

## Comparison of Fatty Acids Profile of Some Freshwater and Marine Fishes

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**Abstract:** The aims of this paper was to investigate the component fatty acids of some freshwater fish species and marine species as well as compare the nutritional quality of freshwater fish with that of marine fish by comparing the levels of essential fatty acids present. The FAME were determined by GC-MS and were identified using retention time locked methods and retention time databases. The fatty acids profiles include minor amounts of odd-number, branched-chain, and even-number fatty acids as well as saturated components, the MUFA and PUFA. The major SFA were C14:0 and C16:0. The C18:1 was the prominent MUFA. The dominant PUFA are of the  $\omega$ -6 series and are found chiefly in C18:2 fatty acids. The essential fatty acids compositions showed prominence in C18:3n-3 and C18:2n-6. The branched chain fatty acids identified C15:0, C16:0, C17:0, C18:2 and C20:0. The overall significance of this study had been its revelation that marine fishes have regular pattern of FA composition are better sources of  $\omega$ -3 EFA while freshwater fish are good source of  $\omega$ -6 EFA. The high percent of branched and saturated FA in freshwater fish gives them an advantage in curing processing.

**Keywords:** Fatty acids, Freshwater, Marine, composition,

### Introduction

Basically there are three major fisheries- marine, freshwater, and diadromous (involving fishes that ascend freshwater streams to spawn, like salmon, or descend from streams to reproduce in the oceans like certain eel).

Seafood resources show a distinct geographic pattern, favouring the eastern edge of oceans because of geostrophic upwelling, the high latitudes through seasonal mixing and recycling of nutrients, and the proximity of large rivers. Therefore the most important fishery centres for the marine species are the northeastern Atlantic, northern pacific, northern Atlantic, and indo-pacific. Marine ecology comprises the ecology of the oceans with their shores and estuaries. The nonliving, or abiotic, materials in a marine ecosystem cycle comprise not only a variety of water-soluble inorganic nutrient salts (nitrates, phosphates, sulphates of Ca, Na, K and dissolved gases) but also organic compounds (amino acids, vitamins, and growth substances).

Freshwater habitats are divided into a lenitic or basin series, such as lakes, reservoirs, ponds and bogs, and a lotic or channel series, such as rivers, streams, brooks, springs, and groundwater. The lotic series is distinguished by a continual flow of water in one direction. The energy of freshwater ecosystems is derived mainly from photosynthesis accompanied by the algae suspended in the water and by the higher plants and algae growing on or in the bottom. A variable proportion of the total energy available is derived from allochthonous organic matter, such as leaves and pollen, produced by terrestrial communities. The major freshwater fishing areas are Asia, Russia, Africa, and Central and Northern North America.

The existing inter and intra species variability in the composition of FA of fish lipids (and of the

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specific PUFA in particular) is usually explained by the existence of a large number of external factors (environment, culturing method, tropic effects) and internal factors (fish species, feeding regime and digestion, life cycle stage, quantitative and qualitative characteristics of lipids-triacylglycerols, phospholipids and their topographical origin- dorsal and ventral part of muscle tissue).

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

The aim of the study reported here was to determine the differences in the quantitative and qualitative compositions of FA in the amounts of saturated, monounsaturated, and polyunsaturated n-3 and n-6 FA between freshwater and marine fishes.

## Materials

### Sample Preparation

Fresh captured *Mormyridae* (*Hyperopisus bebe occidentalis*; MHO, *Mormyrops deliciosus*; MMD and *Mormyrus rume*; MMR), *Cichlidae* (*Oreochromis niloticus*; CON and *Sarotherodon galileus*; CSG), *Clariidae* (*Clarias gariepinus*; CCG and *Clarias anguillaris*; CCA), *Heterobranchus bidorsalis*; CHB, *Centropomidae* (*Lates niloticus*; CLN), *Clariheterobranchus*; CCH and *Characidae* (*Hydrocynus forskalii*; CHF) were obtained from Fishermen at the Kainji Lake Dam site. The fishes were weighed, deheaded, eviscerated and cleaned prior to freezing. In an attempt to obtain a homogeneous sample from each species, their flesh were removed from their backbones, minced, blended and immediately extracted.

