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COMPARATIVE ANALYSIS OF THE PROXIMATE, FATTY ACIDS AND MINERAL COMPOSITION OF SELECTED FISH SPECIES

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Abstract: *Proximate, minerals, fatty acids and cholesterol compositions were determined in five different freshwater fish species (Oreochromis leucostictus, Clarias garipinus, Gynarchus niloticus, Hyperopisus bebe occidentallis and Synodotus clarias) obtained from Shiroro Lake, Niger State, Nigeria using standard analytical methods. The proximate compositions were found to be 39.00 – 51.18% protein, 19.43 – 48.47% fat, 2.13 – 14.74% carbohydrate, 4.91 – 19.59% ash and 4.57 – 5.63% moisture content, with significant difference at $p < 0.05$ among fish species, whereas the fatty acids were found to be composed of 38.93 – 42.81% saturated fatty acids (SFA), 33.63 – 45.37% monounsaturated fatty acid (MUFA) and 7.79 – 26.10% polyunsaturated fatty acids (PUFAs) with significant difference at $p < 0.05$ among fish species. The cholesterol content was significantly higher in Tilapia (Oreochromis leucostictus) with 8.38 ± 0.02 mg/100g and the lowest in Catfish (Clarias garipinus) with 2.74 ± 0.04mg/100g respectively. The mineral composition (Na, Ca, K, Mg, Fe and Zn) in the five species of fish ranged from 31.63 – 49.59mg Na/100g, 8.56 – 33.60mg Ca/100g, 8.68 – 20.69mg K/100g, 6.33 – 11.77mg Mg/100g, 0.07 – 2.05mg Fe/100g and 0.13 – 0.33mg Zn/100g respectively with significant difference at $p < 0.05$ among the fish species. In this study, Gynarchus niloticus and Synodotus clarias have registered the highest amount of poly-unsaturated and the lowest amount of saturated fatty acids and therefore they are recommended for consumption. Notwithstanding other species should also be harnessed for their outstanding mineral contents in order to improve the essential nutrients derivable from them.*

Keywords: *Fish species, cholesterol, proximate composition, minerals, polyunsaturated fatty acids*

1. Introduction

Fish is one of the most diverse groups of animals known to man and there are more species of fish than all other vertebrates. It has remained as one of the most important sources of animal protein due to its availability, being relatively cheap as compared with other sources of proteins such as meat, excellent taste, easy digestibility, lack of any cultural or religious taboos associated to its consumption by any particular ethnic group, high content of essential nutrients and unsaturated fatty acids which are very relevant for functionality of protein in the body [1-4]. Fish lipids act as important sources of energy for the biochemical activities of cell membranes. It is composed of mono and poly unsaturated fatty acids such as omega three (ω-3) and omega six (ω-6) [5, 6] that can help reduce the cholesterol content of the body and the risk of cardiovascular diseases [7-10].
From the nutritive point of view, various studies have been carried out to determine

the proximate composition of fish. The results obtained from these studies, show that fish is composed of between 30 to 90% water [11], 60-75 % protein [12], 30 to 50% lipids [13], 0.1 – 1% carbohydrates [4], essential minerals such as sodium, magnesium, calcium, phosphorus, potassium, iodine and appreciable quantity of vitamins such as A, D, E, K [14]. These proximate compositions according to the report may vary depending on the geographical location, season of the year, the feed intake, sex, species, age and maturity or size of the fish [14]. One of the most common fish species in Nigeria is *Clarias garipinus* (African sharp-tooth catfish) which belongs to the family of *Clarida* and is found in freshwaters, lakes, rivers and swamps. Other species are Tilapia, which belong to the *Tilapine cichlid* family [15], *Hyperopisus bebe occidentallis* (Elephant fish) to the family of *Mormyridae* [16], *Synodontis clarias* (fresh water catfish), to the genus *Mochokidae* [17-18] and *Gynarchus niloticus* (Trunk fish) [16].

This study was aimed at determining the proximate, essential minerals, cholesterol and fatty acids composition of selected fish species obtained from Shiroro River, Nigeria and their oil extracts. These species were selected to be studied due to their economic importance and consumers' demand. Therefore, detailed information about their proximate, essential minerals and fatty acids composition is important from nutritional point of view.

2. Materials and Methods

The fish species, *Oreochromis leucostictus, Clarias garipinus, Gynarchus niloticus, Hyperopisus bebe occidentallis and Synodotus clarias,* used for the study were purchased from fishermen at Shiroro River of Niger State, Nigeria. The fish species were taken to the laboratory and were properly washed with water and lacerated. The non-edible parts were removed and then properly washed again with tap water and rinsed with distilled water. The samples were oven dried at 90°C until a constant weight was obtained. The dried samples were ground and homogenized.

The moisture, ash, raw protein, raw fiber and raw fat contents of the samples were determined in triplicate according to the method describe by the Association of Official Analytical Chemist methods [19]. Carbohydrate contents of the samples were determined by difference as reported by other workers [20]. The concentration of calcium, magnesium, zinc and iron were determined using Atomic Absorption Spectrophotometry after wet digestion using the mixture of concentrated HNO_3 and $HClO_4$. The digestion was done in a fume hood using a hotplate at 70°C. Fatty acid profile was determined using gas chromatography/mass spectrophotometer (GC-MS) after extraction of the sample with a Soxhlet extractor, purification and cleanup using a packed column. Free fatty acid (FFA), saponification value, peroxide value, iodine value, acid value and level of cholesterol of extracted oil in the fish species were determined as described in the Association of Official Analytical Chemist methods [19].

3. Results and Discussion

The result of proximate compositions of the studied fish species per dried weight are shown in the Table 1. From the results, there were significant differences ($p < 0.05$) in the mean concentrations of biomolecules (carbohydrate, fats, protein) and other parameters such as (ash fiber, moisture) between the species of the fish studied. The concentration of raw protein in the fish species was in the following order: *Gynarchus niloticus* (51.1±80.36%)

> *Clarias garipinus* (48.15±0.02%) > *Synodotus clarias* (46.13±0.03%) > *Oreochromis leucostictus* (45.17±0.04%) > *Hyperopisus bebe occidentallis* (39.00±0.02%) respectively. The relatively high raw protein content in the fish species could be attributed to the fact that fish is good source of protein and it is likely to meet the daily protein intake needed by human consumption. Higher protein compositions were observed by several works carried out on Nigeria freshwater fish. Alfa *et al* reported a range of 49.47-61.90 % in studies carried out on fish sold within the Bida market in Niger state, Nigeria [21]. Also, Adesola reported protein compositions between 38 % - 50 % in some important fish species obtained from Lagos, Nigeria [22]. The differences in the protein concentration between fish may be due to their feeding life style or absorption capability and the conversion potentials of the essential minerals in their diets and environment into various biochemical molecules in their body system [23].

