

# Comparative Study of the Physicochemical Properties and Fatty Acid Profile of Oil Extracted from Flesh and Viscera of Farmed Catfish (*Clarias gariepinus*) at Three Different Ages

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**Abstract -** This study ascertained the properties of oil extracted from the flesh in comparison to that from visceral oil sacs (processing waste) of farm-raised catfish harvested at (three) 3 different age brackets. The main aim of this research was to establish any difference between the fatty acid profile of the oil from catfish waste products and the edible parts. Oil was extracted through cold maceration from both flesh and visceral oil sacs of catfish at different ages using n-hexane. The oils were analysed to determine the yield, physicochemical properties, and fatty acid profile. It was found that the visceral oil sacs contained more oil than catfish flesh. A downward trend was observed in the yield of fish oil as the age increased. Results also showed that all catfish oil samples were rich in omega-3 fatty acids, but the samples from the viscera of the 5-month-old fish exhibited 7.2 percent and 11.4 percent concentrations of Eicosapentaenoic Acid (EPA) and Docosahexaenoic acid (DHA), respectively, which were comparable to the Codex Alimentarius standards for anchovy and cod liver oils. There was no significant difference in the omega-3 and omega-6 fatty acid profiles and oil characteristics of fish oil from the visceral sac and flesh of catfish across the three ages. The flesh of catfish contained a similar concentration of omega-3 fatty acids as the visceral oil sacs across different fish ages in the sample set analysed.

**Keywords:-** Catfish; Fish Oil; Fatty Acids; Visceral Oil Sacs; Fish Waste

## I. INTRODUCTION

The Nigerian aquaculture sector has experienced a remarkable growth in the past decades [1]; however, the emphasis has been on raising fish for food. This is most likely due to the fact that domestic production remains less than demand [2]. It has, therefore become a necessity to gather research data to encourage diversification into waste and byproduct utilization, thus promoting a circular economy approach within the aquaculture sector. Access to nutritious food, particularly sources of vital nutrients such as omega-3 fatty acids is known to be crucial for promoting public health especially in countries like Nigeria which face serious challenges in food security and nutrition with 35 %

of children under 5 years old stunted in growth [1]. There is numerous research-based evidence that Omega 3 fatty acids consumption in food, as supplementation or food fortification, can prevent stunting in children under 5 years [3], [4], [5]. Omega 3 fatty acids are also beneficial for proper functioning of the brain and eyes [6], They improve infant and maternal health [7], are beneficial in management of neurodegenerative diseases and mood disorders [8], [9]. They also have anti-inflammatory and immunomodulating properties that make them helpful in management of coronavirus infections [10], [11], [12] and many other health conditions [13], [14], [15] [16]. Nigeria is the highest aquaculture producer in sub-Saharan Africa [17] with catfish as the dominating fish species [17], [18]. Depending on the fish product, fish processing waste ranges from 20 to 80 % of fish weight [19] comprising heads, fins, bones, blood, skin and viscera. The catfish viscera weigh about 14 % of total fish weight [20] with the oil sacs constituting over 30 % of visceral weight [20]. The visceral oil sacs contain over 90 % oil which can be easily recovered without rigorous processes. Catfish processing facilities in Nigeria which produce up to 1 ton of catfish per batch may generate up to 300 kg fish waste and 40 kg visceral oil. Despite this, Catfish oil has not been widely utilized for production of fish oil supplements, as fortification ingredients for human consumption, or use in many other industrial applications.

There is limited research data comparing the essential omega 3 fatty acid composition of fish oil from catfish flesh, and the oil from the visceral oil sacs. There is also insufficient research data on the fatty acid profile of fish oil extracted from farm raised catfish at different harvest ages, to know if the much younger fish contain as much omega 3 fatty acids as the older fish. This study compares the properties and nutritional composition of oil from catfish flesh, which is generally consumed in meals and oil from visceral oil sacs hitherto considered a waste product. The study observes the fatty acid profile of oils from both sources and also reviews same parameters for catfish at different ages, comparing them with the standard fatty acid profile for anchovy and cod liver oils. This information may be useful in the development of standardized processes and quality control measures in Nigeria's catfish industry. Comparing the fatty acid profile of catfish oil with standard

fish oil from common oil fish species such as Anchovy and Cod helps in understanding the potential of catfish oil in fish oil supplement production.

## II. MATERIALS AND METHODS

### ➤ *Sample Collection*

Live catfish at two, five, and eight months were harvested from tarpaulin tanks at a catfish farm in Nnewi Town of Anambra State using plastic baskets. The fish had average weights of 160 g, 267 g, and 1000 g respectively. The fish from each age set were kept alive in plastic bowls containing tap water for about three hours until required for oil extraction.

### ➤ *Sample Preparation*

Each fish set was stunned and de-slimes using common salt, they were thoroughly washed under running water then degutted to separate flesh from waste and to detach the visceral oil sacs. The oil sacs and flesh were washed separately under running water, drained, and weighed. The fish flesh was cut into pieces using sterilized kitchen knives before being pounded in ceramics mortar to further reduce particle size. Visceral oil sacs and mashed fish flesh thus prepared were used immediately for oil extraction.

