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RESEARCH ARTICLE

TOTAL LIPID CONTENTS AND LIPID CLASSES IN COMMERCIAL FISHES OF SELECTED KENYAN FRESHWATERS

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Many societies are becoming more and more reliant on natural products as opposed to synthetic products. This study focused on determining and documenting information regarding total fish lipid contents and classes including the nutritional and dietary data from various commercial fish species found in Kenyan freshwaters. Kenya has a long coastal strip, inland lakes and rivers, which provide the necessary sources of the various aquatic natural products. A total of twelve fish species were sampled from all regions as follows: two from Lake Turkana, six from Lake and four from river Tana and their total lipid contents and lipid classes were analysed. The mean lipid contents ranged between 0.4 – 4.7 % for the fishes from Tana River, 0.6 – 38.8 % for the fishes of Lake Victoria and 3.5 – 14 % for the fishes of Lake Turkana. Triacylglycerides (TAG) was the dominant lipid class ranging between 0.03% for Labeo fish of Tana River and 10.08 % for Labeo fish of Lake Victoria. These were followed by the phospholipids (PL) such as the PE ranging between 0.03% for tilapia fish of Tana River and 3.64 % for Labeo fish of Lake Victoria while PC ranged between 0.02 % for tilapia of Tana River and 0.73 % of catfish of Lake Victoria. In conclusion, most of the freshwater fishes found in Kenya have relatively high total lipid content hence can be used to suppress cardiovascular diseases due to benefits associated with this fish lipids.

Introduction:-

The consumption of marine natural products especially fish has many health benefits especially in terms of helping to reduce the attack by diseases such as coronary heart diseases, high blood pressure and other diseases that result from excessive consumption of foods containing high cholesterol levels. Fish constitute an important source of protein for many people throughout the world, and fish consumption has increased in importance among health-conscious people because it provides a healthy, low cholesterol source of protein and other nutrients. Fishing is also a popular pastime (J. Burger, 2002; J. Burger et al., 2002; J. Burger, Cooper, & Gochfeld, 1992; Knuth, A. Connelly, Sheeshka, & Patterson, 2003) in the rural including in some urban areas (J. Burger, 2002). Fish provide omega-3 (n-3) fatty acids that reduce cholesterol levels and the incidence of heart disease, stroke, and preterm delivery (Joanna Burger & Gochfeld, 2009; Lane, 1996).

Fish provide omega-3 (ω-3) fatty acids that are known to reduce cholesterol levels and the incidence of coronary heart diseases (CHD), stroke, and pre-term delivery (Joanna Burger & Gochfeld, 2009; Hurlbert, Anderson, Sturm, & Hurlbert, 2007).

Aquatic products especially fish may serve a more important role in human nutrition and health than previously realized. Unfortunately, food consumption habits of Kenyans highly depend on their customs (and to some extend on the geographical set-up). For instance, in Kenya the areas towards central province and the Mount Kenya region, there are at present few fish eaters whereas in the lake and coastal regions, fish-meat eating habits is the norm (Huss, 1995).

Consumption of fish in many developed and developing communities around the World has increased greatly in recent years as a result of the nutritional awareness and the literacy level of the consumers in these countries. The consumption of marine natural products especially fish is very beneficial for body health and development since they provide essential nutrients that are unavailable in plant natural products and terrestrial animals. For instance, the human body requirement for the long chain polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are not adequately provided by vegetables or meat from terrestrial sources. The indirect provision through the carbon chain elongation followed by the C-C- desaturation process of the C-18, α-linolenic acid (18:3*n*-3) is generated and is abundantly available in green vegetable, is by the body enzymatic system which is usually not adequate. It is documented that these fatty acids are very essential in the brain's grey matter, in the eye ball's fluid and hence important in the eye's visual acuity, in the development of spermatozoa and in the nervous system. In general however, marine natural products are known to be beneficial in the body particularly in preventing attack by such diseases as the coronary heart diseases, cancer, diabetes, high blood pressure, gout and other diseases that arise as a result of excessive consumption of foods containing high levels of cholesterol.