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Table 1

Proximate compositions of selected Fish species from Shiroro River

Fish specie	Moisture (%)	Raw fat (%)	Raw protein (%)	Raw fiber (%)	Ash (%)	Carbohydrate (%)
Gynarchus niloticus	5.99 ± 0.47^b	$23.34 \pm .20^d$	51.18 ± 0.36^a	0.90 ± 0.01^c	9.01 ± 0.03^c	9.58 ± 0.03^b
Clarias garipinus	4.57 ± 0.02^c	31.79 ± 0.05^b	48.15 ± 0.02^b	1.17 ± 0.02^b	9.89 ± 0.03^a	6.53 ± 0.02^d
Oreochromis leucostictus	6.01 ± 0.02^b	22.43 ± 0.01^e	45.17 ± 0.04^d	2.00 ± 0.21^a	9.65 ± 0.28^b	14.74 ± 0.03^a
Synodotus clarias	7.87 ± 0.02^a	26.71 ± 0.02^c	46.13 ± 0.03^c	1.20 ± 0.10^b	8.59 ± 0.01^d	9.51 ± 0.01^c
Hyperopisus bebe occidentallis	4.60 ± 0.20^c	48.57 ± 0.02^a	39.00 ± 0.02^e	0.85 ± 0.03^c	4.90 ± 0.02^e	2.13 ± 0.02^e

Means (± Standard deviation) on the same column with different superscripts are significantly different ($p < 0.05$)

The order in which the concentration of raw fat in the fish species were significantly higher than the other one was the following: *Hyperopisus bebe occidentallis* (48.57±0.20 %) > *Clarias garipinus* (31.79±0.05 %) > *Synodotus clarias* (26.71±0.02 %) > *Gynarchus niloticus* (23.34±0.2%) > *Oreochromis leucostictus* (22.43±0.0 1 %). Alfa *et al* explained that raw fat in fish varies from each other because of seasonal variation, species, geographical location and variation in their age and maturity [21]. Based on the classification made by Bennion and Scheule, fish species can be categorized as fatty fish if they all have fat content greater than 10 % by weight [24].

The raw fiber content of *Oreochromis leucostictus* (2.00±0.21 %) was significantly higher than those of the other fish species. The fiber contents of *Synodotus clarias* (1.20±0.10%) and *Clarias garipinus* (1.17±0.02 %) were not significantly different from each other but were significantly higher than those of *Gynarchus niloticus* (0.90±0.90 %) and *Hyperopisus bebe occidentallis* (0.85±0.03 %), which also were not significantly different from each other. The raw fiber content of *Gynarchus niloticus* and *Hyperopisus bebe occindentallis* were within the range of the values (0.54 - 0.95 %) obtained by Alfa *et al* for *Clarias garipinus, Synodotus clarias* and *Oreochromis leucostictus* fish species in Bida region of Niger state [21]. Also Taiwo *et al,* who studied cultured and wild *Clarias garipinus* and *Synodotus clarias* fish species reported values that ranged between 0.78 - 1.23% [25]. The order in which the ash contents (index of mineral content of biota) of the fish species were higher than in each other was the following:*Clarias garipinus* (*9*.89±0.03%), *Oreochromis leucostictus* (9.65+0.28%), *Gynarchus niloticus* (9.01±0.03%), *Synodotus clarias* (*8*:59±0.01%), and *Hyperopisus bebe occidentallis* (4.90±0.02%). Low ash content in *Hyperopisus bebe occidentallis* as compared to other species may be due to the lesser amount of skeleton it possesses. The results obtained in this study fall within the ones obtained by Mazumder *et al* for different fish species which ranged between 4.91% and 19.59% [26] and also similar to the work carried out by Alfa *et al* in freshwater fish species from Bida which ranged from (5.32 – 42.44%) in the same species of fish [21]. However, it is higher when compared with the work carried out by Taiwo *et al* and Osibona which ranged between 1.16-1.26 % and 1.22-1.40 % in *Clarias garipinus*I and *Oreochromis leucostictus* respectively [15, 27]. The order in which the carbohydrate composition of the fish species studied were significantly different ($p < 0.05$) from each other was *Oreochromis leucostictus* (14.74±0.03%), *Gynarchus niloticus,* (9.58+0.03%), *Synodotus clarias* (9.51±0.01%), *Clarias garipinus (*6.53±0.02%) and *Hyperopisus bebe occidentallis* (2.13±0.02 %). The results were high, except that of *Hyperopisus bebe occidentallis* which was comparable to that obtained by Alfa *et al* of between 2.69-5.28 % for five freshwater fish species [21]. Also Ayeloja *et al* obtained the range of 2.10-12.57 % for freshwater fish species from the Western part of Nigeria [28]. Instead, the range of 1.95- 11.95 % was obtained by Effiong and Fakunle, in common five fish species from the Kainji Lake [29]. The moisture content which has effects on spoilage ranged from 4.57-7.87 % with maximum value in *Synodotus clarias* (7.87 *%)* which was significantly higher than in other species. The moisture content of *Oreochromis leucostictus* (6.01%) and *Gynarchus niloticus* (5.99%) was not significantly different from each other but significantly higher than that of *Hyperopisus bebe occidentallis* (4.60%) and *Clarias garipinus* (4.57%). *Hyperopisus bebe occidentallis* and *Clarias garipinus* were not significantly different from each other. The values obtained on the basis of dried matter were similar to those obtained by Effiong and Fakunle and Ande *et al* in fish species from the Kainji Lake and River Lafia respectively which gave the range of 5.10 - 10.50% and 5.67 - 9.50% respectively [29, 30]. The values obtained in this study are within the range of 5-8 % for moisture content for fish products on dry basis [31].The results of the proximate parameters obtained in this present study are higher than those reported in *S. nigrita* of 7.09 to 25.46% protein, 5.13 to 11.70%