### ➤ *Oil Extraction*

Extraction of oil from the fish samples was done using cold maceration method with n-hexane as solvent [21]. The mashed fish samples were weighed into labelled, sterilized 1000 ml glass reagent bottles and n-hexane added to each bottle in the ratio of 2:1. The mixture was stirred, covered and stored in dark cupboard for about 50 hours. The stored samples were agitated intermittently during the duration of the storage to ensure adequate solvent exposure to the samples. The cold maceration method combines the advantage of low-temperature extraction with higher batch processing without using any complex tool.

After about 50 hrs., the resultant mixture was shaken thoroughly and then separated using a muslin cloth to remove the coarse fish residue from the filtrate. The filtrate was further filtered using Whatman No. 1 filter paper to remove the remaining fine particles and impurities.

The solvent was then recovered using an IKA rotary evaporator at 40°C, and the rotation speed and time of about 180 rpm and 1 hr. Pale yellow oil was collected in labelled glass sample bottles, weighed and kept in a refrigerator until required for analyses.

### ➤ *Test for Fish Oil Quality*

The physicochemical properties of the fish oil samples were determined at the Pharmaceutical Chemistry laboratory, Nnamdi Azikiwe University, Agulu campus, Anambra State while the fatty acid profile was determined at Springboard Laboratories, Awka, Anambra State.

### ➤ *Determination of Iodine Value*

Each oil sample, (2 g) was weighed to determine the iodine value of the oil using Wiii's Method and the titer

value recorded as (a). Simultaneously, a blank titration (b) was also conducted and the iodine value was calculated using the formula:

$$\text{Iodine Value} = (b - a) \times 1.269 / \text{wt.} \quad (1)$$

Where wt. is the weight (g) of the sample, b is the titer value of blank, while a is titer value of the oil samples.

### ➤ *Determination of Peroxide Value*

Exactly 1 g of the oil sample was weighed into a clean and dry boiling tube and 1 g of powdered potassium iodide and 20 ml of a solvent mixture containing 2 volumes of glacial acetic acid and 1 volume of chloroform were added to the tube. The tube was then placed in a water bath for about 30 seconds until it boiled and the contents poured into a flask containing 20 ml of potassium iodide solution (5 %). The boiling tube was rinsed twice with 25 ml of water, and the washings were transferred to the flask. The resulting solution in the flask was titrated with a 0.002 M sodium thiosulphate solution using starch as an indicator. The volume of the sodium thiosulphate solution used for titration was recorded. A blank titration was simultaneously performed and the volume of thiosulphate solution used for the blank titration was also noted.

$$\text{PV (Meq O}_2\text{/kg)} = (V_b - V_s) \times N \times 1000 / W \quad (2)$$

Where;

- PV is the peroxide value in milliequivalents of active oxygen per kilogram of sample (Meq O<sub>2</sub>/kg).
- V<sub>b</sub> is the volume of the sodium thiosulphate solution used for the blank titration (in mL).
- V<sub>s</sub> is the volume of the sodium thiosulphate solution used for the sample titration (in mL).
- N is the normality of the sodium thiosulphate solution (in mol/L).
- W is the weight of the oil sample used (in grams).

### ➤ *Determination of Saponification Value*

Each oil sample (2 g) was weighed into a conical flask and 25 ml of the alcoholic potassium hydroxide solution added to it. A reflux condenser was attached and the flask heated in boiling water and shaken frequently over about 1hr. Then, 1ml of phenolphthalein (1 %) solution was added and titrated with hot the excess alkali with 0.5 M hydrochloric acid (titration = a ml). Blank titration was carried out at the same time (titration = b ml)

$$\text{Saponification value} = \frac{(b - a) \times 28.05}{\text{wt. (g) of sample}} \quad (3)$$

### ➤ *Determination of Acid Value*

The Acid value of the catfish oil was determined using the ISO 660:20 methods. Exactly 25 ml of diethyl ether was mixed with 25 ml ethanol and 1 ml phenolphthalein solution (1%) and carefully neutralized with 0.1M KOH.

Oil sample (2 g) was dissolved into the mixed neutral solvent and titrated with aqueous 0.1M KOH, shaken constantly until a pink color that persisted for 15 seconds was obtained.

$$\text{Acid value} = \frac{\text{Titer Value (ml)} \times 5.61}{\text{Weight of sample (g)}} \quad (4)$$

#### ➤ Determination Specific Gravity

A 50 ml pycnometer bottle was thoroughly washed with detergent water and petroleum ether, dried and weighed ( $g_1$ ). The dried, empty bottle was filled with water and weighed ( $g_2$ ), then dried again. The dried bottle was filled with the oil sample and weighed ( $g_3$ ).

$$\text{Specific Gravity} = \frac{\text{Weight of sample (g}_3 - g_1)}{\text{Weight of water (g}_2 - g_1)}$$

#### ➤ Determination of Smoke Point

The smoke point of the oil samples was determined using the Cleveland Open Cup method recommended by the American Oil Chemists' Society.

#### ➤ Determination of Fatty Acid Profile

The fatty acid profile of the catfish oil samples was performed using a Buck 530 Gas Chromatograph equipped with an on-column automatic injector, flame ionization

detector, and an HP 88 capillary column (100 m length, 0.25 m film thickness) from CA, USA.

The obtained chromatographs were analyzed, and the peak areas were used to determine the relative abundance of different fatty acids. The fatty acid profile was characterized by the identification and quantification of individual fatty acids present in each sample. The results and chromatogram are shown in chromatograms below.