The mean weights of the fishes were: MHO 250g, MMR 450g, MMD 600g, CLN 500g, CCG 417g,

CON 400g, CSG 300g, CCA 350g, CHB 250g, CCH 200g and CHF 533.33g

### Lipid Extraction

Lipid extractions were performed on minced fish samples (10g each) using the extraction methods of Folch *et al* (1957): chloroform-methanol. Methylene chloride (100 $\mu$ L) and 1 mL 0.5M NaOH in methanol were added to oil extracts in a test-tube and heated in a water bath at 90 $^{\circ}$ C for 10 min. The test tubes were removed from the water bath and allowed to cool before addition of 1 mL 14% BF<sub>3</sub> in methanol. The test tubes are heated again in a water bath for 90 $^{\circ}$ C for 10 min, and cooled to room temperature. One mL distilled water and 200-500 $\mu$ L hexane was added to the test tubes and then FAME was extracted by vigorous shaking for about 1 min. Following centrifugation, the top layer was transferred into a sample bottle for GC analysis.

### Fatty Acids Analyses

The fatty acids profiles were determined in an Agilent Gas Chromatograph, Model 6890N fitted with an Agilent Mass Selective Detector, 5973 series. Separation was carried out in a capillary column (30 x 0.25mm id x 0.25 $\mu$ m DB wax). The starting temperature was 150 $^{\circ}$ C maintained for 2minutes at a heating rate of 10  $^{\circ}$ C/minutes. The total running time was 22 minutes. Helium was the carrier gas while the injection volume was 1 $\mu$ L. The fatty acids peaks were identified using Agilent Technologies software 5988-5871EN.

## Results

Freshwater fish oils are unique in the variety of fatty acids of which they are composed and their degree of unsaturation. There are high levels of  $\omega$ -6 polyethylenic than  $\omega$ -3 polyethylenic fatty acids. The most common fatty acids presented below have an even number of carbon atoms per molecule and seldom contain functionalities other than cis and trans olefinic saturation, which usually occurs in a methylene-interrupted pattern in polyenes.

Long chain fatty acids (Table 1) are ubiquitous constituents noticed. In a given species, saturated and unsaturated fatty acids occur generally side by side, their structures varying widely in chain length and in degree of unsaturation.

## Fatty Acids Profile of Some Freshwater Fish

Table 1: Fatty acids compositions of some freshwater fish species

FATTY ACIDS	CCA	CLN	MMD	MMR	CHF	CHB	MHO	CCG	CCH	CSG	CON
4:0			*	*			*		*		
6:0	*										
10:0	*			*					*		
11:0	*					*					
12:0	*		*	*		*	*		*		
13:0	*			*		*	*				
14:0	*					*		*		*	*
14:1	*							*			
15:0	*			*		*	*	*		*	*
15:1	*			*		*	*	*	*		
16:0		*								*	*
16:1										*	*
17:0										*	*
17:1		*									
18:0	*	*						*		*	*
18:1cis		*						*		*	*
18:1trans	*	*						*			
18:2cis		*	*			*					
18:2trans		*	*				*	*	*	*	*
18:3n-3		*	*			*	*	*		*	*
18:3n-6		*	*					*			
18:4										*	*
20:0		*	*						*		
20:1n-9		*	*						*		
20:2		*	*						*		
20:3n-6		*	*					*	*		
20:3n-3		*	*			*					
20:4n-6		*	*					*	*	*	*
20:5n-3		*	*							*	*
21:0		*	*						*		
21:1						*					
22:0	*										
22:1			*	*			*				
22:2		*	*		*	*					
22:5n-3										*	*
22:6n-6										*	*
23:0	*		*		*						
24:0		*									

