



Comparison of the Oil Composition of *Clarias gariepinus* Collected from Four Lagoons in Lagos, South Western Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Authors AAB and OSM designed the study, managed the analyses and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJACR/2019/v4i430119

Editor(s):

(1) Dr. Endang Tri Wahyuni, Professor, Department of Chemistry, Gadjah Mada University, Indonesia.

Reviewers:

(1) Alok Nahata, Malaysia.

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(3) Ningappa M. Rolli, BLDEA's Degree College, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/54063>

Original Research Article

Received 17 November 2019

Accepted 22 January 2020

Published 07 February 2020

ABSTRACT

Oil composition of *Clarias gariepinus* collected from various locations were studied and compared. Oils contained in the fishes were extracted using Soxhlet extraction method. The physiochemical properties of the oils were determined using official methods of analysis while the fatty acid composition was analysed using Gas Chromatography- Mass Spectrophotometer. The functional groups present in the oils were also detected using Fourier Transform Infra-red Spectroscopy (FTIR). The oil content for the fishes was in the range of 30.65%-40.57%. The oil extracted from *C. gariepinus* collected from Badagry lagoon had the highest peroxide and iodine values (5.12 mg KOH/g and 129 mgI₂/100 g). The fatty acid composition shows that the oils contains large number of essential polyunsaturated fatty acids except for the oil extracted from *C. gariepinus* collected from Ikorodu lagoon that contains large number of monounsaturated fatty acids. The FTIR spectra show the presence of carboxylic acid, methylene, esters, ketone and alcohol functional group. It was deduced from this study that habitat had strong impact on the oil composition of *C. gariepinus*.

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Keywords: *Clarias gariepinus*; location; monounsaturated; polyunsaturated; functional group; habitat.

1. INTRODUCTION

Fish oils are majorly composed of natural bioactive lipids which are commercially used in pharmaceutical and food industries [1]. Fish oil remains the most valuable oil due to its quality and the various health benefits [2]. Fish oil contains majorly polyunsaturated fatty acids and essential fatty acids that cannot be synthesized by the body but can be obtained through diet. Omega-3 fatty acids such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are the most prominent polyunsaturated fatty acids present in fish oil [3]. These fatty acids lower serum triglycerides, prevents cardiovascular diseases, improve learning ability and regulates blood coagulation [4,5]. They secrete eicosanoid which is involved in several metabolic processes of the human body [6].

Clarias gariepinus are readily recognized by their cylindrical body with scale less skin, flattened burning head, small eyes, elongated spineless dorsal fin and four pairs of barbels around a broad mouth. The upper surface of the head is coarsely granulated in adult fishes but smooth in young fishes [7]. *Clarias gariepinus* is indigenous to the inland water of Africa. It is a hardy fish that can be stocked in low oxygen waters making it ideal for culture in areas with limited water supply [8]. They are known to feed on insects, plankton, snails, crabs, shrimp, dead animals, birds, reptiles, amphibians, small mammals, plant matters and other invertebrates [9]. This species is one of the most widely consumed freshwater fish in Nigeria due to its large acceptability [10]. The oil composition of fish varies with species, temperature, salinity, season, size, age, life stage, the type of food they consume and their habitat [11,12]. The aim of this research work is to compare the oil composition of *Clarias gariepinus* collected from Badagry, Ikorodu, Lekki and Epe Lagoon of Lagos State, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Live *C. gariepinus* fishes were collected from landing sites of four different lagoons in Lagos State Nigeria: Badagry lagoon (Longitude 3°0', 3°45'E; Latitudes 6°25', 6°30'N), Ikorodu (Longitude 3°28'29", 4242"E; Latitude 6°33'12", 6792"N) lagoon, Lekki lagoon

(Longitude 4°07'58"E; Latitude 6°30'36"N) and Epe lagoon (Longitude 3°30', 4°05'E; Latitude 6°29', 6°38'N). After collection, samples were transported to the laboratory in an insulated ice box. All the fish samples were beheaded, gutted, washed, cut into pieces to reduce the sizes and dried at 50°C using digital electric oven (J- type) to constant weight. The dried fish samples were pulverized into powdery form using Philips electric blender (HR2118/01 2L/600W) for easy extraction of oil.

