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Analysis of Omega-3 Fatty Acids found in Three Selected Fish Species in NIOMR Fish Farm, Buguma, Rivers State, Nigeria

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Abstract:- The omega-3 fatty acid content (namely EPA and DHA) of *Tilapia guineensis, Sarotherodon melanotheron* and *Mullet* from NIOMR fish farm in Buguma, Rivers State, Nigeria was investigated using GC-MS. The results showed that the composition of EPA was always higher than DHA for the three fish species. The results also showed large quantities of omega-3 fatty acid in the three fish species. Thus, suggesting that the three fish species could be used as good sources of these omega-3 fatty acid.

Keywords:- *Omega-3 Fatty Acid, EPA, DHA, GC-MS, Tilapia Gineensis, Sarotherodon Melanotheron, Mullet.*

I. INTRODUCTION

African Regional Aquaculture Centre (ARAC), Buguma Station is a brackish water fish farm located in Asaritoru Local Government area of Rivers State, Nigeria. It is a research station of the Nigerian Institute for Oceanography and Marine Research (NIOMR) under the Federal Ministry of Agriculture of the Federal Republic of Nigeria. At this research station several ponds are water fed with the tidal movement from the creek. Thus, there is a constant exchange of water between ponds and creek all year round. While fish come from the wild to the ponds, water and fish movement in and out of the pond is controlled by some special screen.

In ancient times fish oil was known to have health benefits. As an example, Hippocrates (460-377BC) who is today described as the father of medicine because of hisuse of observation of clinical signs to draw rational conclusion instead of magic and religious beliefs, used dolphin oil to treat skin diseases (Adam, 1891). In Europe cod liver oil is used as remedy for rickets, tuberculosis, skin wound, joint and muscle pain (Grad, 2004). In modern time fish oil is used in several applications including food and pharmaceutical industries.This is because it is now known to reduce pain and inflammation, improve cardiovascular health, protect from stroke and heart attack, improve brain function and enhance higher intelligence, reduce depression and psychosis, reduce childhood disorder, reduce breast cancer as well as colon and prostate cancer (American Health Association (AHA), 2010).

A major fatty acid component of fish oil is omega-3 fatty acid. Omega-3 fatty acid has gained a lot attention because of its importance. Many of the earlier listed benefits are because of the presence of omega-3 fatty acid (Haglund*et al.,* 1998).It is known to lower triglycerides, reduce heart attack, stroke and abnormal heart rhythm, slow down the hardening of arteries and lower blood pressure. On the other hand, a high dose is known to increase bleeding.

The interest in omega-3 fatty acid was increased by the work of Dyerberg *et al.,* (1978). According to Dyerberg *et al.,* (1978) although Eskimos had a high fatty diet, they had a very low ischematic heart disease. The reason was said to be because of the presence of omega-3 fatty acid present in the fish oil they consumed. This had led to recommendation from health bodies for increased intake of fish oil especially, eicosapentaenoic acid (EPA, see fig.1) and docosahexaenoic acid (DHA, see fig.2).

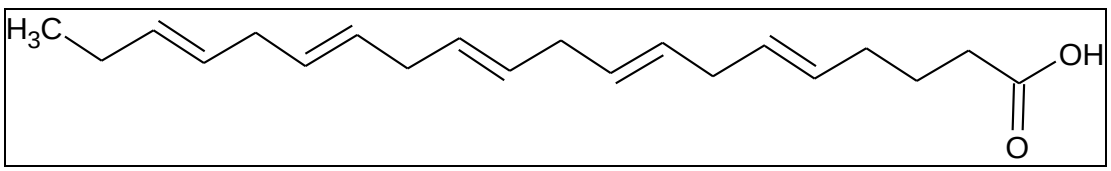

Fig. 1: EPA (C20H30O2)

