

A COMPARATIVE ANALYSIS ON THE FATTY ACID PROFILE OF *Clarias gariepinus* OIL AND COD LIVER OIL

¹ONYECHE V. O & ²OKAKA A. N.C

¹ Aquaculture and Biotechnology, National Institute for Freshwater Fisheries Research, New-Bussa

² Department of Biochemistry, Nnamdi Azikiwe University, Awka.

Email: Vinpella02@rocketmail.com or vinpella02@gmail.com 08035071987

ABSTRACT

Research has shown that polyunsaturated fatty acids are found in fish oil. In Nigeria, majority of omega-3 oil sold are imported due to limited information on the health benefits of freshwater fish oils such as those from *Clarias gariepinus*. Hence oil from *C. gariepinus* was extracted via soxhlet extraction apparatus using petroleum ether as an extracting solvent. The fatty acid profiles of the extracted oil from *C. gariepinus* and that from commercially available Cod liver oil were assayed by gas chromatography. From the results, cod liver oil had a total saturated fatty acid concentration of 7.93 (conc.), while *C. gariepinus* oil was 24.50 (conc.). The polyunsaturated fatty acid concentration detected in *C. gariepinus* oil was 9.06 (conc.) while cod liver oil had 9.20 polyunsaturated fatty acid concentration. Omega 6 fatty acid was detected in *C. gariepinus* oil (0.6116 conc.) whereas none was present in the cod liver oil. The omega 3 fatty acid made up 88.26% of the total unsaturated fatty acid in cod liver oil, and 69% in *C. gariepinus* oil. This study showed that though Cod liver oil has a high concentration of polyunsaturated fatty acid, *C. gariepinus* is also a good source of polyunsaturated fatty acid.

Keywords: Fish oil, omega 3, omega 6.

INTRODUCTION:

Fatty acids are classified according to their degree of saturation – saturated and unsaturated. This classification is based on the number of bonds in the carbon chain. The Saturated Fatty Acid (SFA) contains only a single bond and is chemically the least reactive of the classes while the Unsaturated Fatty Acid (UFA) contains one or more carbon-to-carbon double bonds. Palmitoleic, oleic, linolenic, eicosapentaenoic and docosahexaenoic acids are examples of unsaturated fatty acid which are more predominant in fish oil (Mozaffarian and Rimm, 2006). Fish get their high omega-3 content from eating algae and factors such as the environment (cultured or wild) they are exposed to (Mozaffarian and Rimm, 2006).

Clarias gariepinus, popularly known as Mud Catfish, belongs to the family of the Clariidae and is one of the most cultured and consumed freshwater fishes in Nigeria (Asuwaju *et al.*, 2014).

It is very popular in Nigeria due to its culture characteristic, which has endeared it to many fish farmers (Asuwaju *et al.*, 2014). Cultured *C. gariepinus* contains more omega-6 polyunsaturated fatty acid than the wild one; whereas its' total omega-3 PUFA are lower (Taiwo *et al.*, 2014). These PUFAs may include, Omega-3, 6 and 9. Intensive studies on omega-3, 6, 9 and cholesterol content as well as fatty acids profile of some sea water and freshwater fish had been carried out by some researchers (Ugoala *et al.*, 2008; Adeniyi *et al.*, 2012; Sayad *et al.*, 2016).

It has been established that the marine fish such as Cod and mackerel contain more omega 3

fatty acid than the freshwater fish (Adeniyi *et al.*, 2012). There are several documentations on the health benefits of oil extracted from marine fishes with very little exploitation and attention of the potential benefits of fatty freshwater fishes such as *C. gariepinus* (catfish), which is accessible to Nigerians due to the availability of natural and artificial lakes, streams, rivers and even aquaculture fish ponds. The objectives were to determine the physiochemical characteristics and fatty acid constituent of *C. gariepinus* oil and compare the polyunsaturated fatty acid contents of the crude *C. gariepinus* oil to the commercially available Cod liver oil.

MATERIALS AND METHODS:

Collection of Fish

A commercial available Cod liver oil (seven seas product) and Six (6) specimens of *Claris gariepinus* weighing 2000 g each were purchased from a the market and commercial fish farm respectively in Asaba, Delta State. They were carried to the National Institute for Freshwater Fisheries Research (NIFFR) station, Asaba for identification and finally transported to the Delta State University Faculty Research Laboratory, Asaba Campus.