2.2 Extraction of Oil

The fish oils were extracted according to the method of [13] with some modifications. Pulverized samples (5 g) each were extracted in a thimble of the Soxhlet apparatus with 200 mL n-hexane in triplicate for 6hrs at 65°C. The mixture was concentrated to remove the n-hexane used using evaporator (Heidolph, Germany). The extracted oils were further air-dried to remove residual solvent vapour and measured. The oil yields were calculated as follows:

$$\text{Oil yield} = \frac{\text{weight of the oil}}{\text{weight of the sample}} \times 100$$

2.2.1 Physiochemical properties of *C. gariepinus* oils

The physiochemical properties were determined according to the methods of AOAC [14] with some modifications.

2.2.1.1 Specific Gravity (S.G.)

Empty specific gravity bottles were weighed and labelled as (M_e), the oil samples were dispensed into the specific gravity bottles, weighed and labelled as (M_o). The oils were then substituted with water of the same volume and weighed to give (M_w). Thus, specific gravities of the oil samples were determined according to the formula below:

$$\text{Specific gravity} = \frac{(M_o - M_e)}{(M_w - M_e)}$$

Where

M_o -Specific gravity bottle + oil
 M_e -Empty specific gravity bottle
 M_w -Specific gravity bottle + water

2.2.1.2 Acid value

Mixture of ethanol and diethylether (v/v, 50 mL) with the addition of 3 drops of phenolphthalein indicator was neutralized with 0.1 M KOH solution until a faint pink colour appears. 0.5 g of each of the oil samples was added to the neutralized solution in the presence of 3 drops of phenolphthalein and was finally titrated against 0.1M potassium hydroxide solution till a permanent pink colour was attained. The acid values were calculated as follows:

$$A.V = \frac{\text{Vol. of KOH used} \times \text{mass of KOH}}{\text{Mass of sample}}$$

2.2.1.3 Peroxide value

0.5 mL of the oil was dissolved in a solvent mixture (30 ml of 1:2 acetic acid-chloroform). Potassium iodide (1.3 g) was added to the resulting solution and put in a shaker for 1 min. The mixture was placed in a dark cupboard for 1 hr, after which, 75 mL of distilled water was added followed by 3 drops of starch indicator, the mixture then was titrated against 0.05M sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) till the solution turns to colourless. The same condition solution were prepared but without adding oil sample as a blank solution. The peroxide values were calculated as follows:

$$\text{Peroxide value} = \frac{((S-B) \times N \times 1000)}{W}$$

Where, B= Vol. of standard potassium thiosulfate used for titration of blank, S= Vol. of standard potassium thiosulfate used for titration of sample, N= normality of sodium thiosulfate solution, W= weight of sample.

2.2.1.4 Iodine value

The iodine values of the oil samples were determined by Wijjs method. Oil sample (0.5 mL) each was dispensed into a conical flask and mixed with chloroform (5 mL) and Wijjs reagent (8 mL), (9 mL of iodine trichloride and 10 g of iodine in chloroform (300 mL)/acetic (700 mL) solution). The conical flask was shaken and placed in the dark for 1 hr. After which, 7 mL of potassium iodide and 75 mL of distilled water were added and titrated against 0.05 M sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) solution using starch as the indicator. A blank test was carried out simultaneously without the fat under the same conditions.

$$I.V. = \frac{(\text{Blank-sample}) \times 0.01269}{W} \times 100$$

S= (Vol. of $\text{Na}_2\text{S}_2\text{O}_3$ for blank – Vol. of $\text{Na}_2\text{S}_2\text{O}_3$ for sample)

W= Weight of sample

2.2.2 Determination of fatty acids profile

The fatty acids contained in the oils were determined following the method used by [13] with some modifications. Fatty acids profiles were analyzed by the preparation of Fatty acid methyl esters (FAMES), assisted with a Hewlett Packard HP 6890 Series gas chromatograph coupled with a Hewlett Packard 5973 mass spectroscopy detector (GC-MS) system. 60 μL of oil was placed into 10 mL centrifugal tubes with 1 mL n-hexane and 50 μL of 1 M sodium methoxide (30% methanol in sodium methoxide). Then the mixture was shaken vigorously for 30s using an auto-vortexer (Stuart, UK) and then it was kept for another 2 min at an uninterrupted condition in order to form a bi-layer. The clear upper layer of the mixture containing the FAME (1.0 μL) was pipetted off and injected into the gas chromatography immediately to avoid the reverse reaction. A HP-5 MS capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) was used for the GC system. The temperature program was set up from 50°C to 250°C with 4°C/min, both the injector and detector temperatures were 280°C and He was used as carrier gas.