fat, and 3.40 to 14.23% raw fiber content in *T. mariae* [32]. The variation can be attributed to environmental factors, such as the size of fish and its feeding habits. Other researchers also reported various proximate parameters in fish that were dependent on environmental factors and feeding habits [33]. The mineral and cholesterol concentration in the fish species studied is shown in the Table 2. There were significant differences ($p < 0.05$) in the mean mineral and cholesterol concentration between fish species. The concentration of calcium in fish species ranged from 4.28±0.02 to 16.80±0.01 mg/100g. The concentration of Calcium in *Synodotus clarias* (16.8mg/100g) and *Oreochromis leucostictus* (16.79mg/100g) was not significantly different from each other but was significantly higher than that of the other species. The concentration of calcium in *Clarias garipinus* (8.57mg/100g) was significantly higher than that of *Gynarchus niloticus* (7.73mg/100g) and *Hyperopisus bebe occindentallis* (4.28mg/100g). *Gynarchus niloticus* had its calcium content significantly higher than that of *Hyperopisus bebe occindentallis*. Alfa *et al* reported a lower concentration of calcium (0.003 -0.014 mg/100g) in freshwater fish sold in Bida Markets [21] than that obtained in this study. The calcium concentration of fish species was higher than the recommended standard [31] except for *Hyperopisus bebe occindentallis* that fell below. The magnesium concentration in the fish species ranged from 3.17±0.02 to *5.88±0.04* mg/100g. This falls within the recommended range of 4.5-452 mg/100g [31]. The order of magnesium content in different fish species were *Synodotus clarias* (5.84mg/100g) > *Clarias garipinus* (5.16mg/100g) > *Orechromis leucustictus* (4.03mg/100g) > *Hyperopisus bebe occindentallis* (3.45±0.01 mg/100g) > *Gynarchus niloticus* (3. l7±0.02mg/l00g). These results were lower than those obtained by other researchers [34], who reported a range of mg/l00g to 20 mg/l00g which is also higher than the result obtained by Alfa *et al* of 0.06 mg/l00g to 1.19 mg/l00g [21].

Table 2

Mineral and cholesterol composition of selected fish species from Shiroro River

Fish specie	Ca (mg/100g)	Mg (mg/100g)	Fe (mg/100g)	Zn (mg/100g)	Cholesterol (mg L^{-1})
Gynarchus niloticus	7.73 ± 0.03^c	3.17 ± 0.02^e	0.50 ± 0.02^a	0.08 ± 0.02^b	548 ± 0.02^b
Clarias garipinus	8.57 ± 0.03^b	5.16 ± 0.03^b	0.48 ± 0.01^a	0.06 ± 0.02^b	274 ± 0.04^d
Oreochromis leucostictus	16.79 ± 0.03^a	4.03 ± 0.03^c	0.51 ± 0.04^a	0.17 ± 0.00^a	838 ± 0.02^a
Synodotus clarias	16.80 ± 0.01^a	5.84 ± 0.04^a	0.03 ± 0.02^c	0.14 ± 0.01^a	503 ± 0.02^c
Hyperopisus bebe occidentallis	4.28 ± 0.02^d	3.47 ± 0.01^d	0.21 ± 0.02^b	0.08 ± 0.02^b	509 ± 0.04^c
WHO/FAO, 2011	5.00 – 502.00	50.00 – 451.00	1.00 – 5.60	0.23 – 2.10	≤ 30000

Means (± Standard deviation) on the same column with different superscripts are significantly different ($p < 0.05$)

The iron concentration in the fish species ranged from 0.03±0.02 to 0.51±0.04 mg/100g, which falls below the standard concentration range of 1.0 – 5.6mg/100g [31]. The concentration of magnesium in *Oreochromis leucostictus* (0.51mg/100g), *Gynarchus niloticus* (0.50mg/100g), *Clarias garipinus* (0.48mg/100g) was not significantly different from each other but it was significantly higher than that of

Hyperopisus bebe occidentallis (0.2 1mg/l00g) and *Synodotus clarias* (0.03mg/100g). *Hyperopisus bebe occidentallis* was significantly higher than *Synodotus clarias.* The results obtained were within the range of those obtained by Guerin *et al* in fish from French market which gave a range of 0.13-1.9 mg/100g [35] except that of *Synodotus clarias* which fell below the lowest concentration. From the analysis carried out by Alfa *et al* on fish samples from Bida Markets of Niger State, Nigeria which gave the range of 1.42-2.00 mg/100g [21] resulted higher values than those obtained in this study. The concentration of iron in *Oreochromis leucostictus* was the highest and that of *Synodotus clarias* was the lowest one. The concentration of Zinc in this study ranged from 0.07±0.02 to 0.17±0.00 mg/100g which was below the standard range of 0.23 – 2.1mg/100g [31]. The concentration of Zinc in O*reochromis leucostictus* (0.17mg/100g) and *Synodotus clarias* (0.14mg/100g) was not significantly different from each other but it was significantly higher than that of *Gynarchus niloticus* (0.08mg/100g), *Hyperopisus bebe occidentallis* (0.08mg/100g) and *Clarias garipinus* (0.06mg/100g). The results obtained were lower than those of Tao *et al* who obtained values of 0.64 – 0.81mg/100g in farmed fish in China [36] as well as those obtained by Alas *et al* in fish sampled from Beysehir Lake in Turkey [37] but they fell within the range reported by Guerin *et al* in fish from French market with value ranging from 0.13 – 2.5mg/100g [35].The cholesterol contents in oil extract of the studied fish species in the Table 2 ranged between 274±0.04 - 838±0.02 mg/L. In *Oreochromis leucostictus* registered higher content of cholesterol (838mg/L) while *Clarias garipinus* (274.0mg/L) had the lowest one. *Oreochromis leucostictus* oil had its cholesterol content significantly higher than other fish species. The cholesterol content in *Gynarchus niloticus* (548mg/L) was significantly higher than that of *Hyperopisus bebe occindentalis* (509mg/L), *Synodotus clarias* (503mg/L) and *Clarias garipinus* (274mg/L). *Hyperopisus bebe occindentallis* and *Synodotus clarias* oils were not significantly different in their cholesterol content but they were significantly higher than that of *Clarias garipinus.* The values obtained were all lower than the recommended standard of 30000mg/L [31] which implies that fish species can be recommended as suitable for consumption by humans without any negative implication on their health with respect to total cholesterol. The chromatograms showed that oil in the fish species was made up by long chains of fatty acids, with a minimum carbon chain length of 10 - 22 carbons (Table 3), which is a typical characteristic of fish oil [38]. Each of the fish species was found to be composed of different fatty acids. Still, some fatty acids were not detected in some of the fish species studied.

Fatty and non-fatty acid compositions of oils in the selected fish species are shown in the Table 4. The poly-unsaturated fatty acids (PUFA) content in the fish gave a range of 7.79 - 26.10% which was less than the saturated fatty acids (SFA) of 38.93 - 41.58% and mono-unsaturated fatty acids (MUFA) of 33.63 - 45.37% respectively. The differences in fatty acids composition can be attributed to the influence of environmental factors and nutritional habits of fish [39]. Other researchers have also reported that fresh water fish has lower content of poly-unsaturated fatty acids (PUFA), because freshwater fish feeds largely on vegetation and plant materials [22].

