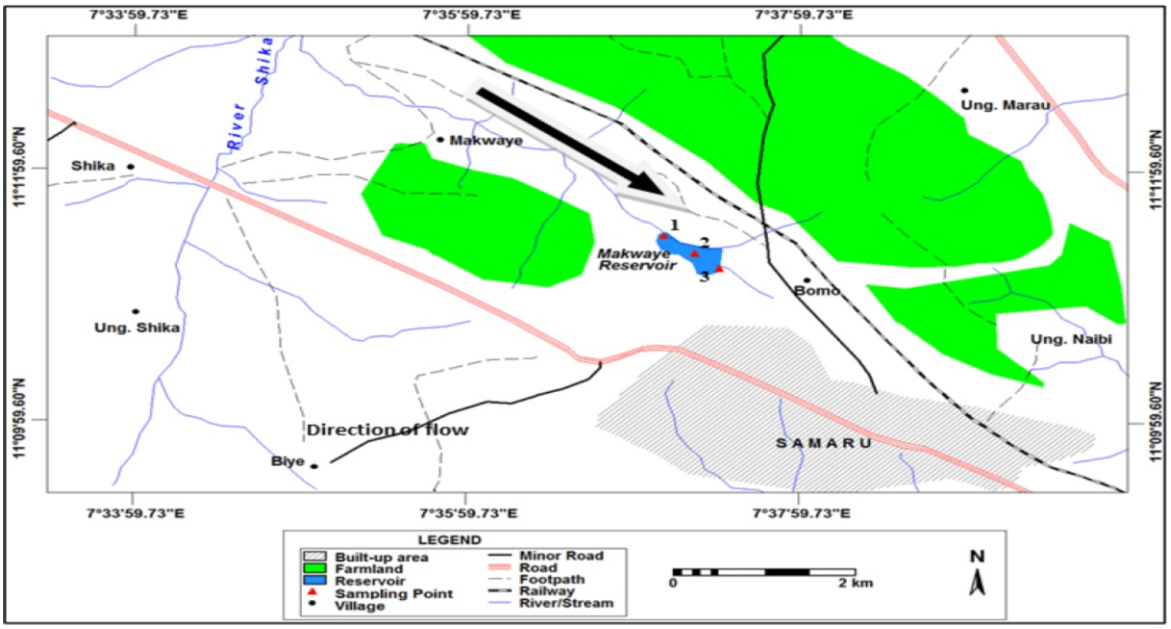

Figure 1: The map of Makwaye Lake in Samaru, Zaria Kaduna state

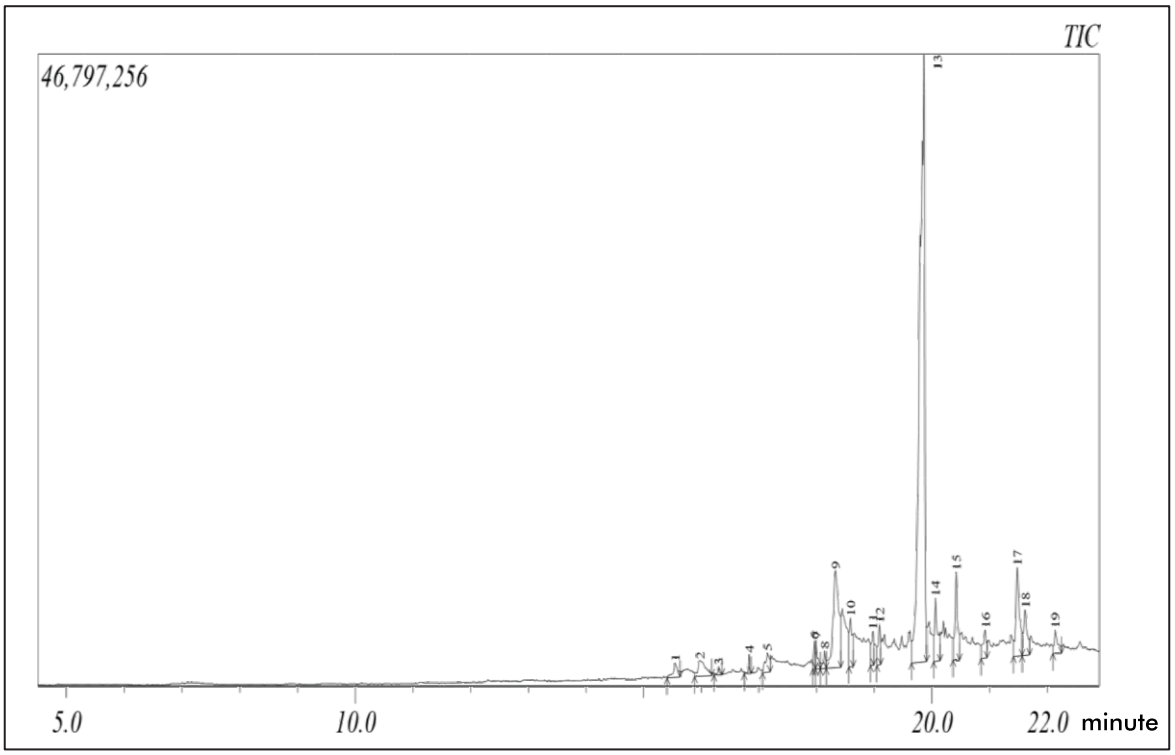

Figure 2: GC-MS chromatograph of *H. bimaculatus*

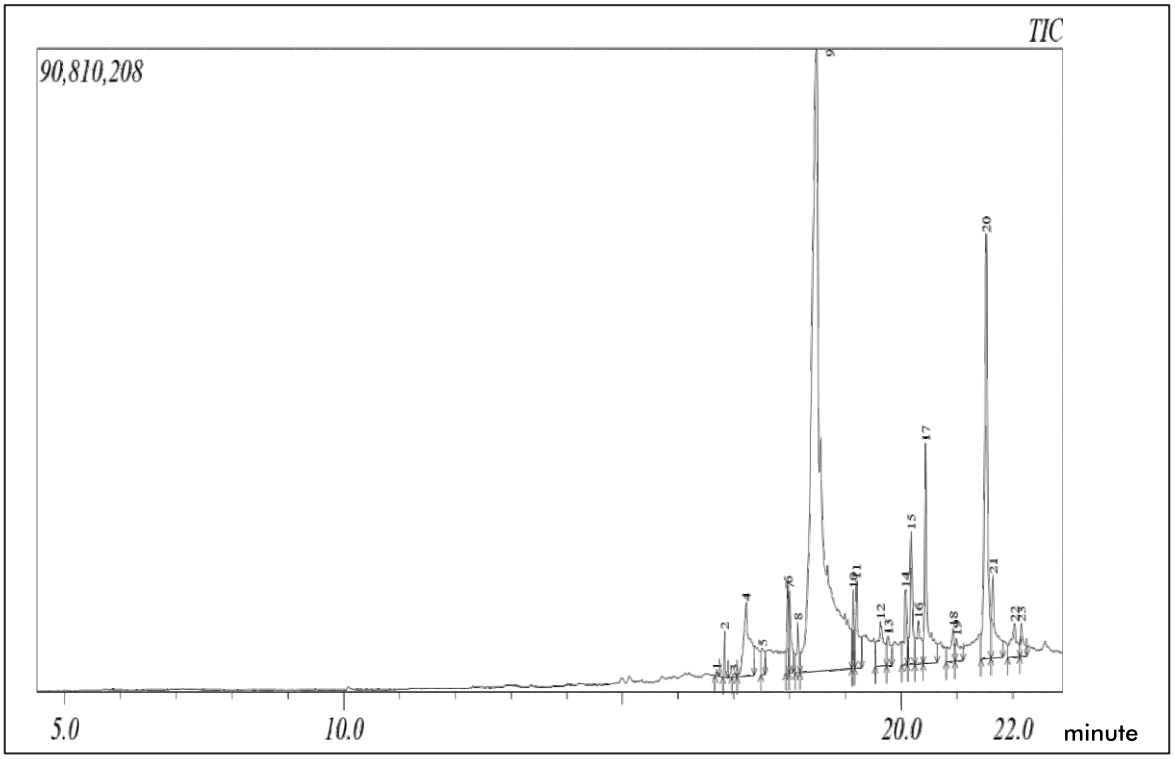

Figure 3: GC-MS chromatograph of *E. niloticus*

Table1: Comparison of individual fatty acid from the fish oil of the two species analyzed from Makwaye, Lake

Fatty acid	Trivial name	Abbreviation	*Hemichromis bimaculatus*	*Eutropius niloticus*
Saturated Fatty Acid				
Cyclopropaneoctanoic acid	*	C11:0	+	+
Tetradecanoic acid	Myristic acid	C14:0	+	-
Hexadecanoic acid	Palmitic acid	C16:0	+	+
Octadecanoic acid	Strearic acid	C18:0	+	+
Monounsaturated Fatty Acid				
9-Pentadecanoic acid	Pentacyclic acid	C15:1	-	+
7-Hexadecenoic acid	cis-Hypogeic acid	C16:1	+	+
2- Hexadecanoic acid	Gaidic acid	C16:1	-	+
9-Octadecanoic acid	Oleic acid	C18:0	+	+
Polyunsaturated Fatty Acid				
9, 12-Octadecadienoic acid	Linoleic acid	C18:2	+	+
8, 11, 14-Eicosatrienoic acid	Dihomo-γ-linolenic	C20:3	+	-