Oil Extraction Using Soxhlet Apparatus

Principles:

Extraction of oil from a solid sample using a solvent via the reflux cycle upon heating on fire.

Procedure: The fishes were cut into bits using a knife, and then washed to remove impurities, and

oven dried (at 105 °C for 48 hours) to remove moist. Oil from the fish was extracted using soxhlet extractor (according to the methods of Folch *et al.*, 1957). The solid sample of the fish was placed into porous timbles with cottonwoods. The extraction was carried out at 70 °C using petroleum ether as extracting solvent for 7 hours, then the solvent was recollected out of the oil. The crude oil was placed in a rotary vacuum evaporator (at 40 °C) allowing the solvent to evaporate to dryness. The resulting crude oil was then stored in the refrigerator to prevent oxidation and rancidity.

Fatty Acid Analysis by Gas Chromatography:

Principles: Fats are converted into corresponding methyl esters by transesterification with methanol, using boron trifluoride - methanol complex (BF₃-MeOH) as catalyst AOAC (2005).

Procedure: The gas chromatography was carried out according to the standard method of AOAC (2005). The result was calculated as both the weight percentage (FA profile) and Conc. (mg/g dry tissue).

Oil Characterization:

The physical and chemical characterization of the crude oil extracted from the *Clarias gariepinus* was assayed for, according to AOAC (2005) methods.

RESULTS:

Table 1: Chemical characterization of crude *C. gariepinus* oil

Physiochemical properties	<i>C. gariepinus</i> oil	Standard value (by ISO, 1988)
Color	Reddish-brown	
Acid value (mg/KOH)	2.8	0.40-4.8
% Free fatty Acid	1.4	1-7
Iodine value (I ₂ /100g)	163.7	135-190
Peroxide value (mEq.)	22	3-20
Saponification value (mg/KOH)	118.93	165-195
Ester value (mg/KOH)	90.93	-
Percentage glycerin (%g)	4.97	-
Density (gcm ³)	0.93	-
Specific gravity (g/ml)	0.875	0.907-0.915
Refractive index (RI)	1.4642	1.473-1400

In Table 1 above, it was observed that some of the results obtained were tolerable to the standard values given by the International Standard Organization .

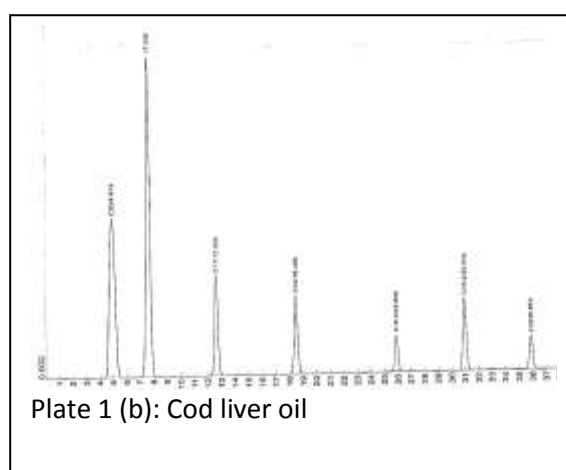
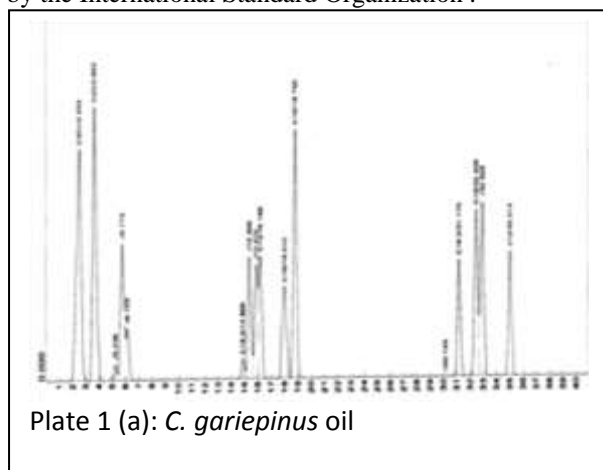


Plate 1 : Fatty acid chromatogram for *C. gariepinus* and Cod liver oil GC result.