2.2.3 Determination of functional group in the oil

The oil samples were injected into the Fourier Transform Infra-red Spectroscopy (FTIR) machine to determine functional groups present in the oils using Shimadzu (Tensor 27) FTIR machine with standard KBr beam splitter. ATR sampling technique was used to detect functional group by recording 60 scan in % transmittance mode in the range of 4000- 500 cm^{-1} .

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of *C. gariepinus* Oils

The oil content was in the range of Badagry>Lekki>Epe>Ikorodu. Their oil content was higher than 15.92% reported by [15] but lower than 48% reported by [13]. The specific

gravity of oil extracted from *C. gariepinus* collected from Ikorodu lagoon (0.9965 g/cm^3) was the highest in all the samples while the oil of *C. gariepinus* collected from Badagry lagoon (0.9950 g/cm^3) was the lowest. The specific gravity of fish oils seems to be higher than those of vegetable oils as the specific gravity of most vegetable oils ranges between 0.89-0.92 at 20°C [16]. The peroxide values of the oil samples were 5.12 mEQ/kg, 3.92 mEQ/kg, 4.23 mEQ/kg and 4.02 mEQ/kg for *C. gariepinus* collected from Badagry, Ikorodu, Lekki and Epe Lagoon respectively. The peroxide values of two of the oil samples falls in the range 3.11-6.90 mEQ/kg reported by [17] and were close to 4.9 mEQ/kg reported by [18]. The peroxide value indicates the amount of primary oxidation products produced during oil extraction. The quality of oil improves with low peroxide value. The presence of high content of polyunsaturated fatty acids in fish oil will make the oil highly susceptible to oxidation [15]. However, the values are within the acceptable limit of 5.0 mEQ/kg reported by [19] except for the oil extracted from *C. gariepinus* collected from Badagry lagoon. This is an indication that this oil is more liable to rancidity than others. The acid value of oil extracted from *C. gariepinus* collected from Ikorodu lagoon (3.57 mg KOH/g) was the highest in all the samples. The acid values fall within the range of 2.08-7.1 mg KOH/g reported by [18] but higher than 2.244 mg KOH/g reported by [15]. It indicates the lower level to which glycerides in the fish oils had been decomposed by lipase action. The lower the acid value of the oil, the few free fatty acids it contains which makes it less susceptible to rancidity [20]. The iodine values show that the fish oils are highly unsaturated except for the oil of *C. gariepinus* collected from Ikorodu lagoon that has an iodine value of $97 \text{ mgI}_2/100 \text{ g}$. Iodine value measures the degree of unsaturation in oil, the higher the iodine value, the more liable to rancidity the oil. Oils rich in unsaturated fatty acids have been reported to reduce heart diseases associated with cholesterol [21].

3.2 Fatty Acid Composition of *C. gariepinus* Oils

Oil extracted from *C. gariepinus* collected from Badagry lagoon had the highest amount of polyunsaturated fatty acids (59.17%). The results for the fatty acid composition of all the

fish oils were closely related to those reported by [17] except for the oil extracted from *C. gariepinus* collected from Ikorodu lagoon that contains large amount of monounsaturated and saturated fatty acids which was closely related to those reported by [22]. The oils extracted from *C. gariepinus* collected from Badagry, Lekki and Epe lagoon contains large percentage of essential polyunsaturated fatty acids. Essential fatty acids (EFA) refer to those polyunsaturated fatty acids (PUFA) that cannot be synthesized by the body which are necessary for health and must be provided in food [15]. The EFA consists of omega-3 (ω -3) and omega-6 (ω -6). It has been estimated that consumption of approximately 900mg of omega-3 fatty acid (i.e., EPA and DHA) per day beneficially affect mortality rates in patients with coronary heart disease (CHD) [23] and could also reduce cancer risk [24,25]. DHA helps in brain memory and performance in early stage and adults to maintain normal functioning of brain and also improves the learning ability [13]. American Heart Association recommends EPA and DHA for the prevention of cardiac death and cardiovascular diseases [23,26]. They promote good heart health by increasing the HDL (good cholesterol) and decreasing triglycerides (fats in the blood).