### Major Fatty Acids Class of Freshwater Fish

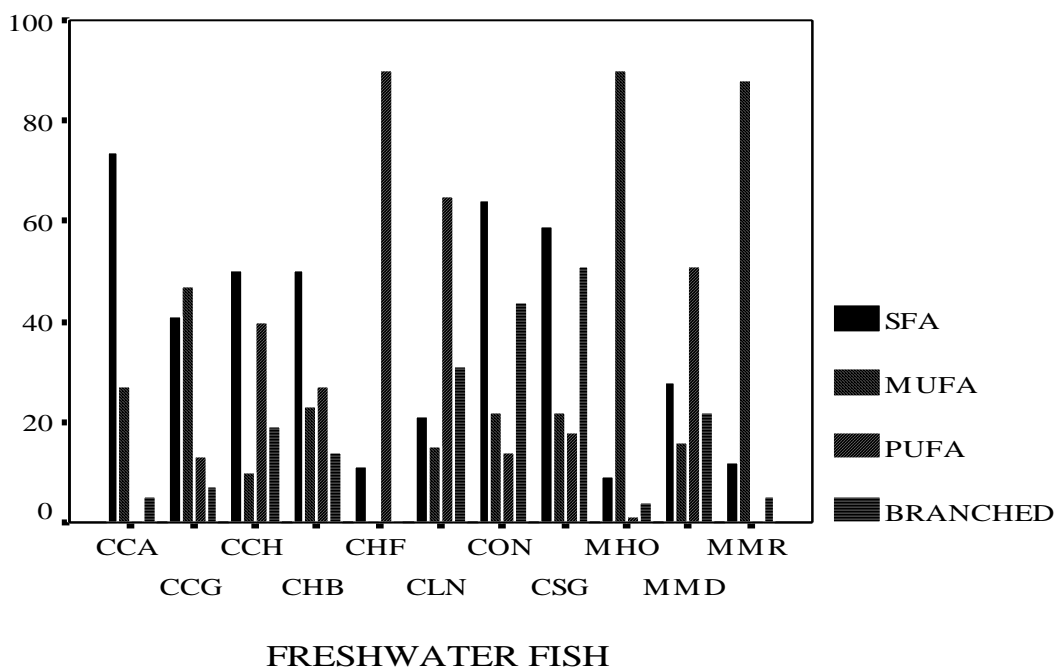


Figure 1: Major Fatty Acids Contents of Some Freshwater Fish

### The Essential Fatty Acids of Freshwater Fish

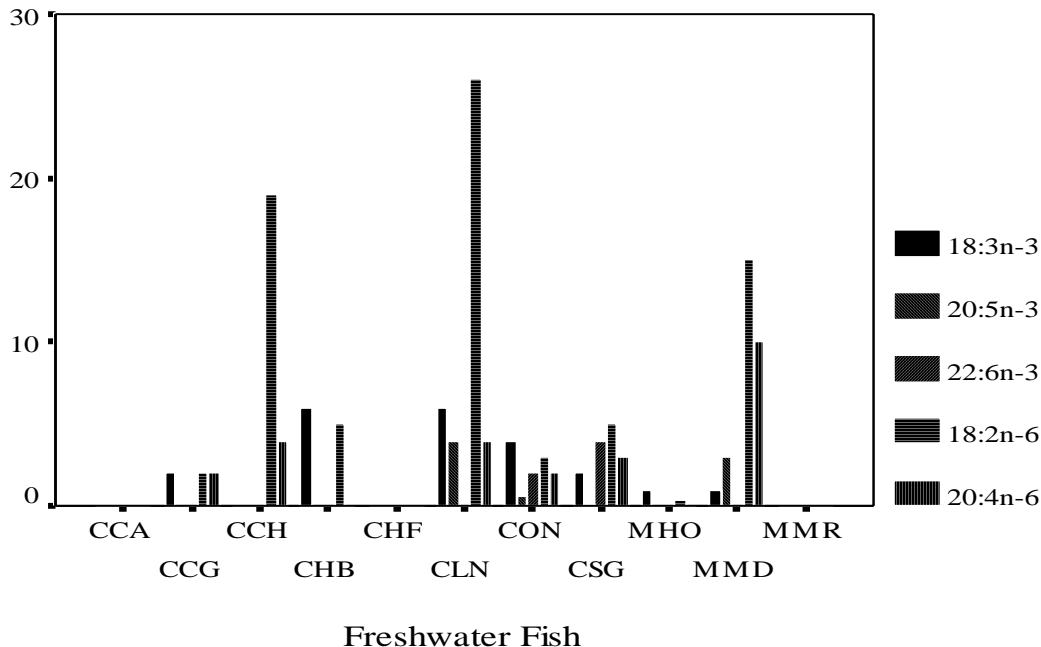


Figure 2: Essential Fatty Acids composition of Some Freshwater Fish

The values of component fatty acids were expressed in moles percent because molar composition is frequently more informative than composition by weight since it expresses the

relative number of molecules of each type of acid present in oil. This expression is very convenient for the determination of the value of each fatty acid.

## Discussion

Triglyceride mixtures from animal sources are usually complex and may contain 10-40 different fatty acids, which could form a possible 1, 000-64, 000 different triglycerides. This complexity, together with the very similar chemical and physical properties of the various molecules, makes the complete analysis of natural triglyceride mixtures extremely difficult. However, there is a huge incentive for fatty acids profile analysis and identification, although, the high dimensionality of the resultant data poses serious challenges.

Detailed information about lipid components and their fatty acids constituents is needed to understand how to diminish oxidative or hydrolytic factors which affect quality of fish. The nature, proportion, and degree of unsaturation of the fatty acids in the lipids are all closely related to the oxidation of the oils. However, the fatty acids composition of the muscle cell membranes are especially important factors in determining the stability because oxidative changes are initiated from the membrane components of muscle (Buckley *et al*, 1989).

Rancidity development is a vital concern to the food industry because it may be used for indexing and assisting in technology development.

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