### III. RESULT AND DISCUSSION

The result on Table 1 below shows the yield of oil from the viscera and flesh of farmed catfish harvested ages 2, 5 and 8 months. As expected, there is generally a lower percentage oil yield from the flesh of catfish (16.26 – 14.19 %) than the visceral sac (83.00 – 74.19 %). The oil yield from catfish at younger ages (2 months and 5 months) were higher than oil yield at older age (8 months).

This observation corresponds to result from previous researcher [22] where volume of oil extracted from the flesh of adult catfish was found to be proportionally less than that from juveniles, suggesting that younger catfish may have more oil yield than older fishes.

Table 1 Percentage of Oil Yield from Visceral Sacs and Flesh of Catfish at Different Ages

Fish Age	2 Months	5 Months	8 Months
% Oil yield from the flesh (F)	16.26	16.95	14.19
% Oil yield from visceral oil sac (V)	83.00	81.6	74.00

Values show the oil yield of farmed catfish harvested ages 2, 5 and 8 months

#### ➤ Iodine Value of Catfish Oil

The mean iodine values for oil samples decreased with age, between 135.528 mg/kg in 2 months flesh and 119.003 mg/kg in 8 months visceral oil sacs. High iodine values imply more unsaturated bonds. Oils with high iodine values are known to improve blood cholesterol levels and so are beneficial for reducing the risk of cardiovascular diseases. Their anti-inflammatory properties reduce the risk of chronic inflammation-related conditions [10], [13]. They are very reactive and can be modified to form other products [23]. reported possible industrial applications through modifications such as epoxidation, sulphonation, hydroxylation, and chlorination for oil samples with high iodine values. The iodine value of oils has also been used to classify oils as drying, semi-drying, and non-drying [24]. Iodine values less than 100 are non-drying oils; 100 to 130 are semi-drying oils and 130 to 190 are drying oils. All the fish samples studied were semi-drying oils except oil samples from the 2months age which showed an iodine value slightly higher than 130. Semi-drying oils are suitable as cooking oils and can also be applied in the production of resins, paints, and varnishes [25].

#### ➤ Peroxide Value of Catfish Oil

The peroxide value (PV) of the oil samples ranged from 5.6 from 5-month-old fish viscera to 12.40 from 5-month-old flesh which is above the maximum standard of 5

recommended by the Codex Alimentarius Commission for fish oils [26]. The PV of the oil sample gives a measure of the first stages of oxidative rancidity [27]. It indicates the number of primary oxidation products produced during the extraction process. Oil refining procedures can break down the peroxides in crude fish oils such that the resultant oils will have a lower peroxide value [20], [28].

#### ➤ Saponification Value of Catfish Oil

Oils from two months of fish flesh showed the highest saponification value of 202.956 mg/kg KOH which is not significantly higher than the value from the oil of the 5 months of flesh and 5 months of visceral sacs. The saponification value of oil from 5 months fish visceral and 2 months fish flesh are consistent with previous literature [29]. The lowest saponification value, 111.586 mg/kg KOH was recorded from the oil from the flesh of 8 months catfish. This value implies that it can be used for making soaps.

#### ➤ Acid Value (AV) of Catfish Oil

The acid value (AV) of an oil sample is the weight of KOH (in mg) needed to neutralize the organic acids present in 1 g of fat. It gives a measure of the free fatty acids (FFA) present in the lipid sample. Apart from the samples in 8-Month viscera which showed a higher value of 4.191, the acid value of other catfish oil samples decreased with increasing fish age. Also, the acid values of all samples were

above 3.0 which is the standard for fats and oils [26] except for the sample from 5 months old fish viscera which is 2.240.

➤ *Specific Gravity of Catfish Oil*

The Specific gravity of oils from the flesh are 0.9, and 0.84, while that from the viscera is 0.8, 0.79 0.81. As seen on Table 2 above, catfish oil samples from the viscera have lower specific gravity (0.84, 0.81, and 0.79) than the oil from fish flesh (0.90, 0.90, and 0.80) across all ages. [30]

Hasan et al., (2016) also reported that edible oils with lower density have better consumer preference.

➤ *Smoke Point of Catfish Oil*

The catfish oil samples all had relatively low smoke points with sample 5MF showing the lowest at 73°C, while sample 5MV showed the highest at 110 °C. Based on this finding, catfish oil is not ideal as a frying oil but for other food preparations that do not require much heating.

Table 2 Physicochemical Properties of Catfish Oil Samples

Sample	Acid Value (mg KOH/g)	Saponification Value (mg KOH/g)	Peroxide Value (Meq/kg)	Iodine Value	Specific Gravity	Smoke Point (°C)
2 Months Flesh	3.93 ± 0.00*	202.96 ± 0.00*	8.60 ± 0.01*	135.52 ± 0.00*	0.90 ± 0.00	85.00 ± 1.00
5 Months Flesh	3.28 ± 0.00	176.50 ± 0.10	12.40 ± 0.02	127.68 ± 0.01	0.84 ± 0.00	73.00 ± 2.00
8 Months Flesh	3.09 ± 0.00*a	111.59 ± 0.00*a	6.60 ± 0.00*a	120.04 ± 0.00*a	0.90 ± 0.00*	105.00 ± 0.00* <sup>n</sup>
2 Months Viscera	3.28 ± 0.00*	142.77 ± 0.00*	7.20 ± 0.02*	128.68 ± 0.00*	0.80 ± 0.00*	80.00 ± 2.00*
5 Months Viscera	2.24 ± 0.00	190.24 ± 0.00	5.60 ± 0.01	124.60 ± 0.00	0.79 ± 0.00	110.00 ± 0.00
8 Months Viscera	4.19 ± 0.00*a	159.49 ± 0.00*a	8.80 ± 0.00*a	119.04 ± 0.00*a	0.81 ± 0.00*	78.00 ± 0.00* <sup>ns</sup>

Values are mean ± standard deviation of triplicate values (n=3). No statistically significant differences were found between the groups based on ANOVA analysis (P > 0.05).