3.3 Functional Groups in *C. gariepinus* Oils

The FTIR spectra of the oils showed the presence of O-H stretch of carboxylic acid which occurs at 3347.1 cm^{-1} , 3011.7 cm^{-1} and 3004.2 cm^{-1} . The spectra showed a very strong band at 1729.5 cm^{-1} which indicates C=O stretch of normal aliphatic esters in all the oils except for the oil extracted from *C. gariepinus* collected from Ikorodu lagoon that showed a very strong band at 1740.7 cm^{-1} indicating C=O stretch of ketone. These results were related to those reported by [15]. The peaks at 1461.1 cm^{-1} , 1408.9 cm^{-1} and 1461.1 cm^{-1} showed the presence of methylene group in all the oils. The bands at 2922.2 cm^{-1} , 2914.8 cm^{-1} , 2851.1 cm^{-1} and 2847.7 cm^{-1} indicate the presence of alkenes functional group which denotes the existence of unsaturated fatty acid in *C. gariepinus* oils [15]. The peaks at 704.5 cm^{-1} and 723.1 cm^{-1} shows the presence of CH_2 rocking motion functional group which showed that long and linear aliphatic hydrocarbon chain exists in *C. gariepinus* oil.

Table 1. Physiochemical properties of *C. gariepinus* oils

Parameters	Badagry Lagoon	Ikorodu Lagoon	Lekki Lagoon	Epe Lagoon
Oil content (%)	40.57±0.04	30.65±0.08	38.12±0.02	36.08±0.05
Specific gravity (g/cm ³)	0.9950±0.01	0.9965±0.06	0.9960±0.005	0.9954±0.03
Acid value (mg KOH/g)	2.35±0.12	3.57±0.015	2.74±0.024	3.28±0.018
Peroxide value (mEQ/kg)	5.12±0.004	3.92±0.02	4.23±0.001	4.02±0.024
Iodine value (mgI ₂ /100 g)	129±0.013	97±0.01	125±0.028	123±0.015

Values are mean ± standard deviation of triplicate determinations

Table 2. Fatty acid composition of *C. gariepinus* oils

Fatty acids	Fatty acid no	Badagry Lagoon (%)	Ikorodu Lagoon (%)	Lekki Lagoon (%)	Epe Lagoon (%)
Lauric acid	C12:0	1.02±0.043	0.67±0.054	ND	ND
Myristic acid	C14:0	6.89±0.051	7.23±0.036	8.01±0.080	6.97±0.043
Pentadecanoic acid	C15:0	0.12±0.029	3.24±0.096	0.31±0.014	1.02±0.022
Palmitic acid	C16:0	1.98±0.043	2.45±0.042	2.67±0.054	ND
Magaric acid	C17:0	0.02±0.008	0.11±0.025	ND	0.03±0.016
Stearic acid	C18:0	3.13±0.042	2.45±0.057	3.19±0.050	1.98±0.016
Arachidic acid	C20:0	0.09±0.022	0.24±0.029	0.05±0.022	1.23±0.022
Behenic acid	C22:0	2.83±0.0222	6.99±0.036	2.87±0.022	3.38±0.022
Lignoceric acid	C24:0	0.02±0.008	9.02±0.022	0.04±0.022	0.15±0.037
Σ%SFA		16.10±0.004	32.40±0.005	17.14±0.014	14.76±0.022
Myristoleic acid	C14:1	5.98±0.043	6.49±0.037	4.71±0.036	6.68±0.022
Palmitoleic acid	C16:1	6.01±0.022	9.01±0.029	6.23±0.022	8.01±0.057
Oleic acid	C18:1	9.28±0.028	30.85±0.036	12.47±0.036	15.12±0.036
Eicosenoic acid	C20:1	0.18±0.025	0.09±0.043	0.28±0.028	1.24±0.029
Erucic acid	C22:1	3.28±0.009	4.02±0.014	ND	3.98±0.051
Nervonic acid	C24:1	ND	0.01±0.00	0.37±0.057	2.45±0.049
Σ%MUFA		24.73±0.004	50.47±0.023	24.06±0.005	37.48±0.024
Linoleic acid (n-6)	C18:2	3.27±0.043	1.87±0.022	5.19±0.0229	4.14±0.033
α-Linolenic acid (n-3)	C18:3	40.12±0.022	6.01±0.057	39.12±0.065	37.54±0.053
γ-Linolenic acid (n-6)	C18:3	ND	0.24±0.059	ND	ND
Stearidionic acid (SDA) (n-3)	C18:4	0.01±0.00	ND	0.12±0.065	1.05±0.057
Eicosatrienoic acid (ETE) (n-3)	C20:3	2.47±0.057	1.57±0.051	1.97±0.022	0.97±0.022
Dihomo-gammalinolenic acid (n-6)	C20:3	ND	ND	1.28±0.014	0.23±0.057
Eicosatetraenoic acid (ETA) (n-3)	C20:4	4.23±0.022	0.99±0.043	3.43±0.079	0.97±0.050
Arachidonic acid (n-6)	C20:4	3.27±0.057	2.98±0.051	5.19±0.037	1.13±0.057
Timnodonic acid (n-3)	C20:5	0.14±0.0216	ND	ND	ND
Heneicosapentaenoic acid (HPA) (n-3)	C21:5	ND	0.23±0.014	0.30±0.093	0.02±0.008
Docosadienoic acid (n-6)	C22:2	2.15±0.046	ND	0.01±0.00	0.01±0.00
Cervonic acid (n-3)	C22:5	ND	1.57±0.022	1.50±0.029	1.15±0.022
Docosatetraenoic acid (n-6)	C22:4	0.12±0.025	0.21±0.01	0.09±0.022	0.25±0.022
Docohexaenoic acid (n-3)	C22:6	3.19±0.036	0.19±0.043	0.10±0.067	0.30±0.037
Tetracosapentaenoic acid (n-3)	C24:5	0.20±0.022	1.27±0.054	0.50±0.014	ND
Σ%PUFA		59.17±0.008	17.13±0.025	58.80±0.022	47.76±0.050
Σ(n-3)		50.36±0.022	11.83±0.034	47.04±0.01	42.00±0.002
Σ(n-6)		8.81±0.030	5.30±0.032	11.76±0.023	5.76±0.033