Table 3

Percentage (%) Fatty acid composition of selected fish species from Shiroro River

FAME Systemic Name	Fish species				
	Gynarchus niloticus	*Clarias garipinus*	*Oreochromis leucostictus*	*Synodotus clarias*	*Hyperopisus bebe occidentallis*
10:0 Decanoic acid	2.01 ± 0.06[a]	ND	ND	ND	1.82 ± 0.03[b]
13:0 Tridecanoic acid	ND	0.55 ± 0.02[a]	0.47 ± 0.02[b]	0.41 ± 0.04[c]	ND
14:0 Tetradecanoic acid	2.76 ± 0.02[c]	1.71 ± 0.01[d]	3.30 ± 0.04[b]	1.40 ± 0.01[e]	4.70 ± 0.01[a]
15:0 Pentadecanoic acid	1.54 ± 0.03[c]	0.83 ± 0.06[d]	2.98 ± 0.01[b]	0.47 ± 0.02[e]	3.35 ± 0.02[a]
16:0 Hexadecanoic acid	14.95 ± 0.02[c]	17.72 ± 0.02[c]	18.84 ± 0.05[b]	22.91 ± 0.02[a]	17.07 ± 0.02[d]
17:0 Heptadecanoic acid	4.52 ±0.02[a]	2.03 ± 0.02[b]	1.27 ± 0.01[c]	ND	0.70 ± 0.02[c]
18:0 Octadecanoic acid	11.07 ± 0.03[d]	14.18 ± 0.01[b]	11.61 ± 0.02[c]	16.39 ± 0.02[a]	8.93 ± 0.02[e]
20:0 Eicosanoic acid	ND	2.36 ± 0.01[b]	ND	ND	2.93 ± 0.03[a]
21:0 Heneicosanoic acid	ND	ND	ND	ND	0.54 ± 0.02[a]
22:0 Docosanoic acid	2.02 ± 0.00[b]	1.28 ± 0.02[c]	ND	ND	2.80 ± 0.03[a]
18:1ω-12 6-Octadecenoic acid	ND	ND	1.09 ± 0.03[a]	ND	ND
18:1ω-9 9-Octadecenoic acid	1.90 ± 0.01[d]	27.89 ± 0.01[a]	23.63 ± 0.02[b]	ND	21.98 ± 0.01[c]
18:1ω-7 11-Octadecenoic acid	23.49 ± 0.04[b]	4.76 ± 0.03[e]	8.24 ± 0.05[d]	35.76 ± 0.03[a]	9.57 ± 0.02[c]
18:1ω-9 12-Octadecenoic acid	8.25 ± 0.04[a]	ND	ND	ND	ND
20:1ω-9 11-Eicosanoic acid	ND	ND	ND	ND	4.86 ± 0.00[a]
22:1ω-9 13-Docosenoic acid	ND	4.10 ± 0.02[c]	4.75 ± 0.02[b]	8.15 ± 0.02[a]	ND
24:1ω-9 15-Tetracosenoic acid	ND	ND	ND	1.46 ± 0.03[a]	1.43 ± 0.02[a]
16:3ω-3 7,10,13-Hexadecatrienoic acid	ND	ND	2.44 ± 0.02[b]	ND	4.51 ± 0.03[a]
18:2ω-6 9,12,15-Octadecadienoic acid	ND	1.49 ± 0.01[b]	2.82 ± 0.03[a]	1.17 ± 0.02[c]	ND
18:2ω-6 9,12-Octadecadienoic acid	9.43 ± 0.03[c]	16.77 ± 0.02[a]	ND	6.16 ± 0.02[d]	11.82 ± 0.02[b]
20:4ω-6 5,8,11,14-Eicosatetraenoic acid	1.58 ± 0.01[c]	1.44 ± 0.04[d]	3.44 ± 0.04[a]	ND	2.36 ±0.03[b]
20:5ω-3 5,8,11,14,17-Eicosapentaenoic acid, EPA	8.70 ± 0.02[b]	ND	8.75 ± 0.03[a]	ND	ND
22:6ω-3 4,7,10,13,16,19-Docosahaenoic acid, DHA	6.39 ± 0.06[a]	2.14 ± 0.01[b]	1.94 ± 0.04[c]	ND	ND

Means (± Standard deviation) on the same row with different superscripts are significantly different ($p < 0.05$)

The highest saturated fatty acid (SFA) was found in the oil *Oreochromis leucostictus* (42.81%) (Table 4) followed by those of *Hyperopisus bebe occidentallis* (41.58%), *Synodotus clarias* (41-28%), *Clarias garipinus* (40-66%) and *Gynarchus niloticus* (38-93%). Of these fatty acids identified from the study, palmitic acid (16:0) was the predominant one. The maximum palmitic acid (Hexadecanoic acid) content was 22.91 % in *Hyperopisus bebe occindentallis* and the minimum was *14.95 %* in *Gynarchus niloticus.* The amount of acid in the oil of *Hyperopisus bebe occindentallis* was significantly higher than that of the other fish species which was significantly higher than in each other in the following order *Synodotus clarias* (18.84 %) > *Clarias garipinus* (17.72%) > *Oreochromis leucostictus*.(17.07%) > *Gynarchus niloticus*. The amount of palmitic acid in fish was within the range of the findings made by Adesola and Rahman *et al* that obtained the concentration range of 10.11 - 21.15 % and 12.7 - 26.6 % in freshwater fish species in Lagos and Malaysia respectively [22, 40].

Table 4

Fatty and non-fatty acid (%) composition of selected fish species from Shiroro River

Fatty acids type	*Gynarchus niloticus*	*Clarias garipinus*	*Oreochromis leucostictus*	*Synodotus clarias*	*Hyperopisus bebe occidentallis*	WHO/FAO, 2011
SFA	38.93	40.66	42.81	41.28	41.58	≤10
MUFA	33.63	36.75	37.84	37.71	45.37	≥12
PUFA	26.10	21.83	19.69	19.39	7.79	≥6
Non-fatty acid	1.34	0.76	0.66	1.62	5.28	-
PUFA/SFA	0.67	0.54	0.46	0.47	0.19	≥0.1
ω-6	11.01	18.21	14.18	6.26	6.62	≥8
ω-3	15.09	3.62	4.51	13.13	1.17	≥2
ω-3/ω-6	1.37	0.20	0.31	2.10	0.18	≤4

SFA: Saturated fatty acid, MUFA: Mono-unsaturated fatty acid, PUFA: Poly-unsaturated fatty acid