Plate 1 show the chromatogram for the fatty acid gas chromatography result of *C. gariepinus* oil (plate 1a) and cod liver oil (plate 1b). Sixteen (16) fatty acids were detected in *C. gariepinus* oil by the chromatogram while seven (7) fatty acids were detected by the chromatogram for cod liver oil.

Table 2: Fatty acid values of *C. gariepinus* oil

Carbon	Common name	Type	Conc. (PPM)	(%) Conc.
C30:0	Melissic Acid	SFA	8.9704	24.378
C18:0	Stearic Acid	SFA	5.0261	13.659
C12:0	Lauric Acid	SFA	3.5212	9.569
C14:0	Myristic Acid	SFA	3.4025	9.246
C16:0	Palmitic Acid	SFA	3.5471	9.639
C16:2	Palmitoleic Acid	n-9 (MUFA)	3.2748	8.899
C20:0	Eicosapentaenoic acid	n-3 (PUFA)	8.4429	22.944
C18:2	Linoleic acid	n-6 (PUFA)	0.6116	1.662
TFA			36.7966	

Where SFA= Saturated fatty acid, MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid and TFA = Total fatty acid.

Table 2 above shows the identified (8) fatty acids and their concentration in *C. gariepinus* oil.

Table 3: Fatty acid values of the COD liver oil

Carbon	Common name	Type	Conc. (PPM)	(%)Conc.
C20:0	Eicosapentaenoic acid	n-3 (PUFA)	9.1963	52.297
C17:0	Margaric Acid	SFA	3.4081	19.379
C16:0	Palmitic Acid	SFA	3.4081	15.067
C16:2	Palmitoleic Acid	n-7 (MUFA)	1.2231	6.9550
C12:0	Lauric Acid	SFA	1.1085	6.3034
TFA			17.5857	

Where SFA= Saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid and TFA = total fatty acid.

Table 3 above shows the identified (5) fatty acids and their concentration in the cod liver oil.

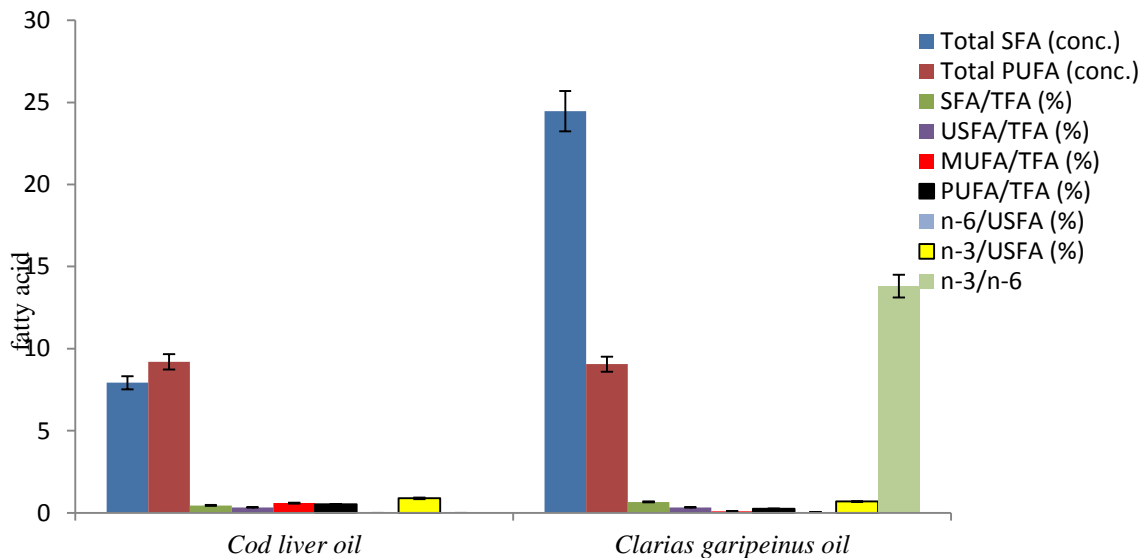


Fig.1 Fatty acid Profile of oil samples

Fig. 1 shows the fatty acid profile of cod liver oil and *C. gariepinus* oil. With *C. gariepinus* having a higher saturated fatty acid than Cod liver oil.