➤ *Fatty Acid Profile of Catfish Oil Samples*

The fatty acid composition of oil samples extracted from the flesh and visceral sac of 2 months, 5 months and 8 months have been presented in the Table 3. Currently, there are no standards for catfish oil, however, standards for common fish oils (cod liver oil and anchovy fish oils) by the codex Alimentarius Commission have been inserted in the table for ease of comparison. Four saturated and 9 unsaturated fatty acids were identified in the catfish samples. Omega 3 fatty acids constituted 36-47%, 26-38% omega 6, 0.095-5.909% omega 9, and 18-26% saturated fats. Palmitic acid and stearic acid are the most abundant saturated fatty acids in the viscera while flesh also has good palmitic acid and some lauric acid.

Alpha-linolenic acid (ALA), Arachidonic acid, Palmitic Acid and Docosahexaenoic acid (DHA) are the significant omega-3 fatty acids in the samples. The most important essential fatty acids, DHA, and EPA constitute 11.423% and 7.279% respectively in the oil sourced from the viscera of the 5-month-old catfish, making it the closest in quality with the standard fish oils compared.

The statistical Analysis of Variance show that both source of fish oil and harvest age of catfish do not impact significant difference in the fatty acid profile of catfish oil samples so consumption of catfish or fish oils from all ages should be encouraged.

Table 3 Fatty Acid Profile of Catfish Oil Samples

Fatty Acid	Category	Oil Samples From Flesh			Oil Samples From Visceral Sac			Codex 2017 Fish Oil Standard	
		2 Months	5 Months	8 Months	2 Months	5 Months	8 Months	Anchovy	Cod Liver Oil
Lauric Acid (C12)	Saturated	2.729	3.546	2.472	0	4.509	0	0	0
Myristic Acid (C14)	Saturated	0	1.227	0	0.699	2.001	1.471	2.7-11.5	2.0-6.0
Palmitic Acid (C16)	Saturated	15.305	11.89	16.677	12.308	8.346	14.568	13.0-22.0	7.0-14.0
Stearic Acid (C18)	Saturated	0	4.52	0	12.808	6.633	10.131	1.0-7.0	1.0-4.0
Oleic Acid (C18:1)	Omega 9	3.966	5.337	3.859	1.785	0.095	2.499	3.6-17	12.0-21.0
Linoleic Acid (C18:2)	Omega 6	0	20.156	0	20.441	26.447	18.04	0-3.5	0.5-3.0
Alpha Linolenic	Omega 3	29.563	11.878	29.112	23.129	20.645	22.925	0-5.0	0.0-2.0

Acid (ALA) (C18:3)									
Eicosadienoic Acid (C20:2)	Omega 6	9.914	0.017	8.119	3.958	0	4.721	0	0
Mead Acid (C20:3)	Omega 9	1.876	0	2.05	2.495	0	2.899	0	0
Arachidonic Acid (C20:4)	Omega 6	28.201	5.849	30.81	7.296	4.868	7.177	0-2.4	1-1.5
Eicosapentano ic acid (EPA) (C20:5)	Omega 3	0.042	3.019	0.044	3.564	7.278	2.426	5.0-26.0	7.0-16.0
Docosahexaen oic Acid (DHA) (C22:6)	Omega 3	8.325	25.166	6.82	4.587	11.422	5.477	4.0-26.5	16-18
Tetracosapent aenoic Acid (C24:5)	Omega 3	0.072	7.389	0.032	6.392	7.751	7.612	0	0
<b>Total Fatty Acids</b>		<b>99.993</b>	<b>99.994</b>	<b>99.995</b>	<b>99.462</b>	<b>100</b>	<b>99.946</b>		
% Saturated FA		18.034	21.183	19.149	25.815	21.489	26.17		
% Unsaturated FA		81.959	78.811	80.846	73.647	78.506	73.776		
% Omega 3		38.002	47.452	36.008	37.672	47.096	38.44		
% Omega 6		38.115	26.022	38.929	31.695	31.315	29.938		
% Omega 9		5.842	5.337	5.909	4.28	0.095	5.398		

List of fatty acids found in catfish oil samples and their abundance as detected using the GC-FID and CODEX Alimentarius standards for Anchovy and Cod liver oils

#### IV. CONCLUSION

Our analysis of the physicochemical properties of catfish oil has revealed its stability and suitability as a cooking oil, although not ideal for deep-frying purposes due to its low smoke point. These properties also suggest catfish oil is well-suited for various industrial applications, such as the manufacture of soaps, body lotions, and several cosmetics products.

Furthermore, the oil extracted from the visceral oil sacs, previously considered waste, has proven to contain similar nutritious components to the oil obtained from the edible flesh, implying that the waste product can be recovered for use for human consumption.