The MUFA content was the highest in *Hyperopisus bebe occidentallis* (45.37%) followed by *Oreochromis leucostictus* (37.84%), *Synodotus clarias* (37.71%), *Clarias garipinus* (*36.75%)* and *Gynarchus niloticus* (33.63%), with the smallest one. The major mono-unsaturated fatty acids were 18:1 *.a-9* and 18: l-7 (Oleic and Vaccenic acid). Similarly, Osibona reported 18:1 *a* -9 and 18:1 ω-7 as major MUFA in freshwater fish species from Lagos, Nigeria [27]. This is contrary to that reported by Kaneiwaa *et al* which were of 18:1 and 16:1 (Oleic and Palmitic oleic) found in freshwater fish species from China [38]. The variation can be attributed to environmental difference. Guler *et al* and Osman *et al* reported that high Oleic acid is a major fatty acid component of freshwater fish species [41, 42]. The maximum Oleic acid (18:1 ω -9, 9-octadecenoic acid) content was found in *Clarias garipinus* (27.89%) and the minimum was 1.90 % in *Gynarchus niloticus.* The highest vaccenic acid (18:1-7, 11-octadecenoic acid) was *35.75* % found in *Hyperopisus bebe occindentallis* and the minimum content was 4.76 % in *Clarias garipinus.* The monounsaturated fatty acids are responsible for physiological activities and also in the reduction of serum cholesterol [25]. Majority of polyunsaturated fatty acids (PUFAs) are essential in carrying out physiological activities in the body. PUFAs of the oil of the samples ranged between C16 to C22 in chain length and are ω-3 and ω-6 type. Freshwater fishes usually contained higher amounts of ω-6 fatty than ω-3 when compared with marine fish [43]. Similar pattern was observed in the fish species studied in this work except in *Gynarchus niloticus* and *Synodotus clarias*, which corresponds to the findings of Aclcman and Mcleod who reported higher omega-3 PUFAs in fish from cold northern freshwaters fish species [44]. It could therefore be deduced that environmental factor plays vital roles in the PUFAs content of fish.

Fish oil goes through oxidative deterioration and hydrolytic spoilage due to the presence of unsaturated fatty acids (mono-unsaturated and poly-unsaturated).

In this present study the oxidative stability of oil in the five (5) species of freshwater fish was determined using different analytical parameters, such as peroxide value, free fatty acid, and saponification, iodine and acid values to assess the quality of fish oil under room conditions. The results are shown in the Tables 5 – 9.

Table 5

Change in oxidative stability of *Synodotus clarias* oil during storage under room temperature

Storage days	Peroxide value ($meqO_2/kg$)	Free fatty acid (%)	Saponification value (mgKOH/g)	Iodine value (mgKOH/g)	Acid value (mgKOH/g)
0	1.32 ± 0.05^d	2.75 ± 0.20^e	160 ± 0.01^e	170 ± 0.02^a	5.50 ± 0.20^e
15	1.40 ± 0.10^d	2.98 ± 0.01^d	165 ± 0.11^d	165 ± 0.01^b	5.96 ± 0.01^d
30	2.71 ± 0.01^c	3.26 ± 0.05^c	168 ± 0.12^c	163 ± 0.01^c	6.52 ± 0.05^c
45	4.50 ± 0.11^b	3.53 ± 0.01^b	172 ± 0.01^b	162 ± 0.04^d	7.05 ± 0.01^b
60	4.93 ± 0.12^a	6.21 ± 0.11^a	199 ± 1.15^a	155 ± 1.00^e	12.42 ± 0.11^a

Means (± Standard deviation) on the same column with different superscripts are significantly different ($p < 0.05$)

Freshness of lipid is determined by its ability to withstand oxidation reaction during storage, measured by its peroxide value [45]. In this study the peroxide values in $meqO_2/kg$ within storage period of 0 to 60 days were *1.32±0.05 - 4.93±0.12* for *Synodotus clarias* (Table 5), 1.89±0.01 - 5.12±110 for *Clarias garipinus* (Table 6), 1.13±0.10 - 3.71±0.01 for *Oreochromjs leucostictus* (Table 7), 1.00±0.10 - 3.80±1.00 for *Hyperopisus bebe occidentallis* (Table 8) and 0.98±0.01 - 3.98±0.03 for *Gynarchus niloticus* (Table 9). In the entire sample an increase in peroxide value with increased storage time was observed except *in Hyperopisus bebe occidentallis* where a decrease of 3.87 $meqO_2/kg$ to3.80 $meqO_2/kg$ occurred between 45 - 60 days of storage, which were statistically comparable. The increase after 15days from the initial day was not significant for all the species except *Gynarchus niloticus*, with significant increase. Alicia *et al* and Gokhan *et al* reported peroxide values of raw fish oil as 3 – 20 $meqO_2/kg$ [46, 47] but the values obtained by the five fish species did not exceed 10 $meqO_2/kg$.

Free fatty acid is the measure of the extent to which by hydrolysis reaction fatty acids are released from their ester linkages with the parent triglyceride molecules [48]. Willy *et al* stated that free fatty acid formation due to the lipid hydrolysis provides suitable means of assessment of oil spoilage during storage [49].

Table 6

Change in oxidative stability of C*larias garipinus* oil during storage under room temperature

Storage days	Peroxide value ($meqO_2/kg$)	Free fatty acid (%)	Saponification value (mgKOH/g)	Iodine value (mgKOH/g)	Acid value (mgKOH/g)
0	1.89 ± 0.01^c	1.58 ± 0.01^e	162 ± 0.12^e	175 ± 0.10^a	3.15 ± 0.01^e
15	2.25 ± 0.12^c	2.70 ± 0.11^d	164 ± 0.01^d	168 ± 0.12^b	5.40 ± 0.11^d
30	2.51 ± 0.10^c	3.25 ± 0.10^c	168 ± 0.12^c	166 ± 0.11^c	6.50 ± 0.10^c
45	3.90 ± 0.10^b	3.49 ± 0.13^b	170 ± 0.10^b	162 ± 0.10^d	6.98 ± 0.13^b
60	5.12 ± 1.10^a	5.89 ± 0.15^a	190 ± 1.12^a	150 ± 0.01^e	11.78 ± 0.15^a

Means (± Standard deviation) on the same column with different superscripts are significantly different ($p < 0.05$

The percentage free fatty acids content of the five fish species during the periods of storage from 0 to 60 days ranged from 2.75±0.20 - 6.2l±0.11 for S*ynodotus clarias* (Table 5), 1.58±0.01 - 5.89±015 for *Clarias garipinus* (Table 6), 1.00±0.01 – 4.57+002 for *Oreochromis leucostictus* (Table 7), l.58±0.0l -2.5l±0.l2 for *Hyperopisus bebe Occidentallis* (Table 8) and 1.07 - 4.42 % for *Gynarchus niloticus* (Table 9). Free fatty acid significantly increased ($p < 0.05$) as the time of storage increased except for *Hyperopisus bebe Occidentallis* where the increase between 30 and 60 days was not significant ($p > 0.05$). Bimbo suggested the maximum acceptable values of 5%, [50] however in this study *Synodotus clarias* and *Clarias garipinus* had values above 5% after 45days and 60 days of storage.