### Fatty Acids Profile of Freshwater Fish

The fish oils (MHO, MMD, MMR, CON, CSG, CCG, CCA, CHB, CLN, CCH and CHF) are unique in their variety of fatty acids (Table 1) of which they are composed and their degree of unsaturation (Figure 1). The fatty acids profiles include minor amounts of odd-number, branched-chain, and even-number fatty acids. These varieties as well as the quantity and quality of fatty acids noticed are due to differences in sub-species, diet, spawning cycle, season and environment.

The saturated components ranged from 9% to 76%. Within these components the major fatty acids were C14:0 and C16:0. The mole percent of each fatty acid seems to vary. The monoene contents ranged from 10% to 90% with C18:1 the prominent

MUFA. PUFA attained the highest value (90%). The branched chain fatty acids identified are C15:0, C16:0, C17:0, C18:2 and C20:0. These fatty acids were 51%, 44% and 31% in *Sarotherodon galilaeus*, *Oreochromis niloticus* and *Lates niloticus* respectively and were the highest noticed. This high level of branched chain fatty acids in these species has an important advantage. Branched chain fatty acids influence lower melting point, lower cholesterol levels, provide energy, and form an integral part of biomembranes. Branched fatty acids because of their high temperature stability play an important role in the finished product of hydrogenated fish oils. Esterification of branched chain fatty acids to cholesterol causes the fatty acids to stimulate protein synthesis. The branched chain esters influence some ribosomal functions which are necessary for peptide elongation (Hradec *et al*, 1974).

In all the fish species analysed, the dominant PUFA are of the  $\omega$ -6 series and are found chiefly in C18:2 fatty acids. The essential fatty acids compositions showed prominence in C18:3n-3 and C18:2n-6. C22:6n-3 was noted in the tilapia species.

CCA has more of saturated fatty acids than CCG and their hybrid CCH. CCG has more MUFA while CCH is more of PUFA (Figure 1). MMR and MHO contain more of MUFA while MMD is highest in PUFA contents. The tilapia species (CSG and CON) contain more of saturated lipids but comparable amounts of MUFA, PUFA as well as  $\omega$ -3 fatty acids. *Lates niloticus* and *Hydrocynus forskali* have their lipid content being more of PUFA. *Heterobranchius bidorsalis* is about 50% of SFA.