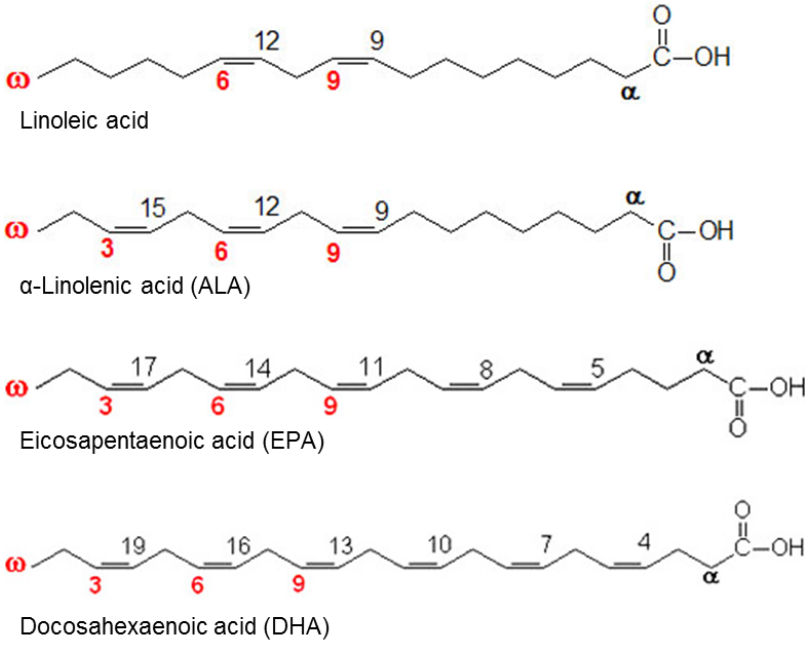

Figure 1: Structures of some fatty acids

These essential lipids are available abundantly in marine natural products. However, the levels of these lipid components in the fish and other marine animals are dependent on the environment and the food available for a particular species. The lipid contents, the classes and fatty acid composition of fishes in fresh and marine waters of Kenyan Indian Ocean, lakes and rivers have not been determined.

This research work aimed at determining the level of total lipid content and classes in the fish species commonly found in selected Kenyan freshwaters. A total of twelve fish species were sampled from all regions as follows: two from Lake Turkana (namely Tilapia and Mudfish), six from lake Victoria (namely Tilapia, Nile perch, Mudfish, Catfish, Labeo and Bonny fish) and four from river Tana (namely Tilapia, Choka, Labeo and Bonny fish) and analyzed.

Fish lipids constitute a wide range of constituents. Lipids are the building blocks of the fats or fatty substances found in animals and plants. They are microscopic layered spheres of oil which, in animals are composed mainly of fats and oils, waxes, phospholipids, steroids (like cholesterol), and some other related compounds. All lipids are hydrophobic(Keriko et al., 2010; Shirai, Suzuki, Toukairin, & Wada, 2001). In this study, the lipid content and classes for common fishes found in Lake Turkana, Lake Victoria and river Tana were determined.

Materials and methods:-

Reagents and standards:-

Analytical grade (AR) chemicals were used throughout the study without any further purification. Standards were procurement from Sigma Aldrich Company. To prepare all the reagents and calibration standards, double glass-distilled water was used. All the experiments were carried out in triplicate. The results were reproducible within ±3% error limit.

Sampling of fish from Kenyan fresh waters:-

Sampling was done in January 2011 and august 2012 from 3 different stations (Lake Turkana, Lake Victoria and river Tana). Common fish species found in each region were sampled and stored in a cold box at -4^{o}C to prevent autolytic post-mortem changes after rigor mortis during transportation to the laboratory where they were placed in clean well-labelled polyethylene bags before preserving them in a refrigerator. Fish were purchased directly from fishermen. Although a variety of freshwater fish species inhabits these freshwater bodies, the common ones; a total of twelve fish species were sampled from all regions as follows: two from Lake Turkana (namely Tilapia and Mudfish), six from lake Victoria (namely Tilapia, Nile perch, Mudfish, Catfish, Labeo and Bonny fish) and four from river Tana (namely Tilapia, Choka, Labeo and Bonny fish) were sampled in the present work. The fish samples were put in a cool box filled with ice and transported to JKUAT laboratory.

Sample preparation:-

Physical parameters such as weight, width and length of each fish specimen were recorded as shown in Table 1. The muscles were removed separately and subjected to extraction protocol as reported by Folch (Folch, Lees, & Stanley, 1957; Keriko et al., 2010).

Lipid extraction:-

The muscle of each fish specimen was separated, weighed and then minced. The tissue was solvent extracted with chloroform/methanol (2:1). After dispersion, the whole mixture was agitated for 20 min. in an orbital shaker at room temperature.