DISCUSSION:

To ascertain the quality and identification of the oil, it was necessary to carry out the physiochemical characterization on the extracted *C. gariepinus* oil. The results of the oil (*C. gariepinus*) characterization

in Table 1 show that the percentage free fatty acid is 1.4% which is below standard value of 2 % to 5% for fish oil. The content of FFA in *C. gariepinus* oil might have been affected by extraction process parameters such as temperature, and oxygen exposure. Idun-Acquah *et al.*, (2016) reported 0.21% free fatty acid in *Lutjanus fulgens* before frying and its free fatty acid became 2.4% after frying.

Iodine value (IV) measures the degree of unsaturation of fatty acids in fats and oils. It is also an essential mineral that when inadequate or unavailable causes goiter. Molla and Asaduzzaman (2016) reported 10.85 (mg I/g oil) iodine value and 13.95mg KOH/g acid value in Shoul fish oil. Norziah *et al.* (2009) had 23.7mg KOH/g acid value in fish oil. The acid value (2.8mg/KOH) and iodine value (163.7I₂/ 100 of sample) were within the standard value for fish oil (0.40-4.8 mg/KOH and 135 – 190 I₂/ 100g respectively) suggested by the International Standard Organization (1988). The high iodine value obtained in this study agrees with the findings of Abdulkadir *et al.* (2010) who had IV of 187.11 (I₂/ 100 of sample) in fish oil.

Peroxide is a chemical compound that contains oxygen atoms in the group -O². Bimbo (1998) reported that peroxide value (PV) of crude fish oil was between 3 and 20 meq/kg. Norziah *et al.* (2009) recorded 9.9 meq/kg peroxide value in fish oil. Molla and Asaduzzaman (2016) reported peroxide value to be 7.91 (meq O₂/Kg) in Shoul fish oil. In this study, the PV was found to be 22 meq/kg, which is slightly above acceptable limit of 20 meq/kg. This is an indication that the oil from *C. gariepinus* has a high lipid oxidation rate. Several factors such as exposure to oxygen, heat, and light can accelerate the oxidative processes in oils.

Saponification is the breaking down of neutral fat into glycerol and fatty acids by alkali treatment. Norziah *et al.* (2009) reported 295.4 mg KOH/g saponification value in fish oil. Molla and Asaduzzaman (2016) reported 146.94 mg KOH/g in Shoul fish oil. The saponification value of *C. gariepinus* oil was 118.93 (mg/KOH). This is below the standard value range for fish oil (165-195 mg/KOH) given by the International Standard Organization (1988). The low saponification value might be as a result of the relatively few number of carboxylic functional groups in the long-chain fatty acids found in the oil. The refractive index of 1.4642 was obtained. Nonetheless, the acceptability limit for refractive index is 1.4 - 1.473 (International Standard Organization, 1988). The refractive index did not exceed 1.473 in this study. This agrees with Oladapo and Awojide (2015) who obtained specific gravity and refractive index of 0.854 g/ml and 1.457 respectively in catfish oil.

Twenty seven fatty acids were obtained from the muscles of *C. gariepinus* oil by Osibona *et al.* (2006) while thirteen fatty acids were identified in *C. gariepinus* oil by Sayem *et al.*, (2016). Effiong

and Fakunle (2012) obtained twenty fatty acids in *C. gariepinus* oil and out of which five were identified as lauric acid (37.24), palmitic acid (12.86), stearic acid (1.332), oleic acid (6.04) and linoleic acid (13.52). Although the concentrations of fatty acids observed by Effiong and Fakunle (2012) vary with this present study, it might be as a result of multiple factors such as the size of the fish and the part where the oil was extracted from. As in this case, the oil was extracted from both visceral and muscle tissues while Effiong and Fakunle (2012) was from the visceral only. A total number of 16 fatty acids were detected in *C. gariepinus* oil by gas chromatography (Plate 1a). Eight of which were identified as melissic acid (C30:0), stearic acid (C18:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:2), eicosapentaenoic acid (C20:0) and linoleic acid (C18:2) at a concentration of 8.97ppm, 5.03ppm, 3.52ppm, 3.40ppm, 3.55ppm, 3.28ppm, 8.44ppm, and 0.61ppm respectively. The presence of these fatty acids agrees with reports of Osibona *et al.* (2006), Effiong and Fakunle (2012) and Sayem *et al.* (2016).