CCA, MMR, CHF and CCH have undetectable levels of  $\omega$ -3 fatty acids while  $\omega$ -6 fatty acids were not detected in CCA, MMR, CHF, CHB, and MHO. Wild fish has low  $\omega$ -3:  $\omega$ -6 ratios. This is needed to reduce high levels of  $\omega$ -6:  $\omega$ -3 in most human diets. The essential fatty acids (figure 2) were lacking in CCA, MMR, and CHF. DHA ( $\omega$ -3) were absent in all the species except the tilapias. The tilapia species contain all the essential fatty acids although they vary in composition. The degree of unsaturation of fish oils vary with seasons. It rises as the water temperature falls and vice versa (IFFO BULLETIN No.18). 9,12-octadecdienoic acid contents in CLN and CCH as well as MMD are high in that order. The oils were characterized by low levels of  $\omega$ -3 PUFA.

## Comparison of Freshwater Fish and Marine Fish

Table 2: Fatty acids compositions of some marine fish species

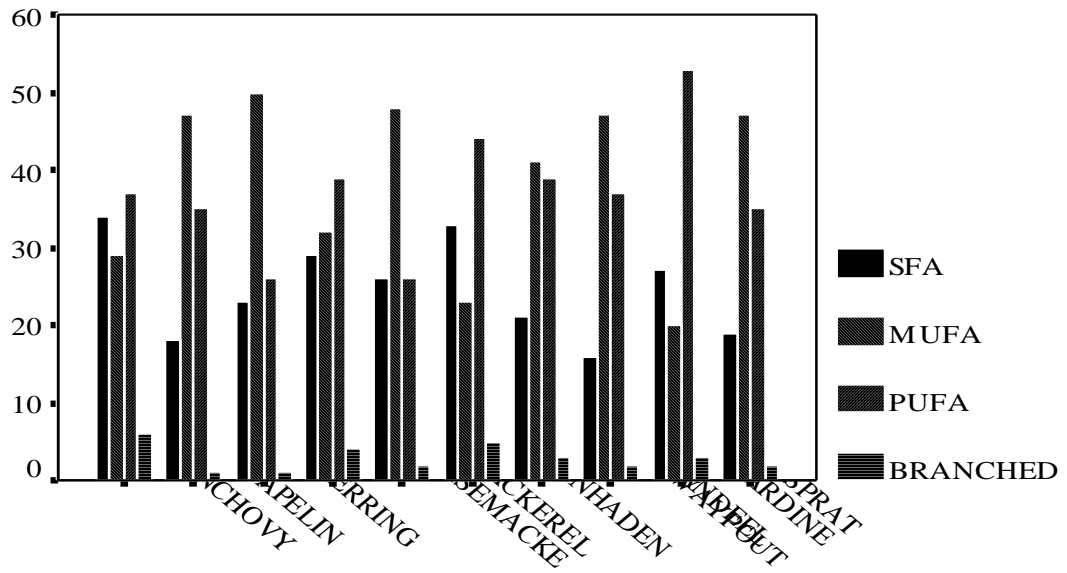
FA	Anchovy	Horse mackerel	Menhaden	Sardine	Capelin	Herring	Mackerel	Norway pout	Sand eel	Sprat
14:0	*	*	*	*	*	*	*	*		
14:1	*		*							
15:0	*	*	*							
16:0	*	*	*	*	*	*	*	*	*	*
16:1	*	*	*	*	*	*	*	*	*	*
16:2			*							
16:3		*	*							
16:4		*	*							
17:0	*	*	*							
18:0	*	*	*	*	*	*	*	*	*	*
18:1	*	*	*	*	*	*	*	*	*	*
18:2	*	*	*	*	*	*	*	*	*	*
18:3	*	*	*	*	*	*	*	*	*	*
18:4		*	*		*	*	*	*		
20:0		*		*						
20:1	*	*	*		*	*	*	*	*	*
20:2					*	*	*	*		
20:3		*								
20:4	*	*	*		*	*		*		
20:5	*	*	*	*	*	*	*	*	*	*
21:5		*	*							
22:0		*								
22:1	*	*	*	*	*	*	*	*	*	*
22:2					*	*		*		
22:4										*
22:5	*	*	*		*	*	*	*	*	*
22:6	*	*	*	*	*	*	*	*	*	*