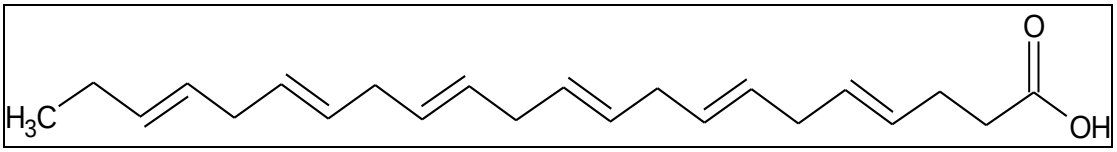

Fig. 2: DHA (C22H32O2)

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Two components of omega-3 fatty acid are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These two long chain omega-3 fatty acids are known to prevent coronary heart disease (Moghadasian, 2008).Both EPA and DHA have their last methyl group three carbons away from a double bond and are classified as essential fatty acids.Omega-3 fatty acids have 18-22 carbon atoms and can form 3-6 double bonds.EPA and DHA are said to be polyunsaturated fatty acids (PUFA)as a result and are also referred to as long-chain omega-3 fatty acids. On the other hand, alpha-linolenic acid (ALA) is referred to as short-chain omega-3 fatty acid because it has 18 carbon atoms. However, while EPA and DHA are found in fish ALA is found in plants. Chia seed and flaxseed are rich in ALA. It is well documented that ALA is formed in the choloplasts of plants.

Studies show that fish species in which these fatty acids are found do not produce these fatty acids in their bodies but accumulate these fatty acids in their bodies by eating microalgae or have preyed on other fish species that have already accumulated these omega-3 fatty acids. This is why fatty predatory fish such as shark are likely carriers of large amounts of omega-3 fatty acids.On the other hand, these predatory fish species are also large carriers of toxic organometallic compounds for example, organomercury which also accumulate in their bodies over time. Therefore, the consumption of these fatty predatory fishes must be controlled (FAO, 2020). According to Innis *et al.*, (1995), the amount of EPA and DHA found in the fish depends on whether the water is fresh or brackish.

II. MATERIALS AND METHOD

Agilent 6890N Gas Chromatographed with Agilent 5975 Mass Selective Detector was used. The materials used are auto sampler vials, 150 µL vial inserts, and crimp seals, vial crimper and decrimper, 2.5mL airtight syringe, 3mL disposable hypodermic syringe, 10 micro-liter autosampler syringe, supelco capillary column (hp-innowax, Agilent, 100 m×0.25 mm, i.d. 0.20µm) centrifuge.

III. REAGENTS

The reagents used are petroleum ether (Optima Grade), Air–Zero gradeNitrogen gas (UHP grade), n-hexane, methanol, potassium hydroxide, sulfuric acid and FAME standard (internal standard).

A. Sample Preparation

The fish was first cut into small pieces, and the fillets were then separated from the bones. The head and internal organs were removed, and the fish was washed under a running tap to remove any blood. The fillets were then dried in an oven at 70°C for 15 hours. After drying, the fillets were ground into a fine powder using a B725 warring blender and sieved through a plastic sieve to ensure the powder was homogeneous.

B. Microwave-assisted extraction process

A Binatone RTC-FA 500 microwave laboratory system was used for the extraction process. The powdered sample (10g) was mixed with ethanol (75ml) in a round-bottom flask. It was then shocked for 1 minute to allow uniformity and expansion of the powdered particles' surface area. Different extraction times (30minutes) were first examined, keeping other factors constant. Microwave power was set at 600W and temperature at 50°C. The extraction was carried out in a closed vessel with a solvent system of ethanol, and no evaporation was observed. The extraction was carried out in a closed vessel with a solvent system of ethanol, and no evaporation was observed. The extract obtained from the sample was filtered with a Whatman No1 filter paper on a Buchner funnel under a Buchi Vac P-250 pump. The residue left behind after the extraction was then removed. The filtered extract was then evaporated in a 100ml round-bottom flask using a SHATI Rotavapor R-2000 rotary evaporator at 35 °C to remove excess solvent.