KEYS – Fatty acid absent
+ Fatty acid present

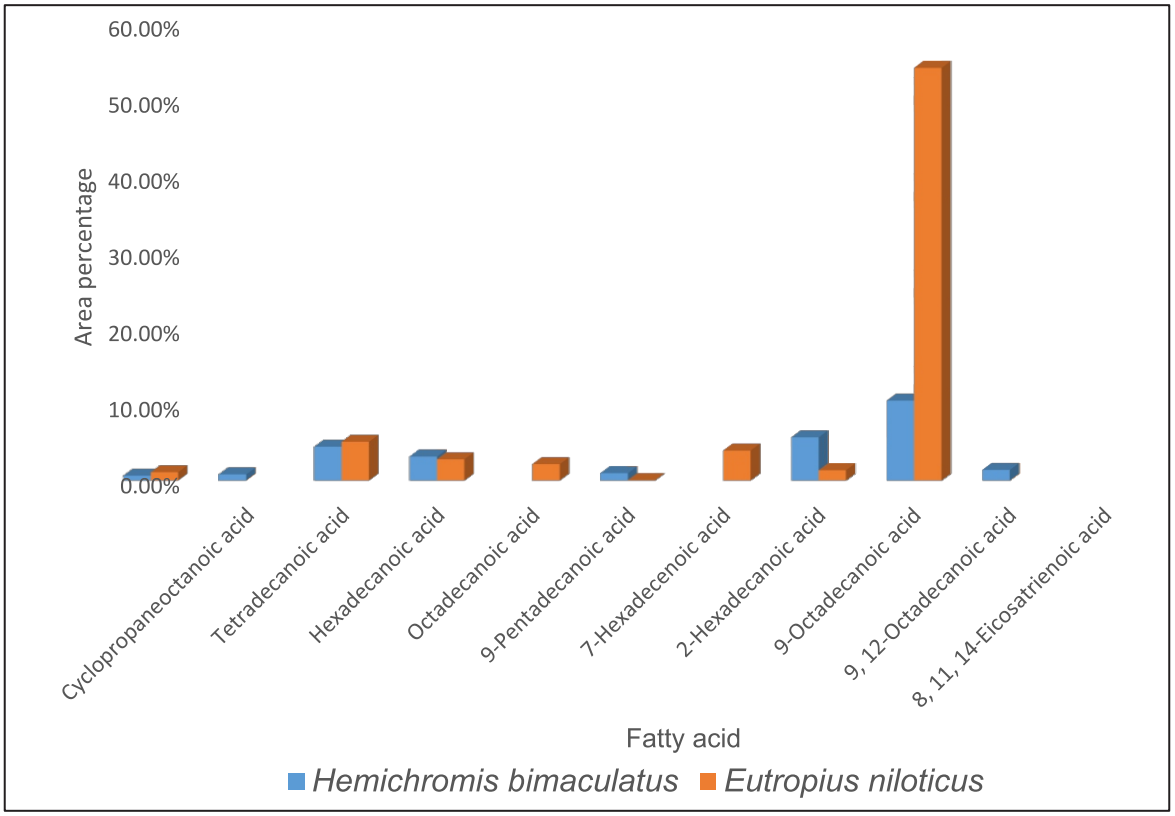

Figure 4: Concentration of individual fatty acid of *H. bimaculatus and E. niloticus* from Makwaye Lake in Samaru, Zaria.