In terms of fish age, our research demonstrates that there is no significant difference between the oil obtained from different ages analyzed. This suggests that the harvest age can be lowered to 2 months without compromising the omega-3 fatty acids available to consumers, particularly when addressing malnutrition in a particular region and the need for producing catfish oil as supplementation to boost infant and maternal health.

Finally, a comparison was made between the EPA and DHA content of catfish oil samples and the CODEX Alimentarius standards for fish oil. The results revealed a significant similarity, particularly in the oil extracted from 5-month-old catfish. This suggests that catfish oil could potentially serve as a source of these essential fatty acids and

be considered a viable raw material for local fish oil supplement production.

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APPENDIX

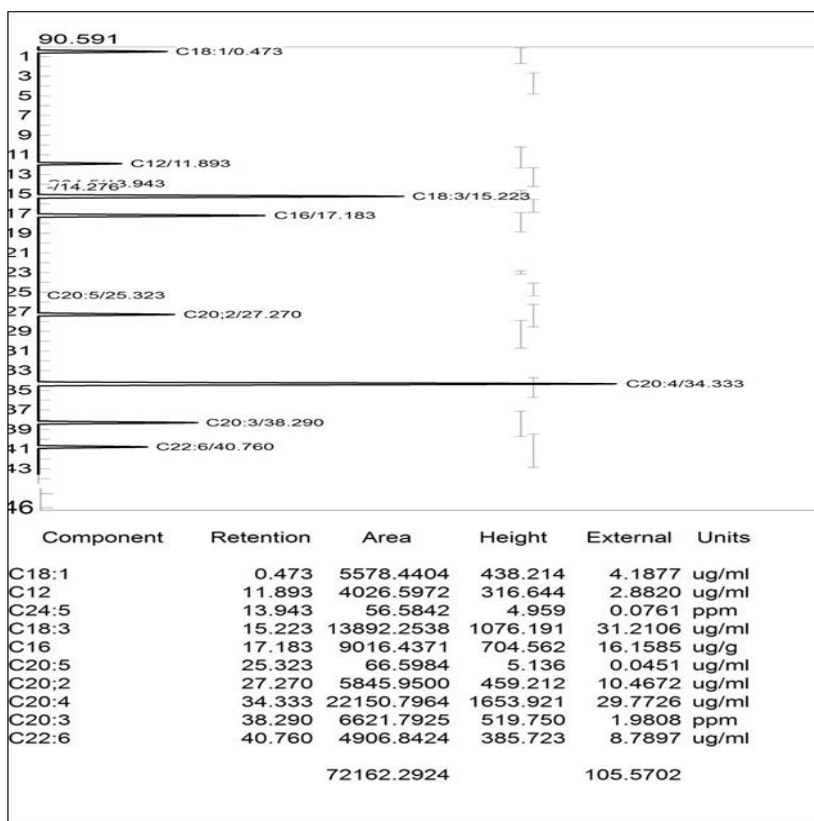


Fig 1 Chromatogram of Fatty Acid Profile of Fish Oil from Flesh of 2 Months Catfish

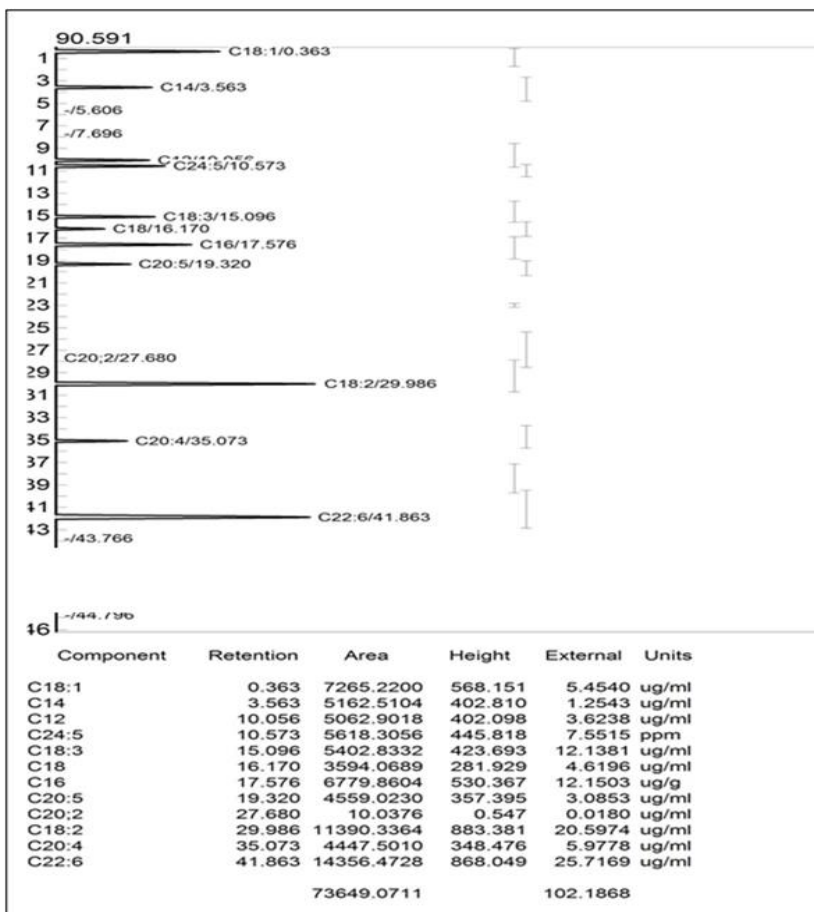


Fig 2 Chromatogram of Fatty Acid Profile of Fish Oil from Flesh of 5 Months Catfish