C. GC-MS Analysis

The lipids were extracted and converted to FAMEs using a process known as transesterification. The FAMEs were then analyzed by gas chromatography-mass spectrometry (GC-MS). The GC-MS system consisted of an Agilent 6890 gas chromatograph with a 5973 mass spectrometer detector and a 60 m x 0.25 mm i.d. 0.25 µm/MS DB-WAX capillary column. The temperature program was as follows: the initial oven temperature was 200°C.The initial oven temperature was 200°C was held for 1 minute, and then increased to 230°C at 1.5°C min-1, and finally held for 10 minutes. The injector was set at 250°C, and the detector at 280°C. Nitrogen was used as the carrier gas at a flow rate of 1mL min-1. The split ratio was 50:1, and the sample size was 1µL. Peaks were identified by comparison with a standard mixture of FAMEs and by interpretation of their mass to charge ratio.

IV. RESULTS AND DISCUSSION

The results obtained are presented below:

Table 1: Omega-3 fatty acid percentage composition of the fish species

Omega-3 fatty acid	**Fish Species % Composition**		
	T. guineensis	*S. melanotheron*	*Mullet*
EPA	48.51	52.17	42.77
DHA	31.68	33.53	24.82

The chromatograms obtained from the analysis of the fish species are presented in figures 3, 4 and 5.