The homogenate was filtered to recover the liquid phase. The interface was rinsed twice with methanol/chloroform (1:1). After centrifugation and siphoning of the upper phase, the lower chloroform phase containing lipids was filtered off and the trace water was removed from the extract by passing it through a filter paper containing anhydrous sodium sulphate (Na_2SO_4),(Sigma-Aldrich, Germany). The individual lipids were recovered after evaporating the solvent under vacuum in a rotary evaporator (Folch et al., 1957).

The lipid extracted was weighed and transferred into a vial and any remaining solvent was left to evaporate in the fume chamber. The dry extract was covered with a foil paper and was stored in a fridge. This procedure was repeated for all the fish species sampled.

Lipid Column Fractionation:-

The crude total lipids were separated into classes on silica-gel packed columns (Merck and Co., Kieselgel 60, 70-230 mesh ASTM), and a quantitative analysis of the lipid constituent was performed using gravimetric analysis of fractions collected from column chromatography using Saito and Murata (Saito & Murata, 1998) protocol.

The first eluate (dichloromethane: n-hexane, 2:3, v/v) was collected as a mixture of steryl esters (SE), wax esters (WE), and glyceryl esters (GE) (Fraction 1). This was followed with pure dichloromethane eluting a mixture of glyceryl esters (GE) and triacylglycerides (TAG), (Fraction 2). Dichloromethane: diethyl ether (35:1, v/v) eluting a mixture of triacylglyceryl esters (TAGE) and diacylglyceryl esters (DAGE), (Fraction 3) while dichloromethane: diethyl ether (9:1, v/v) eluting the diacylglyceryl esters (DAGE) and sterols (ST), (Fraction 4); dichloromethane: methanol (9:1, v/v) eluting the free fatty acids (FFA), (Fraction 5); dichloromethane: methanol (1:1, v/v) eluting Phosphatidylethanolamine (PE), (Fraction 6) while dichloromethane: methanol (1:5, v/v) eluting other lipids such as

inositol, sphingolipids, (Fraction 7) and finally dichloromethane: methanol (1:20, v/v) eluting the Phosphatidylcholine (PC), (Fraction 8) respectively (Keriko et al., 2010).

After fractionation, the individual lipids in each class were identified using standards by comparison of their relative fraction (Rf) values using thin-layer chromatography (TLC on pre-coated aluminium sheets, Merck and Co., Kieselgel 60 F_{254}, thickness of 0.25 mm) and sprayed with 12-Molybdo (IV) phosphoric acid n-hydrate (Phosphomolybdic acid, BDH Chemicals Ltd., Poole, England) dissolved in water (Dittmer & Lester, 1964; Rouser, Siakotos, & Fleischer, 1966). All lipid fractions were dried under the vacuum rotary evaporator and stored in the freezer.

Statistical analysis:-

One-way ANOVA was applied to analyse the significant differences among sampling stations for different lipid levels (Hair, 2006). Turkey'st-test was also performed to identify the homogeneous type of the data sets (Hair, 2006). Pearson correlation matrix was also calculated for different metals in sediments to trace the common sources of pollutants. SPSS® statistical package (Window Version 22.0) was used for data analysis and later confirmed by Origin 8.5 (Creswell, 2013). All statements reported in this study are at the $P < 0.05$ levels (Creswell, 2013; Hair, 2006).

Results and Discussion:-

The major lipid storage sites in fish vary in different species. However, lipids are primarily located in the subcutaneous tissue, belly flap, muscle tissue, liver, mesenteric tissue, and in the head (Zhou, Ackman, & Morrison, 1995). The amount of lipid dispersed through the flesh decreases from the head to the tail, where the muscles used for swimming are located. There is also a considerable amount of lipid located in the myosepta surrounding the muscles (Zhou et al., 1995). The white muscle is responsible for rapid bursts of activity such as sudden fast swimming, while the dark muscle is used for steady, sustained swimming.

Season, habitat, gonad maturity, sex, diet and stomach fullness, health and preservation methods (Tesch, 1998), may influence the length-weight relationships, although they are not considered in the present study. Comparing average body weights and lengths of the whole fresh fish analysed in this study with literature references (Heydarnejad, 2009; Ouattara, Ouattara, & Gour, 2007), it is clear that values are within the reported limits.