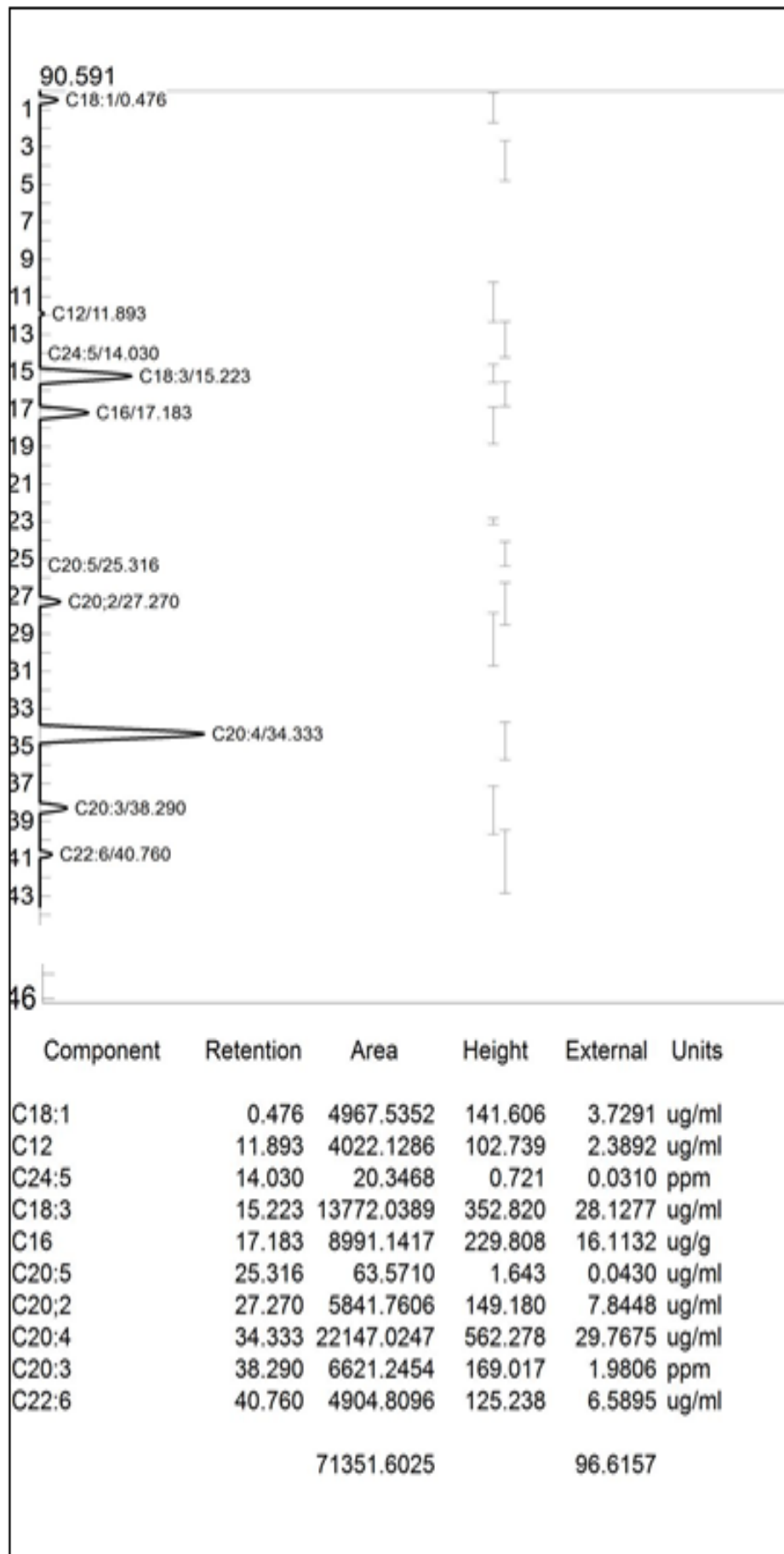


Fig 3 Chromatogram of Fatty Acid Profile of Fish Oil from Flesh of 8 Months Catfish