A total of 7 fatty acids were detected in Cod liver oil by gas chromatography (Plate 1b) and 5 fatty acids were identified; they include, eicosapentaenoic acid (C20), margaric acid (C17:0), palmitoleic acid (C16:2), palmitic acid (C16:0) and lauric acid (C12:0) at a concentration of 9.20 ppm, 3.41 ppm, 3.41 ppm, 1.22 ppm and 1.11 ppm respectively. Oladapo and Awojide (2015) reported the presence of myristic, palmitic, stearic, oleic, linoleic and linolenic acids in commercial cod liver oil. The comparative analysis on the fatty acid profile of *C. gariepinus* oil and Cod liver (fig. 1), adduced that *C. gariepinus* may be considered as a very good source of polyunsaturated fatty acids (9.06) although Cod liver oil had a total PUFA concentration of 9.20. The high levels of PUFA in *C. gariepinus* oil, adduce that it is very susceptible to oxidative deterioration at varying velocities, strongly depending on the storage conditions and fatty acid profile. Palmitoleic Acid (MUFA) was detected in *C. gariepinus* oil. It helps reduce the risk of cardiovascular diseases and stroke by increasing high density lipoprotein cholesterol (HDLC) and decreases low density lipoprotein (LDLC), which causes heart attack and stroke in humans (Sayad *et al.*, 2016).

Oladapo and Awojide (2015) reported a total unsaturated fatty acid of 68.06% and 70.8% in cod liver oil and catfish oil respectively. In this study, the total concentration of unsaturated fatty acid is 10.42 (59.24%) in cod liver oil and 12.33 (33.51%) in *C. gariepinus* oil. Oladapo and Awojide (2015) reported 24.30% and 24.56% total saturated fatty acid in cod liver oil and catfish oil respectively, whereas in this study, the concentration of total saturated fatty acids obtained were 7.93 (45.06%) and 24.5 (66.49%) in cod liver oil and *C. gariepinus*

oil respectively. Margaric acid was the only saturated fatty present in the Cod liver oil and absent in *C. gariepinus* oil, while *C. gariepinus* oil had omega 6 (Linoleic acid) present, but was none detected in Cod liver oil. Linoleic acid synthesises DGLA (Dihomo- γ -linoleic acid) which reduces inflammation but DGLA is broken-down to AA (arachidonic acid) which promotes inflammation, hence too much has been shown to lead to cancer, asthma and depression (Eguchi *et al.*, 2011). The 66.49% SFA and 8.90% obtained in this study vary greatly from the 38.86% SFA and 42.83% MUFA in cultured *C. gariepinus* oil reported by Taiwo (2014).

The n-6/n-3 ratio in phospholipids modulates the balance between prostanoids derived from AA and EPA (Sayad *et al.*, 2016). In this study, omega-6 was not detected in cod liver oil while the oil from *C. gariepinus* had a ratio of 0.07:1 omega-6/omega-3. The balance between omega-6 and omega-3 in biological membranes is regulated based on dietary supply. According to nutrition experts, a diet composed of omega-6/omega-3 in a ratio of less than 5:1 is highly recommended (Waleed *et al.*, 2017).

CONCLUSION:

Although the fatty acid constituent of fishes are as a result of their environment and the food consumed, *Clarias gariepinus* oil is as rich in unsaturated fatty acid contents as Cod liver oil and if the crude *C. gariepinus* oil is purified and processed, it can serve similar function in terms of dietary supplements as the Cod liver oil.

REFERENCES:

Abdulkadir, M., Abubakar, G.I and Mohammed, A. (2010). Production and characterization of oil from fishes. *Journal of Engineering and Applied Sciences*, **5** (7), 1-5.