Table 7

Change in oxidative stability of *Oreochromis leucostictus* oil during storage under room temperature

Storage days	Peroxide value ($meqO_2$/kg)	Free fatty acid (%)	Saponification value (mgKOH/g)	Iodine value (mgKOH/g)	Acid value (mgKOH/g)
0	1.13 ± 0.10^c	1.00 ± 0.01^e	175 ± 0.01^e	165 ± 0.06^a	2.00 ± 0.01^e
15	1.22 ± 0.02^c	1.95 ± 0.05^d	180 ± 0.05^d	163 ± 0.03^b	3.89 ± 0.05^d
30	1.95 ± 0.11^b	2.75 ± 0.03^c	181 ± 0.01^c	159 ± 0.01^c	5.50 ± 0.03^c
45	3.69 ± 0.10^a	3.91 ± 0.01^b	183 ± 0.20^b	152 ± 0.01^d	7.81 ± 0.01^b
60	3.71 ± 0.01^a	4.57 ± 0.02^a	230 ± 0.01^a	139 ± 1.00^e	9.14 ± 0.13^a

Means (± Standard deviation) on the same column with different superscripts are significantly different ($p < 0.05$)

Saponification value (SV) is an indication of the molecular weights of triglycerides of oils. High Saponification value indicates high proportion of low fatty acids since saponification value is inversely proportional to the average molecular weight or length of fatty acids [51]. Therefore the shorter the average chain length (C4 - C12), the higher the Saponification value [52]. The Saponification values in mgKOH/g of analyzed fish oil significantly increase ($p < 0.05$) with increase in storage period. The saponification values of the fish species within the storage periods of 0 to 60 days ranged from 160±0.01 - 199±1.15 for *Synodotus clarias* (Table 5), 162±0.12 – 190±1.12 for *Clarias garipinus* (Table 6), 175±0.01 - 230±0.01 for *Oreochromis leucostictus* (Table 7) and 172±0.01-201±0.12 for *Hyperopisus bebe Occindentallis* (Table 8). It is very possible that the end product of oxidation, such as aldehydes and ketones may have contributed to the increase in saponification value, which may be responsible for high value observed in the last three species of fish. The values obtained were between l60.00 - 203.00 mg KOH/g. These values are within the recommended range of 195 -205 mg KOH/g for oil [53]. The values show that the oils are well suited for soap making.

Table 8

Change in oxidative stability of *Hyperopisus bebe ocidentallis* oil during storage under room temperature

Storage days	Peroxide value ($meqO_2$/kg)	Free fatty acid (%)	Saponification value (mgKOH/g)	Iodine value (mgKOH/g)	Acid value (mgKOH/g)
0	1.00 ± 0.10^c	1.58 ± 0.01^c	172 ± 0.01^e	185 ± 0.04^a	3.15 ± 0.01^b
15	1.37 ± 0.12^c	2.37 ± 0.10^b	175 ± 0.10^d	183 ± 0.01^b	4.74 ± 0.10^a
30	2.82 ± 0.03^b	2.40 ± 0.02^{ab}	178 ± 0.02^c	180 ± 0.01^c	4.81 ± 0.02^a
45	3.87 ± 0.11^a	2.44 ± 0.10^{ab}	179 ± 0.01^b	173 ± 0.12^d	4.89 ± 0.10^a
60	3.80 ± 1.00^a	2.51 ± 0.01^a	201 ± 0.12^a	169 ± 0.10^e	5.02 ± 1.00^a

Means (± Standard deviation) on the same column with different superscripts are significantly different ($p < 0.05$)

The iodine value, defined as the measure of unsaturation in an oil sample, is a useful tool in detecting the level of spoilage in oil. The iodine values of the oils in each of the fish species significantly decreased as the storage time increased from 0 to 60 days. The iodine value of oils as the storage time increased to 60 days decreased from 170.00±0.02 to

155.00±1.00 for *Synodotus clarias* (Table 5), 175.00±0.10 to 150.00±0.01 for *Clarias garipinus* (Table 6), 165.00±0.06 to 139.00±1.00 for *Oreochromis niloticus* (Table 7), 185.00±0.04 to 169.00±0.10 for *Hyperopisus bebe Occidentallis* (Table 8) and 162.00±0.10 to 143.00±0.10 for *Gynarchus niloticus* (Table 9). The percentage decrease was calculated to be 8.65 % for *Hyperopisus bebe Occidentallis,* 8.82 % for *Synodotus clarias,* 11.73 % for *Gynarchus niloticus,* 14.29 % for *Clarias garipinus* and *15.75 %* for *Oreochromis niloticus.* The decrease in iodine value shows the decrease in the degree of unsaturation during the storage of oils [49].

Table 9

Change in oxidative stability of *Gynarchus niloticus* oil during storage under room temperature

Storage days	Peroxide value (meqO2/kg)	Free fatty acid (%)	Saponification value (mgKOH/g)	Iodine value (mgKOH/g)	Acid value (mgKOH/g)
0	0.98 ± 0.01[e]	1.07 ± 0.01[e]	175 ± 0.01[e]	162 ± 0.10[a]	2.14 ± 0.01[e]
15	1.21 ± 0.01[d]	1.99 ± 0.20[d]	176 ± 0.10[d]	160 ± 0.02[b]	3.98 ± 0.20[d]
30	1.90 ± 0.11[c]	2.57 ± 0.09[c]	179 ± 0.05[c]	159 ± 0.10[c]	5.14 ± 0.09[c]
45	3.02 ± 0.10[b]	3.48 ± 0.12[b]	180 ± 0.20[b]	154 ± 0.05[d]	6.95 ± 0.12[b]
60	3.98 ± 0.03[a]	4.42 ± 0.02[a]	216 ± 0.10[a]	143 ± 0.10[e]	8.83 ± 0.02[a]

Means (± Standard deviation) on the same column with different superscripts are significantly different (p < 0.05)

The Acid value is the measure of free fatty acid in oils. The acid values in mgKOH/g of oils in the five fish species significantly increased ($p < 0.05$) with increase in the storage time from 0 to 60days. The acid values for each of the oils from day 0 to the 60[th] day ranged from 5.50±0.20 to 12.42±0.11 for *Synodotus Clarias* (Table 5), 3.15±0.01 to 11 78±0.15 for *Clarias garipinus* (Table 6), 2.00±0.01 to 9.14±0.13 for *Oreochromis leucostictus* (Table 7), 3.15±0.01 to 5.02±1.00 for *Hyperopisus bebe occidentallis* (Table 8) and 2.14±0.01 to 8.83±0.02 for *Gynarchus niloticus* (Table 9). The suitable limit for acid value is 7 - 8mgKOH/g as reported by De-Koning [48]. The study revealed that the acid values of the oils of *Synodotus clarias, Clarias garipinus* and *Oreochromis leucostictus* exceeded the maximum limit after 60 days of storage. The acid value of oils within the storage period of 45days in all the fish species fell within the maximum limit reported by De-Koning [54], while after 60 days of storage, the oil of *Hyperopisus. bebe occidentallis* was below this limit.