If normal growth patterns are present, then there is no cause for alarm. However, indications of stunted growth could be as a result of greater ecological problems such as pollution, variation of food supply and density of fish population per unit volume of water.

Table 1. Physical parameters of fish species

FISH SPECIES			LENGTH	WIDTH	HEIGHT	WEIGHT
Scientific name	**Common name**	**Location**	**(cm)**	**(cm)**	**(cm)**	**(g)**
Labeo gregorii	Choka	River Tana	30.1±1.6	4.7±0.5	7.7±0.5	251.5±44.7
Oreochromis spilurus	Tilapia		16.5±0.7	2.9±0.2	6.2±0.2	78.7±6.5
Schilbe intermedius	ButterFish		19.4±1.4	2.5±0.3	4.8±0.3	62.2±15.9
Labeo cylindricus	Labeo		16.9±0.1	3±0.1	3.8±0.2	46.3±1.9
Protopterus aethiopicus	Mudfish	Lake Victoria	53.8±1.1	9.7±0.1	7.5±0.2	943.7±43.3
Oreochromis variabilis	Tilapia		30.8±0.6	4.8±0.3	12.3±0.8	636.5±51.3
Clarias gariepinus	Catfish		53.9±1.1	9.8±0.1	7.6±0.2	953.7±41.7
Chiloglanis somereni	Labeo		16.9±0.3	3.4±0.2	4.3±0.6	55.8±12.2
Schilbe intermedius	Butter Fish		19.6±1.8	3.2±0.5	4.9±0.4	64.5±24.8
Lates niloticus	Nile Perch		36.1±0.6	4.4±0.5	10.2±0.2	495.6±9.0
Protopterus aethiopicus	Mudfish	Lake Turkana	73.7±11.2	13.9±2.4	7. 5±0.5	1207.7±59.9
Oreochromis niloticus	Tilapia		24.4±1.7	3.2±0.4	9.2±0.7	202.4±8.9

Numbers represent means ± standard errors of six replicates

The relationship between length and weight has been referred to by Haimovici and Velasco (Haimovici & Velasco, 2000) and da Costa and Araújo (Vicentini & Araújo, 2003) as a very important key which has widely been used in fish biology with several purposes. This useful tool provides important information concerning the structure and function of fish populations (Anderson & Gutreuter, 1996; Gerow, Anderson-Sprecher, & Hubert, 2005). Moreover, it is used to predict weight from length measured in yield assessment (Ecoutin, Albaret, & Trape, 2005), to calculate the standing crop biomass (MartinSmith, 1996), to evaluate the index of well–being of the fish population (Bolger & Connolly, 1989), to assess growth rates and age structure of the fish population (Kohler, Casey, & Turner, 1995), to make morphometric comparisons between species and populations (Goncalves et al., 1997; Holcik & Mihalik, 1996) and life history comparisons between regions (Petrakis & Stergiou, 1995).

The lipid contents of fishes from selected Kenyan freshwater:-

Table 2 provides the quantitative estimates of the total lipids extracted from the muscles of twelve (12) freshwater fish species. The total lipid content in these species ranged between 0.4 ± 0.01% and 38.8 ± 0.34% in the muscle of the wet tissues. Catfish from Lake Victoria was the biggest in size it recorded the highest total lipid content in its muscle tissue while tilapia from river Tana was the smallest and recorded the lowest total lipid content in its muscle tissue.

(Kamal, Khan, Rahman, & Ahamed, 2007; Suaib et al., 2013) found out that the muscle lipid contents among seven freshwater fishes from the River Mouri, Khuina, Bangladesh ranged between 3.5 ± 0.92% in *Heteropneustes fossilis* to 7.9 ± 1.91% in *Clarias batrachus*. The lipid contents of some selected muscle fishes from Mymensingh District, Bangladesh ranging between 1.87 and 9.55% was reported. Our results were within these ranges.

In another study, (Mangold, 2012) observed that in three fish studied, the range of total lipid varied from 2.5 ± 0.5% in Catfish muscle to 8.9 ± 0.8% in Dogfish muscle and Basking shark muscle 4.9 ± 0.6%. These figures were too low compared to the results we obtained.

(Hamed, Salem, Ali, & Sheshtawi, 2011)reported the total lipids in some Bangladeshi zeol fish muscle ranging from 2.18 and 9.38% which supported the results obtained in the present study. Fishes with lipid content in the muscles below 5% are considered lean (Ackman, 1989)and hence the four freshwater fish species from river Tana, two from lake Victoria and one from Lake Turkana indicate lean specimen. The low concentrations of lipid in the muscles of this species could be due to the use of fat reserves during normal activities such as spawning.