Values courtesy of IFFO Fish oil bulletin no. 18

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

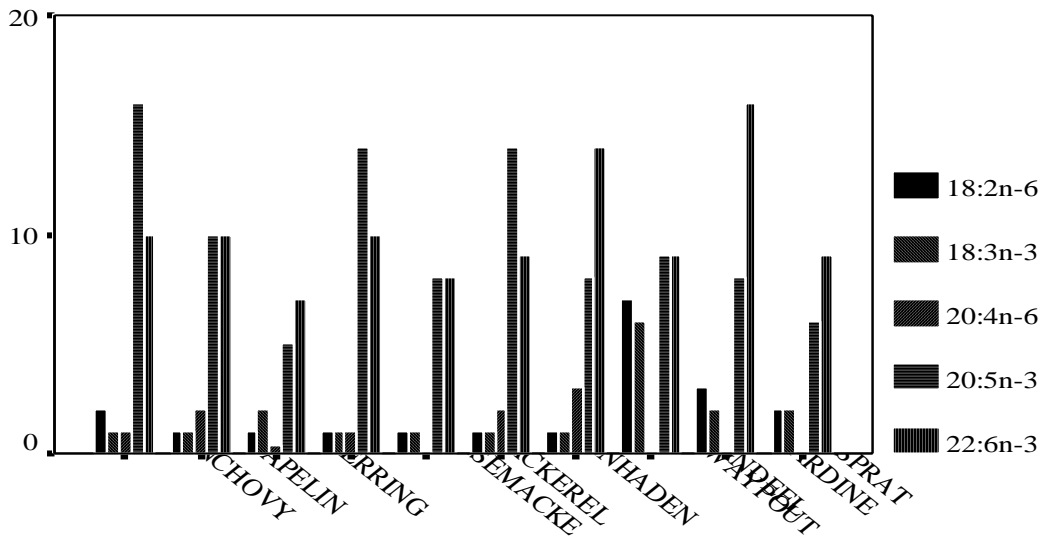
the higher level of unsaturation of the former. A study of Table 1, however, shows an irregular pattern of FA composition

Branched chain fatty acids identified in marine fish (Figure 3) are C16:0, C18:2 and C20:0 as against C15:0, C16:0, C17:0, C18:2 and C20:0 in the freshwater species (Figure 1). The content of these fatty acids in freshwater species ranged between 5-51mole% while in the marine species, it is between 14-21%. This may suggest that the freshwater fish oils may be better fats after hydrogenation.



MARINE FISH

Figure 3: Major Fatty Acids of Some Marine Fish



MARINE FISH

Figure 4: Essential Fatty Acids of Some Marine Fish

Our results revealed that freshwater fish is more of  $\omega$ -6 series of the PUFA while the marine is more of  $\omega$ -3 series (Figures 3 and 4). The prominent  $\omega$ -3

being C22:6 while the C18:2 are for the  $\omega$ -6 series. This may suggest that the dietary essential fatty acids requirements for marine fish for  $\omega$ -3 PUFA

may be higher than that of freshwater fish. The nutritional requirement of marine species for long chain PUFA-3 may not be met by 18:3n-3 due to limited capacity for chain elongation and desaturation (Cowey *et al*, 1976; Deshimaru *et al*, 1982). The PUFA-3 seems to be more potent than 18:3n-3 as sources of EFA (Takeuchi and Watanabe, 1977). However, only CLN seems to compare well with marine species on C20:5n-3 content. The marine species are better in 22:6n-3. The freshwater species are in C18:2n-6 as well as in C20:4n-6 contents. The latter fatty acid is a major constituent of membrane lipids (phospholipids) and is the principal precursor by enzymatic action of hormone-like compounds known as eicosanoids including the prostaglandins (prostanoids, isoprostanes, and isofurans). The eicosanoids produced from C20:4n-6 cause the strongest inflammatory response in humans. Inflammation is one of the body defense mechanisms that reduce the spread of infection.