Adeniyi, S., Orjiekwe, C., Ehiagbonare, J. and Josiah, S. (2012). Nutritional composition of three different fishes (*C. gariepinus*, *M. electricus* and *T. guineensis*). *Pakistan Journal of Nutrition*, **11** (9), 793-797.

AOAC (2005). Official methods of analysis, Association of Official Analytical Chemists (15th ed.). *Macmillan publisher company U.K.*

Asuwaju F.P, Onyeché V.O, Ogbuebunu K.E, Moradun H.F and Robert E.A (2014). Effect of feeding frequency on growth and survival rate of *C. gariepinus* fingerlings reared in plastic bowls. *Journal of Fisheries and Aquatic Science*, DOI: 10.3923.

Bimbo AP, (1998). Guidelines for characterizing food-grade fish oils. *Inform*, **9**, 473-483.

Effiong, B. and Fakunle, O. (2012). Fatty acid composition of catfish (*Clarias gariepinus*) viscera oil. *Journal of Fisheries and Aquatic Science*, 1-5.

Eguchi R., Scarmagnani F., Cunha C., Souza G., Pisani L., Ribeiro E., Nascimento C., Spadari-Bratfisch R., and Oyama L. (2011). Fish oil prevents glucose intolerance and hypercorticoesteronemia in foot shock-stressed rats. *Lipids in Health and Disease*, **10**, 71.

Folch, J., Lees, M. and Sloane S. H., (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, **226**, 497-509.

Idun-Acquah N., Obeng G.Y. and Mensah E. (2016). Repetitive Use of Vegetable Cooking Oil and Effects on Physico-Chemical Properties – Case of Frying with Redfish (*Lutjanus fulgens*). *Science and Technology*, **6**(1), 8-14.

International Standard Organization (1988). Animal and vegetable fats and oils; determination of saponification value. *International Standard Organization*, **3657** (2), 1-2.

Molla R., and Asaduzzaman A. K. M. (2016). Nutritional status, characterization and fatty acid composition of oil and lecithin isolated from freshwater fish Shoul (*Channa striata*). *International Journal of Nutrition and Food Sciences*, **5**(1), 9-15.

Mozaffarian, D. and Rimm E.B., (2006). Fish intake, Contaminants and Human health-evaluating the risk and benefits. *Journal of the American medical association*, **295**, 1885-1899.

Norziah M.H., Nuraini, J. and Lee, K.Y. (2009). Studies on the extraction and characterization of fish oil from wastes of sea food processing industry. *Asian Journal of Food and Agro-Industry*, **2** (4), 959-973.

Oladapo A.O. and Awojide S.H (2015). Quality evaluation of oil extracted from catfish and mackerel as compared with commercial cod liver oil. *Journal of Food Chemistry and Nutrition*, **3** (1), 13-1.

Osibona A.O, Kusemiju K. and Akande G.R. (2006). Proximate composition and fatty acids profile of African Catfish. *Journal of Ife and physical sciences*, **3**, 1-5.

Sayad, M.D., Hasanul, K., Aninda, C., Ruhul A., and Sohel H. (2016). Investigation of nutritional status of the butter catfish *Ompok bimaculatus*: An Important Freshwater Fish Species in the Diet of Common Bangladeshi People. *International Journal of Nutrition and Food Sciences*, **5** (1), 62-67.

Sayem, A., Habib A., and Sarkar P. (2016). Extraction and identification of PUFA from African Catfish (*Clarias gariepinus*) Skin. *International Journal of*

- Fisheries and Aquatic Studies*, **4(4)**, 312-314.
- Taiwo, O.E., Usman, K., Ogono, T.H. and Osoniyi, R.O. (2014). Proximate and Lipid Profile Analysis of Cultured and Wild African Catfish, *C. gariepinus* (BURCHELL), *Ife Journal of Science*, **16**, 1.
- Ugoala, C., Ndukwe G. and Audu T. (2008). Comparison of fatty acids profile of some freshwater and marine fishes. *Journal of Food Safety*, **10**, 9-17.
- Waleed A. K., Hu C., Nadeem K., Amjad I., Shan-Wu L., and Farooq S. (2017). Bioengineered Plants Can Be a Useful Source of Omega-3 Fatty Acids, *Biomedical Research International*, **1**, 9.