Table 2. Fish lipid content

SAMPLING SITE	FISH SPECIES	n	OIL CONTENT (%)
River Tana	Tilapia	3	0.4±0.0
	Bonny Fish	3	2.0±0.4
	Choka	3	4.7±0.8
	Labeo	3	2.1±0.8
Lake Victoria	Mudfish	3	38.7±0.2
	Tilapia	3	2.1±0.5
	Catfish	3	38.8±0.3
	Labeo	3	25.2±3.9
	Nile Perch	3	0.6±0.1
	Bonny Fish	3	11.0±1.8
Lake Turkana	Mudfish	3	14.0±2.6
	Tilapia	3	3.5±0.3

Numbers represent means ± standard errors of three replicates

Lipid classes in the total lipids of the various fish species:-

Lipid classes in the muscle tissue:-

Generally, all fish muscle tissue contained both neutral and polar lipids mainly triacylglycerides (TAG) and phospholipids (PL) as shown in Table 3. The neutral lipid classes of all the twelve fish species under study were quite low as compared with the phospholipids classes. Triacylglycerides are the storage lipids in almost all commercial fish species (Norton & MacFarlane, 1999). The highest percentage of triacylglycerides (TAG) being recorded in the Labeo from Lake Victoria (10.08 ± 4.288%) and the least percent was in Labeo from river Tana

(0.03 ± 0.042%). The phospholipids had the highest amounts of all the lipid classes fractionated catfish from lake Victoria had the highest amount of Phosphatidylcholine (PC) in the muscle (3.36±2.189%). Phosphatidylethanolamine (PE) lipid class compositions in the muscle tissue of different types of fish under study were found not to be significantly differently ($p > 0.05$). Their mean values ranged between 0.02 ± 0.006% and 3.36 ± 2.189% in tilapia from river Tana and Labeo from Lake Victoria respectively.

Table 3.Percentage lipid classes present in the muscle tissue of different fish species.3

Fishes & Location	WE (%)	TAG (%)	DAGE (%)	ST (%)	FFA (%)	PE (%)	SL (%)	PC (%)
Choka, Tana	0.65±0.61	1.82±1.28	0.17±0.01	0.11±0.01	0.10±0.01	0.08±0.01	0.02±0.00	0.15±0.01
Bonny Fish, Tana	0.09±0.03	1.04±1.033	0.07±0.03	0.03±0.00	0.06±0.01	0.12±0.02	0.02±0.00	0.07±0.01
Tilapia, Tana	0.02±0.01	0.13±0.08	0.01±0.01	0.01±0.00	0.02±0.00	0.02±0.00	0.01±0.00	0.02±0.00
Labeo, Tana	0.02±0.00	0.03±0.04	0.05±0.06	0.06±0.01	0.08±0.01	0.09±0.01	0.11±0.01	0.12±0.01
Tilapia, Turkana	0.78±0.89	0.98±0.99	0.44±0.06	0.08±0.29	0.15±0.01	0.09±0.02	0.04±0.01	0.13±0.01
Mudfish, Turkana	7.43±2.43	3.36±2.56	1.36±0.03	0.20±0.01	0.33±0.01	0.30±0.02	0.75±0.02	0.31±0.02
Mudfish, Victoria	28.60±5.35	6.50±1.55	1.81±0.03	0.41±0.64	0.24±0.01	0.28±0.025	0.12±0.02	0.72±0.01
Nile Perch, Victoria	0.04±0.03	0.26±0.25	0.11±0.08	0.06±0.06	0.09±0.01	0.02±0.00	0.04±0.01	0.07±0.01
Catfish, Victoria	28.69±0.33	6.52±1.58	1.81±0.04	0.41±0.02	0.24±0.01	0.28±0.01	0.12±0.01	0.73±0.01
Bonny Fish, Victoria	0.32±0.19	5.28±0.72	0.64±0.01	0.89±0.05	1.27±0.06	1.23±0.01	1.02±0.00	0.30±0.02
Labeo, Victoria	7.23±3.72	10.08±0.29	2.82±0.71	0.70±0.09	0.24±0.06	3.36±0.01	0.28±0.01	0.24±0.01
Tilapia, Victoria	1.16±1.08	0.40±0.04	0.17±0.08	0.05±0.22	0.02±0.00	0.08±0.02	0.05±0.00	0.15±0.03