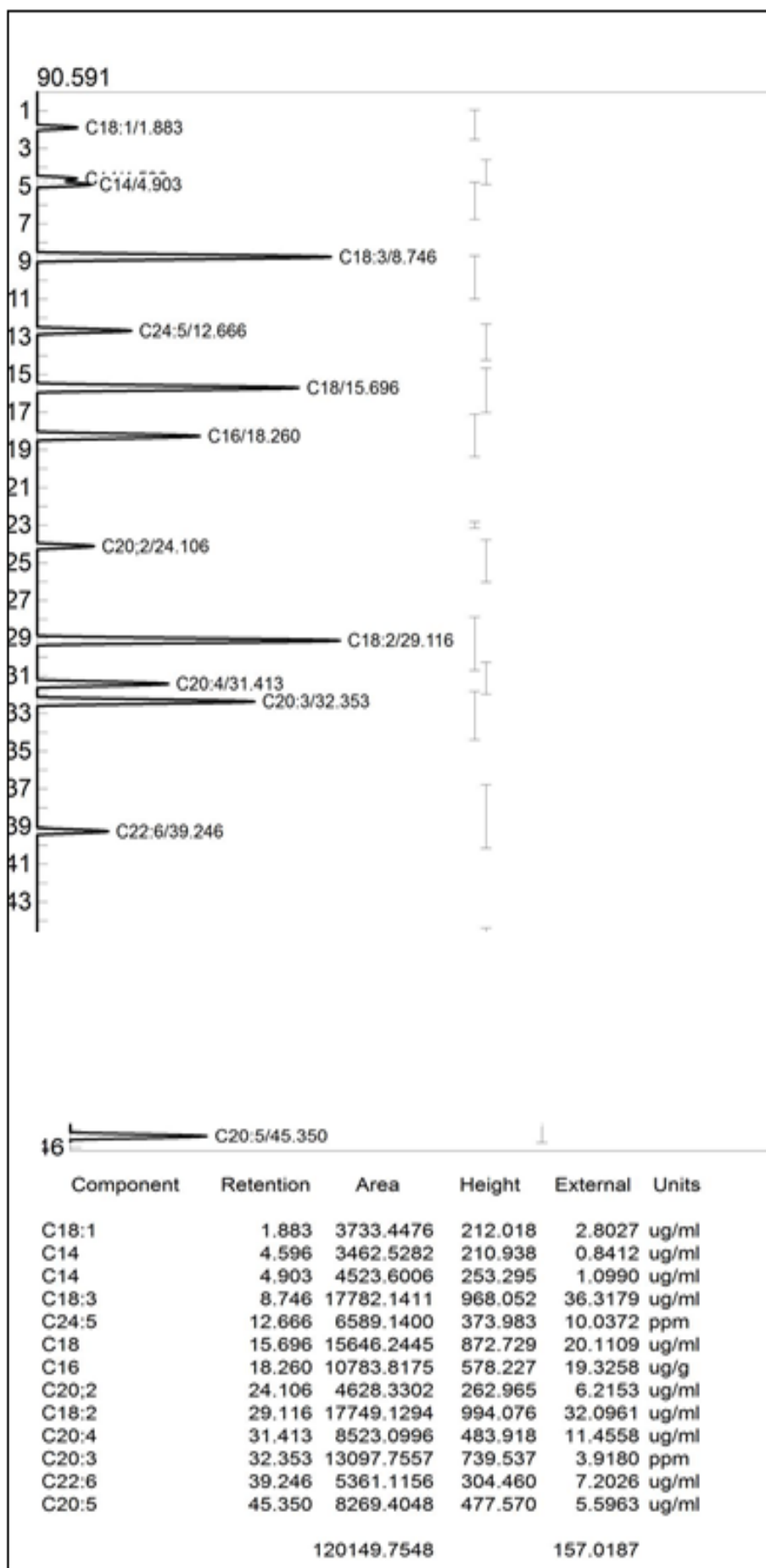


Fig 4 Chromatogram of Fatty Acid Profile of Fish Oil from Viscera of 2 Months Catfish