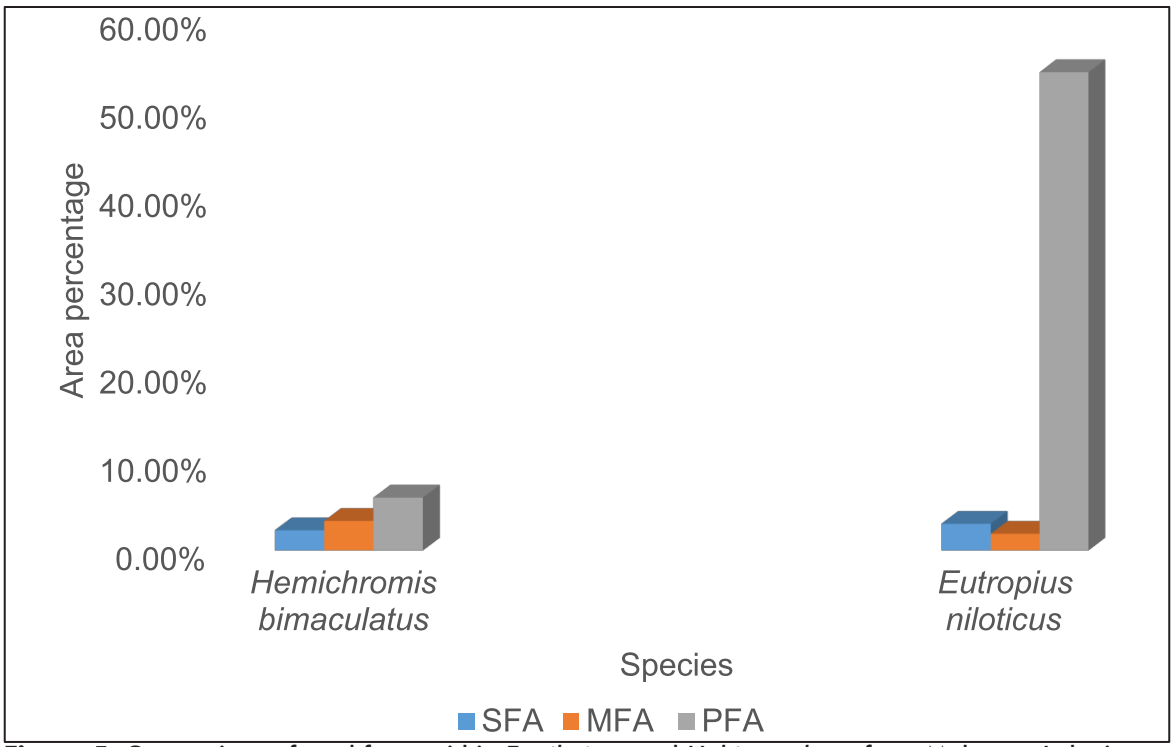

Figure 5: Comparison of total fatty acid in *E. niloticus* and *H. bimaculatus* from Makwaye Lake in Samaru, Zaria.

DISCUSSION

A total of sixteen fatty acid were detected in the two fish species studied and out of which six were present in both species i.e. Cyclopropaneoctanoic acid (C11:0), palmitic acid (16:0) and Strearic acid (18:0) from saturated fatty acid group while Palmitoleic acid (C16:1), Oleic acid (18:1) from monounsaturated fatty acid group then Linoleic acid (C18:2) from polyunsaturated fatty acid group were present in both species. Higher content of fatty acid is present in *E. niloticus* compared to that of *H. bimaculatus* which indicate the probability of its high value in the fish species (15). This findings correlate with the report from other authors on similar studies that indicated the dominance of this fatty acid in freshwater fish species (15) (16).

Among the two species, the most dominant fatty acid was Linoleic acid in *H. bimaculatus*. The high percent of branched and saturated fatty acid in freshwater fish gives them advantage in curing processing. This research was conducted in winter season, and it has been reported by other authors that the degree of unsaturation of fish oil vary with seasons. Branched chain fatty acids influence lower melting point, lower cholesterol levels, provide energy, and form an integral part of bio membranes. Because of their high temperature stability, they play an important role in the finished product of hydrogen fish oils. Esterification of branched chain fatty acid to cholesterol causes the fatty acid to stimulate protein synthesis. Branched chain ester influences some ribosomal function which is necessary for peptide elongation (17) (16).

Fatty acid composition is important as aspect of quality of raw material, sensory attributes and storage ability. The two species had high saturated fatty acid especially cholesterol, it is reported that high proportion of saturated fatty acid could account for low iodine value in the oil and 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 (18).

Saturated fatty acid was higher than the unsaturated fatty acid in *H. bimaculatus* while in *E. niloticus* unsaturated fatty acid was higher than saturated fatty acid. The variation in the concentration of fatty acid in the species may be attributed to factors such as temperature and diet among others (19). Diet generally has a major effect on the fatty acid composition of lipid, as they are mostly obtained from plant food sources (especially algae), other factors that my influence fatty acid composition includes size, age, reproductive status, geographical location and season (16) (20).

CONCLUSION

The SFA component for *H. bimaculatus* were Cyclopropaneoctanoic acid (C11:0), Myristic acid (C14:0) Palmitic (C16:0) and Strearic acid (C18:0) while MFA component were cis-Hypogeic acid (17:1) and Oleic acid (C18:1) and PUFA component were Linoleic acid (C18:2) and Dihomo-γ-linolenic acid (C20:3). While in *E. niloticus* SFA component were Cyclopropaneoctanoic acid (C11:0), Palmitic acid (C16:0) and Strearic acid (C18:0) while MFA component are cis-Hypogeic acid (C17:1), Gaidic acid (C16:1) and Oleic acid (C18:1) and PUFA component were Linoleic acid (C18:2) being the only polyunsaturated acid group that was found in both species. *H. bimaculatus* had a total fatty acid concentration of about 11.62% whereas *E. niloticus* had a total fatty acid concentration of about 58.99%.

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