4. Conclusion

There were some levels of significant differences in the proximate minerals, fatty acids and cholesterol compositions between the different freshwater fish species analyzed in this study. The protein content of the fish species, per dried weight, was generally high, with *Gynarchus niloticus* having the highest concentration of above 50% and *Hyperopisus bebe occidentallis* the lowest one, below 40%. This implies that all the fish species can be used as protein supplements. The fish species were also found to be the oily fish with low saturated fatty acids and high polyunsaturated fatty acid. They are also rich in omega-6 and 3 fatty acids and therefore they can be recommended for human consumption especially *Gynarchus niloticus* and *Synodotus clarias* species. Also, *Oreochromis leucostictus* has the highest cholesterol content while *Clarias garipinus* species has the lowest one. The values are general lower than those recommended by the FAO /WHO as regards the toxicity limit of cholesterol.

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6. References

[1] JITENDER K. J., PAL A. K.., DEVIVARAPRASAD R., SAHU N. P., VENKATESHWARLU G. & VARDIA H. K.., Fatty Acids Composition of Some Selected Indian Fishes. *Africa Journal of Basic & Applied Sciences,* 4(5), 155-160, *(*2012).

[2] STONE N. J., Fish consumption, fish oil, lipids, & coronary heart disease. *Circulation, (*2010).

[3]. DESLYPERE J. P., Effect of fish consumption compares to intake of fish oil, *Bibliotheca Nutrition et Dieta,* 46, 53 – 69, (1990)

[4]. EYO A. A., Fish processing in the tropics. University of Ilorin Press, Nigeria, 153-189, (2001)

[5] KNUTH B., CONNELLY N. A., SHEESHICA J. & PATTERSON J., Weighing health benefits and health Risk information when consuming sport caught fish. *Risk Analysis,* 23, 1185- 1197, (2003)

[6] DARREN J. H. & BRUCE J. H., Omega-3 fatty acids from fish oils and cardiovascular disease. In: molecular and cellular biochemistry. Kluwer Academic Publishers, Netherlands, 2004, 263, 217-225.

[7] WANG C., HARRIS W.S., CHUNG M., LICHTENSTEIN A.H., BALK E.M., KUPELNICK B., JORDAN H.S & LAU J., Omega-6 Fatty Acids from Fish or Fish-Oil Supplements, but not Alpha-linolenic Acid Benefit Cardiovascular Disease Outcomes in Primary and Secondary Prevention Studies: A Systematic Review. *America Journal of Clinical Nutrition*, 84(1):5-17 (2006)

[8] DHANAPAL K.., REDDY A.D., & REDDY G.V.S., Beneficial effects of fish oil and its applications in human health. *Int. J. Med. Bio.*, 17(12): 137-156, (2011)

[9] BHASKAR N., MIYASHITA K. & HOSOKAWA M., Physiological effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) – A review. *Food Rev Int,* , 22: 291-307, *(*2006) .

[10] DAVIGLUS M., SHEESHKA J. & MURKIN E., Health Benefits from Eating Fish. *Comments Toxicology*, 8, 345-348, (2002)

[11]. WHEATON F. W. & LAWSON T. B., Processing aquatic food products. New York; John Wiley, (1985)

[12]. SUZUKI T., Krill and Krill Protein. Processing Technology. Applied Science Publishers, London, (1981)

[13]. DREVON C. A. ω-3 Fatty acids in health disease, Fish fats and your health. Proceedings of the International Conference on fish lipids and their influence on human health, *SuanØy Foundation,* 6965 Svanybukt, Norway, 19-25, (1989).

[14]. SYVAOJA E. L., SALMINEM K.., PIIRONEN V., VARO P., KEROJOKI O & KOIVISTOINEN P., Tocepherols and Tocetrienols in Finnish foods: Fish and Fish Products. *Journal of American Oil Chemical Society*, 62(8), 1245 – 1248, (1985)

[15] IDODO-UMEH G., Freshwater fisheries of Northern Nigeria. (Taxonomy, Ecological Notes, Diet and Utilization), ISBN 978-8052-01-0 (2003)

[16] FROESE R. & PAULY D., *Tilapia jallae* (Boulenger 1896). Fish Base, (2015).

[17] AYOOLA S. A. & ABOTTI C. E., Morphology of Abba Knife Fish *(Gymnarchus niloticus).* World Journal of Fish and Marine Science, 2(5), 354-356, (2011)

[18] STEPHEN K.., STUMBAUER C., VERHEYEN E., MEYER A., & SALZBURGER W., Mitochondrial phylogeny and phylogeography of East African squeaker Catfishes (*Siluriformes: Synodontis*). BMC Evolutionary Biology, 6, 49, (2006)

[19] AOAC, Official methods of analysis, 18th Edition. Method *960.52A.* Association of Official Analytical Chemists, Washington DC, (2007).

[20] ENYISI 1., UMOH V. J., WHONG C. M. Z., ABDULLAHI 1.O. & ALABI O., Chemical and Nutritional Value of Maize and Maize Products Obtained from Selected Markets in Kaduna State, Nigeria. *African Journal of Food Science and Technology, 5,* 100-110, (2014)

[21] ALFA Y. M., NDA-UMAR U. I., SALIHU A. B. & NMA N. Y., Proximate composition and Minerals components of some species of fish sold in Bida fish market. *International Journal of Current Research in Chemistry and Pharmaceutical Sciences,* 1(8), 19- 24, (2014)

[22] ADESOLA O. O., Comparative study of proximate composition, amino acid and fatty acid of some economically important fish species in Lagos, Nigeria. *African Journal of Food Science,* 5, 581 – 588 (2011)