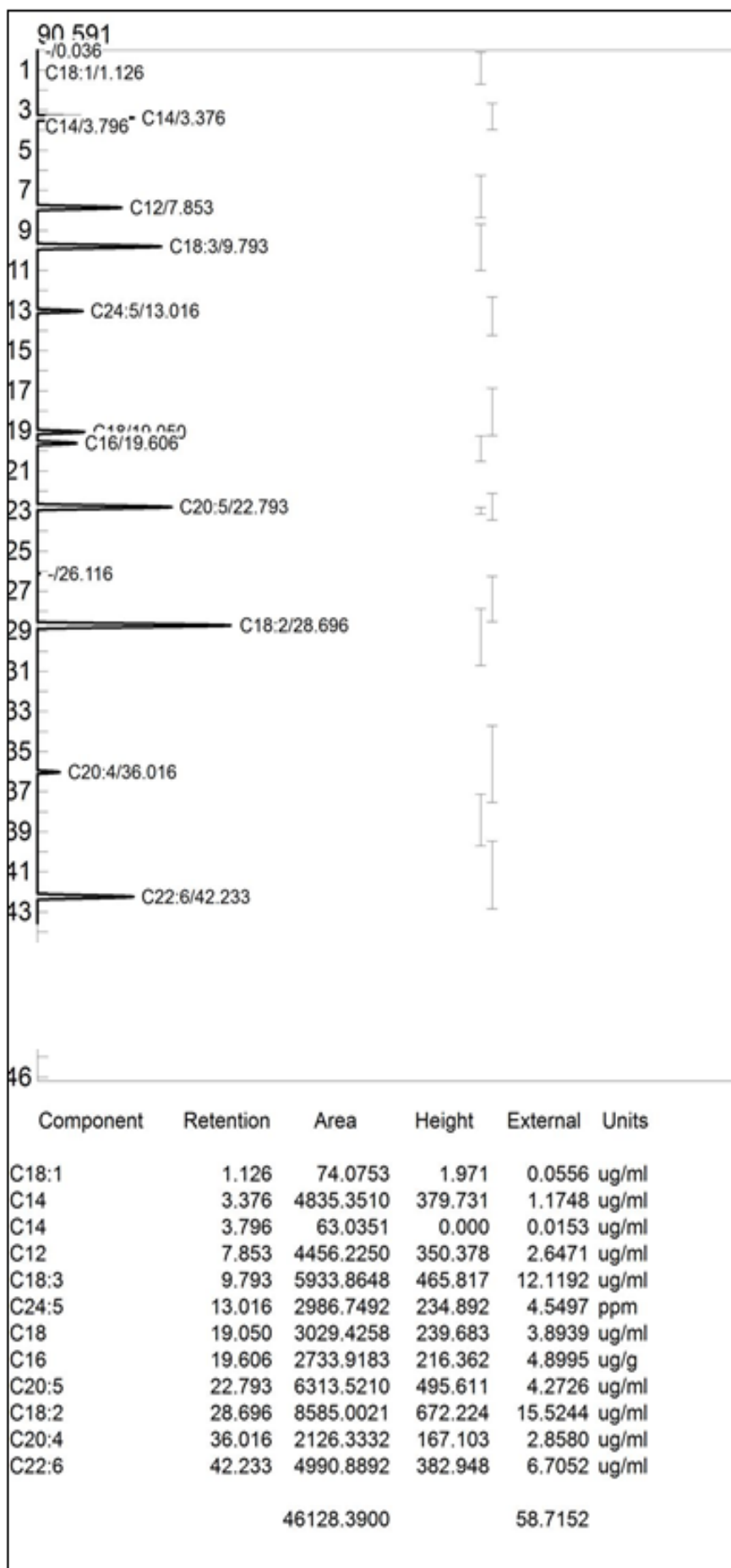


Fig 5 Chromatogram of Fatty Acid Profile of Fish Oil from Viscera of 5 Months Catfish

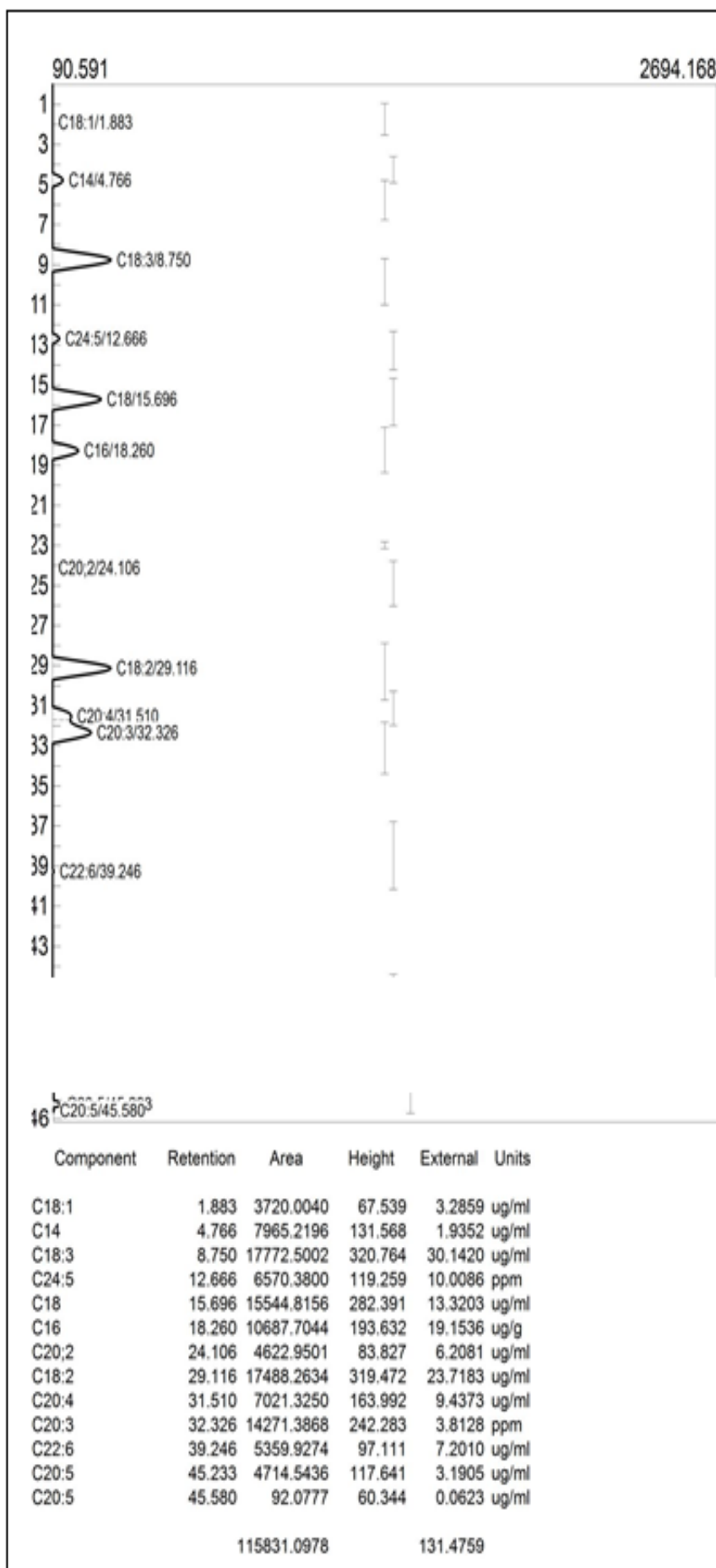


Fig 6 Chromatogram of Fatty Acid Profile of Fish Oil from Viscera of 8 Months Catfish