#### **Fatty Acids Profile and Lipid Deterioration during Processing and Storage**

Fish oils (Tables 1 and 2) contain highly unsaturated fatty acids which are susceptible to oxidation. High percentage of unsaturated fatty acids as well as the lowered energy of activation in the initiation of free radical formation make the oils most reactive. The oils must therefore be hydrogenated or interesterified to modify such fatty acids before the oil can be used for normal commercial food purposes: salad oils, frying fats, industrial margarines and shortenings, low calorie spread to make bread, pastries, cakes, cookies, biscuits and synthetic creams. They could also be used in the production of emulsifiers for food applications. The broad spectrum of components fatty acids as well as the consequent triglycerides will make these fish oils when hydrogenated to solidify in the  $\beta'$  category. These oils will therefore influence any blends of which they form part of, and, also make the blends to crystallize in the  $\beta'$  form, thus giving the desired characteristics to the product produced (IFFO BULLETIN No. 18). Fatty acids composition is the surest method of determining the selectivity of a hydrogenation reaction. Therefore fatty acids profiles aids in determining oils suitable for the production of solid fats for industrial uses.

High contents of PUFA in CLN, MMD, CHF and CCH (Figure 2) and in the marine species (Figure 4) increase the degree of unsaturation of these fish oils and may increase their susceptibility to lipid oxidation. Such fish may not therefore be suitable for preservation through smoking, frying and sun drying except if antioxidants are used. Large or large oily fish require longer periods of processing, therefore cold smoking technique (45-60°C) could

be recommended for such fish species. Also, knowledge of the degree of unsaturation (Figures 2 and 4) aid in the careful control of the hydrogenation conditions to ensure that oxidation products responsible for the typical fishy taste and smell eliminated and a fat of satisfactory oxidative stability produced for margarines and shortenings through the elimination of 6, 5, and 4 double bond acids as well as the trienes. PUFA serve as precursors for fish flavours (Josephson, 1991). Low levels of PUFA ensure low fishy flavour development. However, some fish lack the substantial concentration of nutritionally desirable 5,8,11,14,17-eicosapentaenoic and 4,7,10,13,16,19-docosahexaenoic acids that could be in the edible muscle tissue (Figures 2 and 4).

High proportion of SFA accounts for low iodine value (Figure 1 and 3). However, substantial amount of 9,12-Octadecadienoic acid and 9-Octadecenoic acid (Figure 2 and 4) necessitates high iodine value. 9-Octadecenoic acid and 9,12-Octadecadienoic acid presences encourages lower melting point. 9,12-Octadecadienoic acid and 9,12,15-Octadecatrienoic acid content among other factors (viscosity, colour, iodine value and peroxide value) determine oil quality for industrial purposes.

Oils rich in  $\omega$ -3 PUFA (Figures 2 and 4) containing 9,12-Octadecadienoic acid and 5,8,11,14-eicosatetraenoic acid are essential fats because their constituent nutrients are not synthesized by the body but are required for tissue development. The very long PUFA found predominantly in fish and fish oil,  $\omega$ -3 fatty acids have been associated with reduced risk for coronary heart disease. However a negative health aspect of markedly increasing PUFA would be associated with increased consumption of lipid oxidation products, increased in vivo production of lipid oxidation products and depletion of tissue levels of vitamin E.

Oxidation of lipids is of economic and nutritional significance to the food industry as well as consumers. It may result in sensory changes (flavour and aroma) and loss of nutritional value (Essential fatty acids, fat-soluble vitamins: A, D, E, K), production of primary and secondary oxidation products (hydro peroxides, free radicals, epoxides, etc). These products, like most common oxidation products of cholesterol could cause atherosclerotic injury, potent inhibitors of sterol biosynthesis or be carcinogenic (Fontana *et al*, 1993).

#### **Conclusion**

The aims of this paper was to investigate the component fatty acids of some freshwater fish

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

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