[23] ADEWOLE O. S., FAWOLE O. O. &

OMOTOSHO J. S., Concentration of selected elements in some freshwater fishes in Nigeria. *Science Focus,* 4, 106 – 108, (2003)
[24] BENNION M & SCHEULE B., *Introductory Foods;* Prentice-Hall, New Jersey, USA, 575, (2000)
[25] TAIWO O. E., USMAN K., OGONO T. H. & OSONIYI R. D., Proximate and lipid profile Analysis of cultured wild African catfish, *clarias gariepinus* (burchell), *Ife Journal of Science*, 16(1), 50-52, (2014)
[26] MAZUMDER M. S. A., RAHMAN M. M., AHMED A. T. A., BEGUM T. & HOSSAIN M. A., Proximate composition of some small indigenous fish species (SIS) in Bangladesh. *International Journal of Sustainable Crop Production,* 3(3), 18-23, (2008)
[27] OSIBONA A. O., Comparative study of proximate composition, amino and fatty acids of some economically important fish species in Lagos, Nigeria. *African Journal of Food Science,* 5(10), 581-588, (2011)
[28] AYELOJA A. A., GEORGE F. O. A., DAUDA T. O., JIMOH W. A. & POPOOLA M. A., Nutritional composition of captured *Clarias garipinus* and *Oreochromis niloticus. International Research Journal of Natural Science*, 1(1), 9 – 13, (2013)
[29] EFFIONG B. N. & FAKUNLE J. O., Proximate and Mineral Content of Traditional Smoked Fish Species from Lake Kainjj, Nigeria. *Bulletin of Environmental Pharmacology and Life Sciences,* 1, 43-45, (2012)
[30] ANDE S., LEKE I., ENEJI I. & YAKUBU S., Proximate analysis of smoked and unsmoked fish (catfish and Tilapia) in Ombi River Lafia, Nassarawa State, Nigeria. *Elixir Food Science,* 53, 11801 – 11803, (2012)
[31] FAO/WHO, Human Vitamin and Mineral Requirements Rome: FAO, (2011)
[32].IJEOMA P. O., SANNI O. A., EGUN N. K. & WILFRED-EKPRIKPO C. P., The effect of actellic dust treatment on the proximate and mineral composition of *Synodontis nigrita* and *Tilapia mariae , Food and environmental safety,* 18(1), , 60 – 66, (2019)
[33] SIVAKOVA B. & BLAZHEKOVIKJ – DIMOVSKA D., The impact of different diet and environmental conditions on chemical composition of Rainbow trout (*Oncorhynchus mykiss Walbaum*, 1792) from Macedonian Aquaculture facilities, *Journal of Faculty of Food Engineering, 15(3),* 227 – 233, (2016)
[34] ADENIYI S. A., ORJIEKE C. L., EHIAGBONARE J. E. & JOSIA S. J., Nutritional Composition of Three Different Fishes *(Claria gariepinus, Malapteru, electricus'* and *Tilapia Guineensis) Pakistan Journal of Nutrition,* 11, 793-797, (2012)
[35] GUERIN T., CHEKRI R., VASTEL C., SIROT V., VOLATIER J. L, LEBLANC J. C. & NOEL L., Determination of 20 trace elements in fish and other seafood from the French market. *Food Chemistry,* 127,934 -942, (2011)
[36] TAO N. P., WANG L. Y., GONG X. & LIU Y., Comparison of nutritional composition of Farmed puffer fish muscles among *Fugu Obsurus, Fugu fladivus* and *Fugus rubripes*. *Journal of food composition analysis,* 28, 40-45, (2012)
[37] ALAS A., OZCAN M. M. & HANNAKAYA M., Mineral Contents of Head, Caudal, Central Fleshy Part and Spinal Colum of Some Fishes. *Environmental Monitoring Assessment,* 186, 889-894, (2014)
[38] KANENIWA M., MIAOB S., YUANB C., JIDAC H. & FUKUDAD Y., Lipid components and enzymatic hydrolysis of lipids in muscle of Chinese freshwater fish. *Journal of the American Oil Chemical Society,* 77: 825-830, (2008)
[39] KERIKO J. M., CHEGE C.W., MAGU M.M., MWACHIRO E.C., MURIGI A. N., GITHUA M. N., KARERU P.G., Fish Lipid Contents and Classes of Selected Fish Species found in Lake Naivasha (Kenya) and the Fish Feeding habits of the Lakes inhabitants. *Africa Journal of Pharmacy and Pharmacology,* 4(10): 745-753, (2010)
[40]. RAHMAN S. A., HUAH T. S., HASSAN O. & DAUD N. M., Fatty acid composition of some Malaysian freshwater fish. *Food Chemistry,* 54(1), 45—49, (2015)
[41]. GULER G. O., KIZTANIR B., AKTUMSEK A., CITIL O. B. & OZPARLAK H., Determination of the Seasonal changes on total fatty acid composition and co-3/ w-6 ratios of carp (*Cyprinus carpio L.*) Muscle lipids in Beysehir Lake (Turkey). *Food Chemistry,* 108, (2008)
[42] OSMAN H., SURIAH A. R. & LAW E. C., Fatty Acid Composition and Cholesterol Content of Selected Marine Fish in Malaysian Waters. *Food Chemistry,* 73, 55-60, (2001)
[43] MUHAMAD N. A. & MUHAMAD S., Fatty Acids Composition of Selected Malaysia Fishes (Komposis Asis *Le'rnakllcan Terpilh Malaysis). Sains Malaysiana,* 41(1), 81-94, (2012)
[44] ACKMAN R. G. & MCLEOD C., Lipids and fatty acids of five freshwater food fishes of India. *Journal of Food Lipids,* 9, 127 – 145, (2002)
[45] MALHEIRO R., RODRIGUES N., MANZKE G., BENTO A., PEREIR J. A. & CASAL S., The use of olive leaves and tea extracts as effective antioxidants against the oxidation of soybean oil

under microwave heating, *Industrial Crops and Products,* 44, 37– 43, (2013)

[46] ALICIA R., VANESA L., ANGELICA L., VILMA Q., JULIA V. & SANTIAGO P.A., Development of Lipid Changes Related to Quality Loss during the Frozen Storage of Farmed Coho Salmon (*Oncorhynchus kisutch*). *Journal of the American Oil Chemists Society,* 84, 727-734, (2007)

[47] GOKHAN B., MUHAMMET B. & HIKMET K., Seasonal Changes in Proximate Composition of Anchovy and Storage stability of Anchovy Oil. *Journal of Food Quality,* 31, 503-513, (2006)

[48]. SALHIN M. A. & ABDURAHMAN M. A., Determination of Free Fatty Acids in Palm Oil Samples by Non Aqueous Flow Injection Using Salicyaldehyde-2,4-Dinitrophenylhydrazone as Colorimetric Reagent. *Chemical and Materials Engineering,* 1(3), 96-103, (2013)

[49] WILLY P. W., ABDULAN A. S., FATIMA B., ABUBAKAR N. B. S., ZAMARI B. I. & HAMID A. A., Lipid quality deterioration of bagriclac Catfish *(Mystus nemurus)* during Storage *Research Journal of Biological Sciences,* 4, 525-530, (2009)

[50]. Bimbo A. P., Guidelines for Characterizing Food-Grade Fish Oils. Hertfordshire: UK, , 9(5). 1998

[51]. MUHAMMAD E., AL-MAQBALY R. & MANSOUR M.H., Proximate composition, amino acid and Mineral contents of five commercial Nile fishes in Sudan. *Africa journal food sciences,* 10, 650-654, (2011)

[52]. TAMZID H. M., ALAM M. T. & ISLAM M. A. U., Physico-chemical and Nutritional studies of *Terminalia belerica Roxb* Seed oil and seed kernel. *Journal of Bio-Science*, 15, 117 – 126, (2007)

[53]. AOCS, Official methods and Recommended Practices of the American Oil Chemist Society (5^{th} ed.) Champaign, 2009.

[54]. DE-KONING A .J., The free fatty acid content of fish oil, part V. The effect of microbial Contamination on the increase in free fatty acid content of fish oils during storage at *25°C. Fat/Lipid*, 101(5), 184-186, (1999)