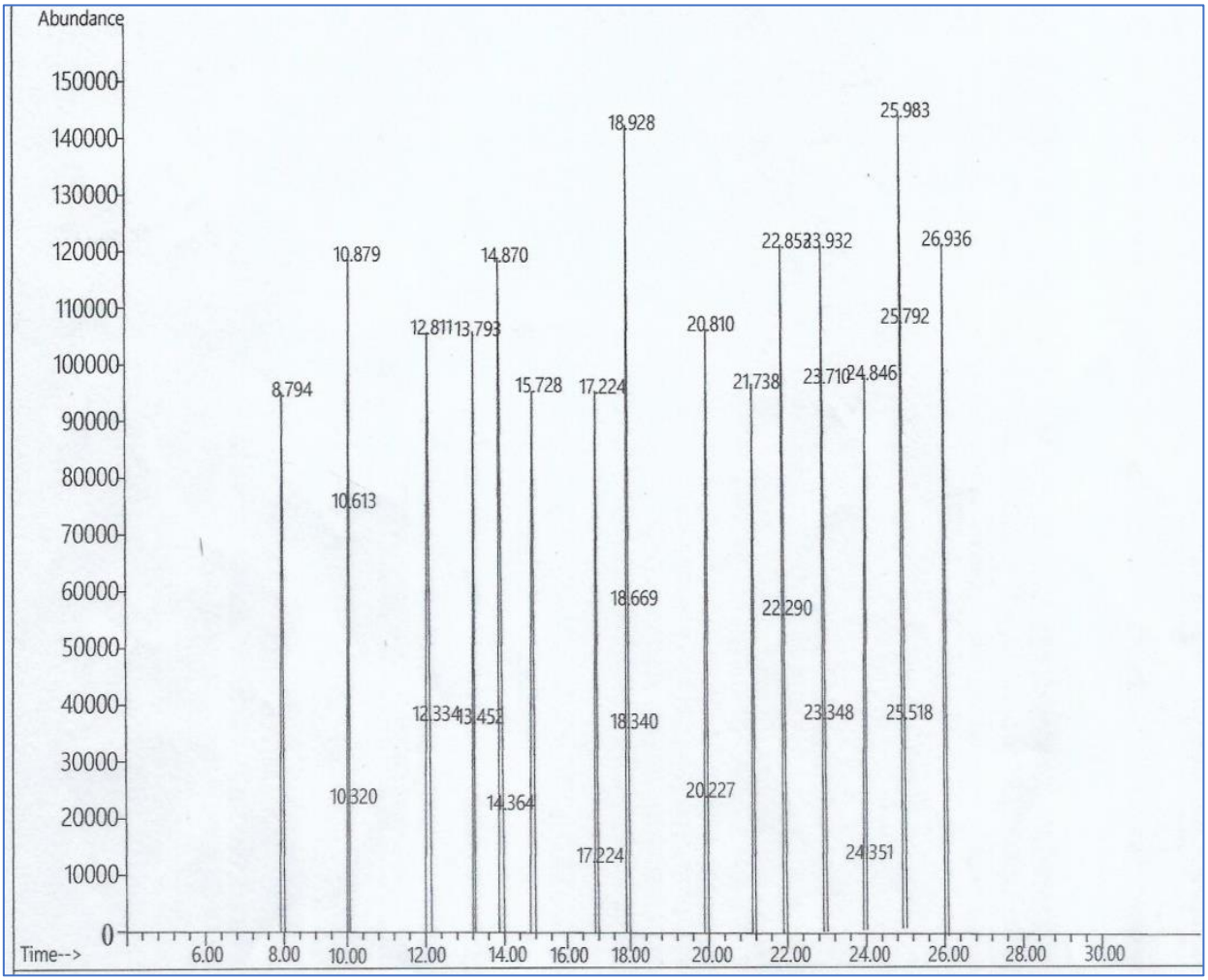

Fig. 3: Chromatogram of Tilapia guineensisGC-MS analysis

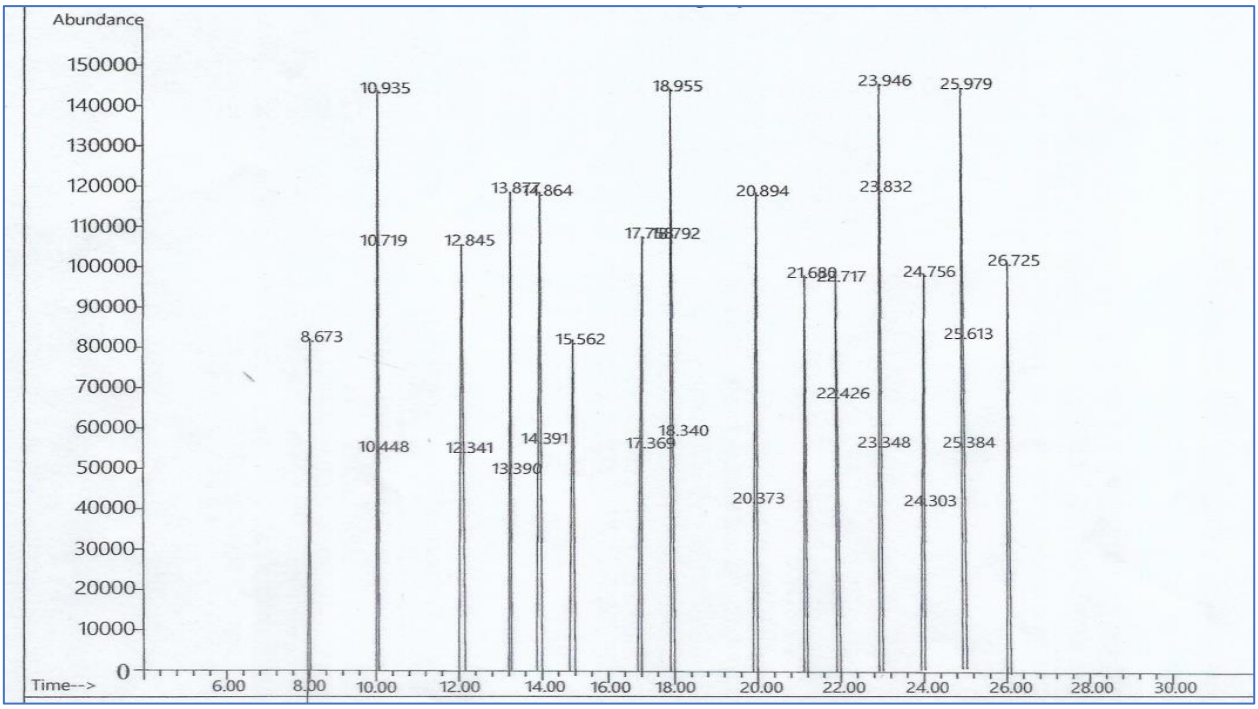

Fig. 4: Chromatogram of *Sarotherodon melanotheron* GC-MS analysis

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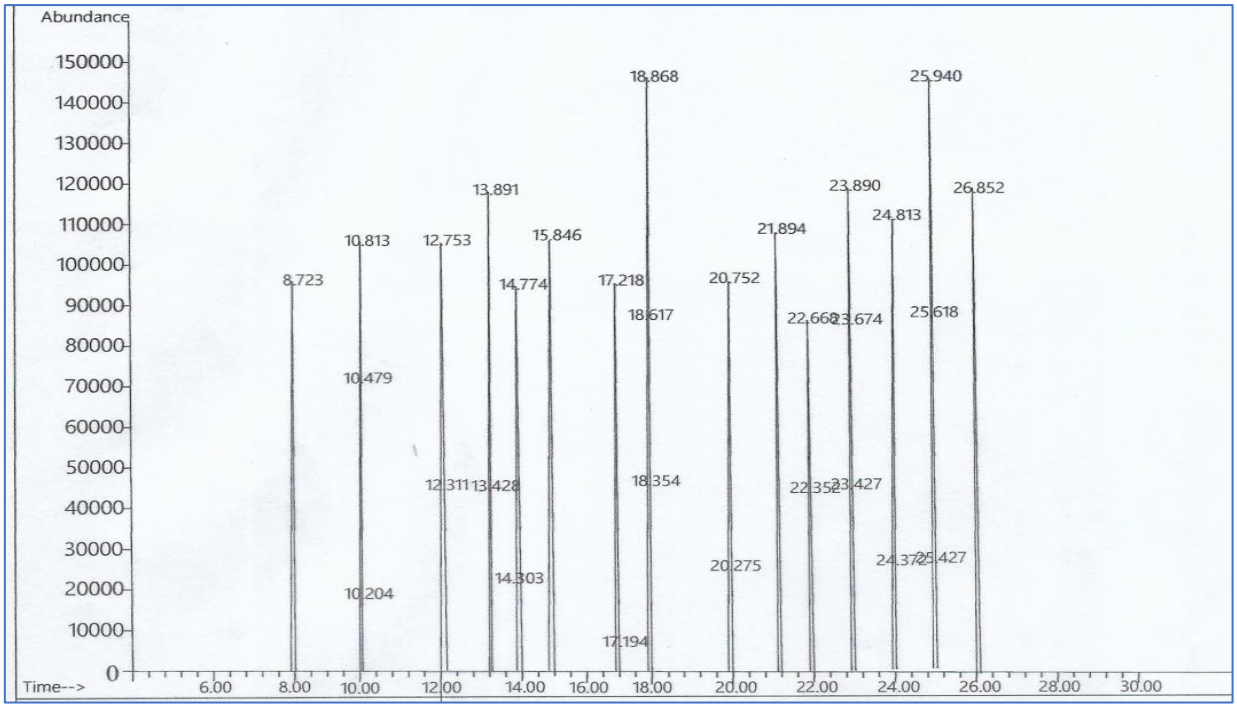

Fig. 5: Chromatogram of Mullet GC-MS analysis

From the results obtained in Table 1, the percentage composition of EPA was always higher than that of the DHA for each of the fish species. This corroborates the findings of Julius (2019).Furthermore, the composition of both EPA and DHA for the three fish species were high. Thus, suggesting that these three fish species could be used as sources of these very important omega-3 fatty acid.

V. CONCLUSION

The results show that the composition of EPA was always higher than DHA for the three fish species. The results also showed large quantities of omega-3 fatty acid in the three fish species. Thus, suggesting that the three fish species could be used as good sources of these omega-3 fatty acid.

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