Values are mean ± standard deviation of triplicate determinations

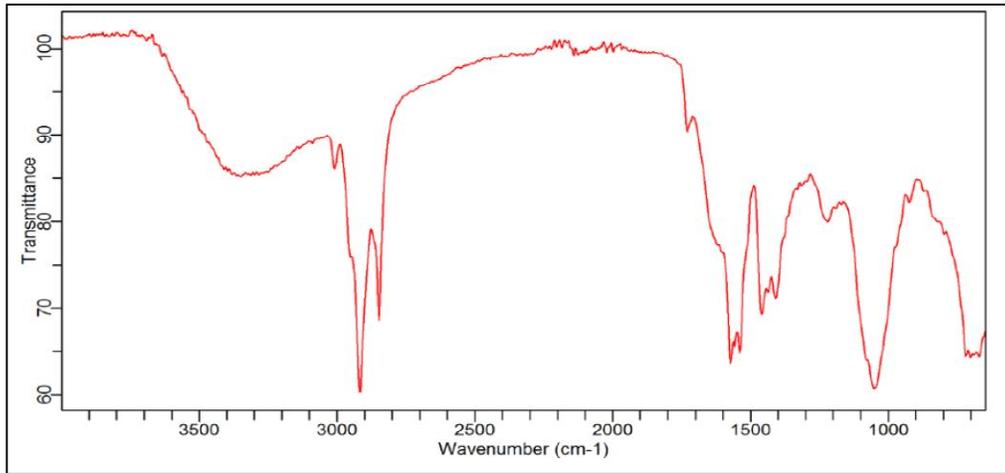


Fig. 1. FTIR spectra of the oil of *C. gariepinus* collected from Badagry Lagoon

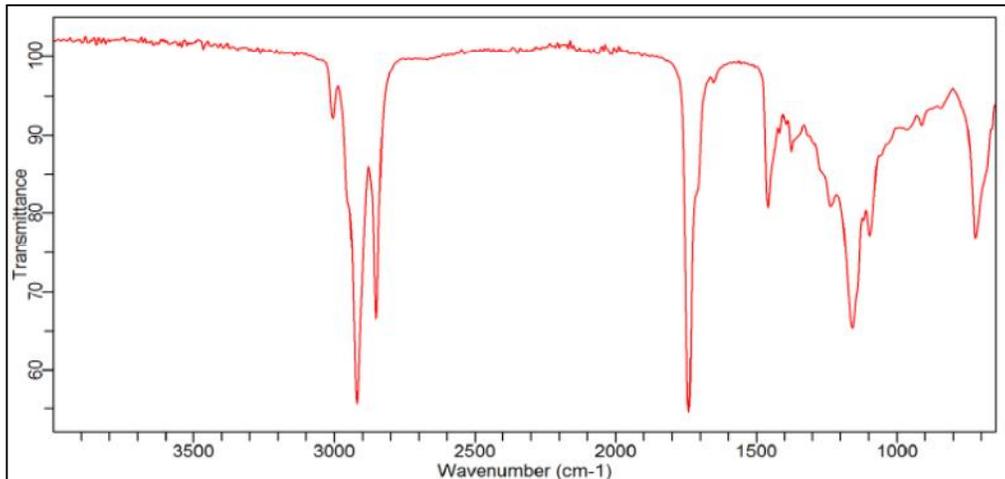


Fig. 2. FTIR spectra of the oil of *C. gariepinus* collected from Ikorodu Lagoon

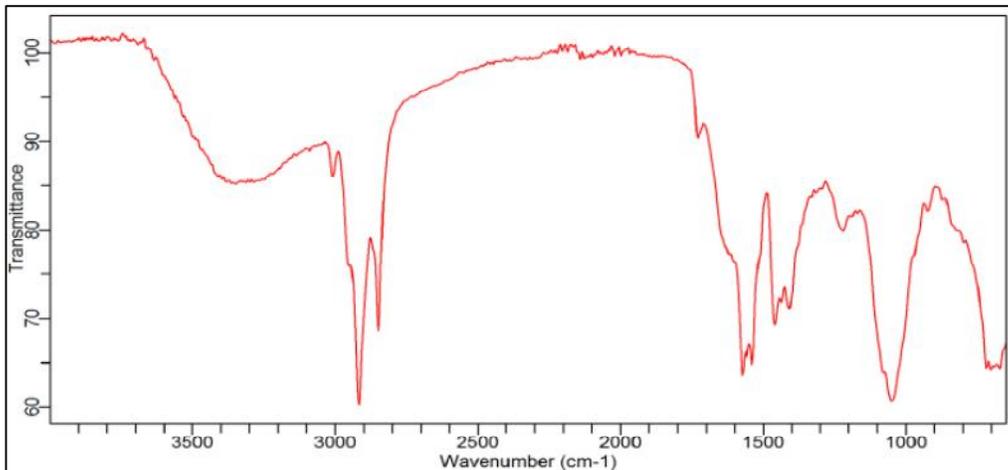


Fig. 3. FTIR spectra of the oil of *C. gariepinus* collected from Lekki Lagoon

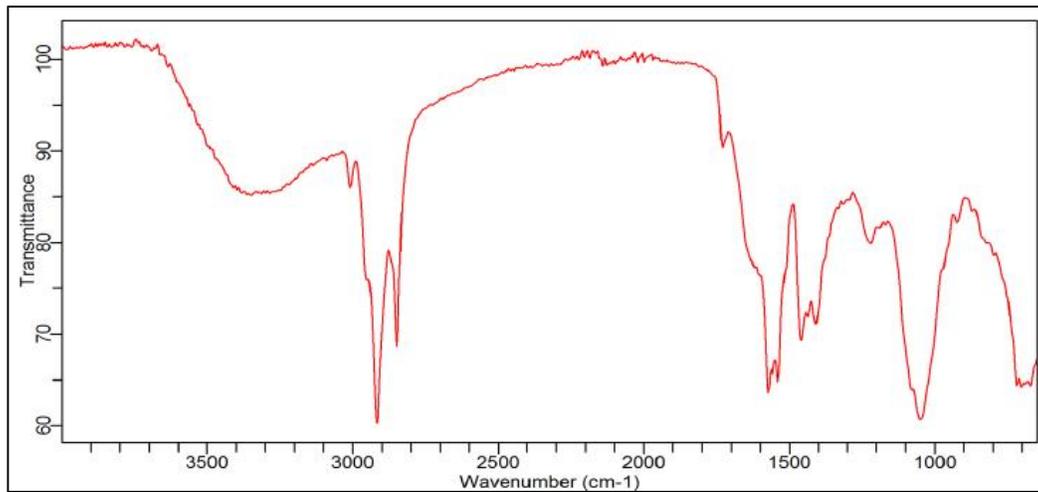


Fig. 4. FTIR spectra of the oil of *C. gariepinus* collected from Epe Lagoon

4. CONCLUSION

Fish oil composition varies according to species, season, size, age, life stage, diet and their habitat. This study was based on the compositional differences of the oil from *C. gariepinus* fish species samples taken from different lagoons within the Lagos metropolis. Data gathered from this study showed that not all *C. gariepinus* oils have polyunsaturated fatty acids as their major fatty acids while some of them contain large percentage of monounsaturated fatty acids as shown in the oil extracted from *C. gariepinus* collected from Ikorodu lagoon. Further studies would be made to determine which factor has the greatest effect on the oil composition of *C. gariepinus*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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