Numbers represent means ± standard errors of six replicates

Key: WE = Wax esters, TAG = Triacylglceride, DAGE = Diacylglceride esters, ST = Sterol, FFA = Free fatty acids, PE =Phosphatidylethanolamine, SL = SphingoLipids, PC = Phosphatidylcholine

Triacylglycerides (TAG):-

The depot subcutaneous fats in fishes are mostly composed of TAGs (neutral lipids consisting mainly of saturated and monounsaturated fatty acids – MUFA). When fishes are exposed to migration, they actively use the saturated fatty acids as energy source and hardly use the polar lipids which mostly comprise the phospholipids as a result; they are conservatively stored in the tissues and cell membranes (Qi, Seo, Jiang, Carpentier, & Deckelbaum, 2006).

Whilst undergoing normal swimming, rapid outbursts when escaping a predator or when chasing preys, during spawning and the development of gonads, these fishes over a short period of time feed little, a condition that is similar to that of the long migration distances (Lovern, 1956). This suggests that these fishes do not simply incorporate the total lipids of their feed fish in the same ratios, but selectively accumulate the polar lipids and actively use the saturated and monounsaturated fatty acids. There are two advantages for the selective use of the saturated and monounsaturated fatty acids, because they are dispensable for these fishes and an effective high calorie energy source as they are saturated with hydrogen (Sargent et al., 1999).

Sterols:-

Sterols (ST) were quite low in all specimens as compared to the other lipid classes. The highest percentage was observed in the bonny fish from Lake Victoria muscle (0.89 ± 0.534%) while the lowest was in the muscle of tilapia from river Tana(0.012 ± 0.007%).

Fish sterol (ST) content is usually relatively stable and ranges between 40 and 60 mg per 100 g (0.04 – 0.06%) edible fish muscle (Krzynowek, Murphy, Pariser, & Clifton, 1990).

General Observations:-

Generally, triacylglycerides (TAG) and phospholipids were present in high proportions when compared to the wax esters, diacylglycerides (DAGE) and sterols.

Worthy of noting was the high levels of free fatty acids (FFA) in the muscles. They may be a result of decomposed products of glyceride derivatives such as triacylglycerides and phospholipids created by enzymatic reactions during storage (Bligh & Scott, 1966; Lu, Ludsin, Fanslow, & Pothoven, 2008).

Wax esters (WE) may also be a major component of some species' muscles and/or livers. Wax esters are deemed most stable and are more suitable for long-term storage of energy compared to short-term energy stores such as triacylglycerides. In addition, wax esters aid in buoyancy and occur in higher amounts in some deep-water fish (Phleger, Nichols, & Virtue, 1997).

Conclusion and recommendations:-

The data confirm the suitability of several species of freshwater fish as foodstuffs, assessments of dietary intake of trace metals in areas where fish form a significant component of the diet should take this into account in both typical diets and diets of critical groups.

In addition, most of the freshwater fishes found in Kenya have relatively high total lipid content hence can be used to suppress cardiovascular diseases due to benefits associated with this fish lipids. Consequently, all fish muscle tissue contained both neutral and polar lipids mainly triacylglycerides (TAG) and phospholipids (PL). The neutral lipid classes of all the twelve fish species under study were quite low as compared with the phospholipids classes. Triacylglycerides are the storage lipids in almost all commercial fish species. More so, the beneficial effects of fish oil are attributed to their eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are polyunsaturated omega-3 fatty acids (omega-3 PUFAs) formed from alpha-linolenic acid. These fatty acids are produced by monocellular algae or phytoplankton, which represent the beginning of food chain, and accumulate in the flesh of fish. This is evident especially due to the high level of stearic acid and palmitic acid in all the four lipid classes analysed for fatty acid composition.

Based on the data provided above, Consumption of fishes with high levels of total lipids is recommended due to their nutritional and beneficial effects. However frequent monitoring of the levels of chemical pollutants such as heavy metals to ensure food safety because such metals have been shown to bioaccumulate in the aquatic organisms such as fish.

I therefore recommend further research to analyse all the fish species not analysed in this project in order to provide comprehensive information on all the fish species found in all freshwater bodies founding Kenya and the rest of the world.

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