***Original Research Article***

# Fatty Acid Profile of Atlantic horse mackerel (*Trachurus trachurus*) oil obtained using different extraction methods

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**ABSTRACT**

The effect of three extraction methods which include soxhlet, gas and charcoal powered smoking kiln on extraction of oil from *Trachurus trachurus* were investigated. The oils obtained through these methods were evaluated chemically to determine its quantity and quality in terms of fatty acid composition using standard laboratory methods. The results obtained indicated that the highest oil yield (14.0%) was recorded in oil extracted with soxhlet method, while the lowest (0.93%) in oil extracted with charcoal powered kiln extraction method. Fatty acid profile of the oils showed the existence of sixteen fatty acids in all the extraction methods with stearic acid (53.62%) as the predominant saturated fatty acid (SFA); oleic acid (7.05%) as the foremost monounsaturated (MUFA) and linolenic acid (23.31%) as the major polyunsaturated fatty acid (PUFA). The fatty acid composition illustrated that extracted fish oil are very healthy in terms of total PUFA content although they should be consumed carefully considering the shelf life, as PUFA oxidizes very rapidly. The Soxhlet methods consistently recorded the highest oil yield, and can be used to extract oil for industrial use. While, the gas powered kiln can be used to extract oil for consumption purpose.

*Keywords: [Extraction, Fish oil, Fatty acid, Shelf life]*

## 1. INTRODUCTION

Fish is the best sought after and economical source of animal protein in Nigeria with about 75% of total annual catch consumed locally (1). Consumption of fish and fishery product in Nigeria is one of the highest in the world; per capita consumption in 2008 was about twice the average of the world (2). It also links with other sectors of the economy in providing raw materials, particularly for fish processing establishments, while engaging the services and products of other areas to operate (3)

The consumption of a considerable amount of fish has been on the increase because of its nutritional values as well as the increase in consumer awareness of its role in human health and disease prevention (4, 5). Fish is highly susceptible to deterioration immediately after harvest and in order to prevent economic losses, processing and preservation of fish are of utmost importance. Refrigeration, freezing, canning, smoking, salting, and drying are all preservation and processing techniques that have been used to enhance the shelf life of fish. (6). Due to the epileptic supply of power in Nigeria, smoking and drying are two of the most common ways to add value to fish thereby eliminating moisture and minimizing the reactions that cause perishability. In addition to this, some improved smoking kilns are attached with oil collectors to retain oil during smoking.

Fish oil remains the most valuable oil due to its quality and the various health benefits (7) and are commercially used in pharmaceutical and food industries (8). According (9), fish oil contains majorly polyunsaturated fatty acids and essential fatty acids that cannot be synthesized by the body but can be obtained through diet which lower serum triglycerides, prevents cardiovascular diseases, improve learning ability and regulates blood coagulation. They also secrete eicosanoid which is involved in several metabolic processes of the human body (10). Public awareness of the importance of taking omega-3 fatty acid-rich fish oil has increased, which has been linked to an increase in fish oil demand for the food sector and also pharmaceutical use (11). This study aimed to quantify and qualify fish oil from Atlantic horse mackerel (*Trachurus trachurus*) obtained using different extraction methods

## 2. MATERIAL AND METHODS

### 2.1 Experimental Location

The experiment was conducted at the Fish Processing Unit, African Regional Aquaculture Centre, Aluu in Port Harcourt.

### 2.2 Source of Fish Materials

The fish samples were purchased from Creek Road Market in Port Harcourt and were identified using keys of (12) on marine and brackish water fishes. These species is widely distributed migratory fish species in the North Atlantic (13). They are beneficial commercial species, targeted by purse seines, midwater trawls and long lines.

### 2.3 Sample Preparation

20kg was weighed, descaled and washed in clean water to remove sand, debris and impurities without removing the viscera. The fish was again rinsed in fresh water, cut in bits (the fillet was cut into small pieces to attain large surface area for drying processes), it was then spread in trays and taken for smoking in the kilns. (14).

### 2.4 Experimental Procedure

Two modern NIOMR kilns powered by Charcoal and Gas and the Soxhlet apparatus were used for the experiment to extract oil from the fish samples. The kilns comprised of a smoking chamber, a fan to distribute heat, a thermometer to control temperature, and a chimney to filter air, NIOMR's fish smoking kiln reduces cooking and smoking time from four days to just four hours. One of the kiln was powered with charcoal and the other one with gas. They were chosen because they are efficient and most farmers are using it to extract oil from fish at a local level. The fish was dried inside the oven for three hours at temperature of $100\pm5^0$C and then maintained at $55\pm5^0$C until constant weight was reached (15). Oil collection commenced after 3 hours of continuous heating in the kiln. Clear oil (supernatant) was collected from the kiln oil collector as sample A1 while the sludge (sediment) was labeled sample A2. The oil was collected from the oil collector attached to each oven. The oil was decanted into a clean plastic bottle and taken to the laboratory for analysis.

### 2.5 Oil Extraction Using Soxhlet Apparatus

**Soxhlet Apparatus is an** apparatus for use in extracting fatty or other material with a volatile solvent (such as ether, alcohol, or benzene) consisting of a vertical glass cylindrical extraction tube that has both a siphon tube and a vapor tube, that is fitted at its upper end to a reflux condenser and at its lower end to a flask so that the solvent may be distilled from the flask into the condenser whence it flows back into the cylindrical tube and siphons over into the flask to be distilled again.

**Procedure**: The fishes were cut into bits using a knife, and then washed to remove impurities, and sun dried to remove moist. Oil from the fish was extracted using soxhlet extractor (according to the methods of 16). The solid sample of the fish was placed into porous timbles with cotton wools. The extraction was carried out at $70^0$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 $40^0$C) allowing the solvent to evaporate to dryness. The resulting oil was collected and then stored in cool place to prevent oxidation and rancidity.

### 2.6 Oil Clarification:

Clarification was done to separate the oil from its entrained impurities. The fluid extracted out of the press is a mixture of fish oil, water, cell debris, and non-oily solids. The fluid was allowed to stand undisturbed to settle by gravity so that the oil, being lighter than water, will separate and rise to the top. The clear oil was decanted into a reception container and sieved.

### 2.7 Packaging and Storage:

Clean, dry sealed plastic bottles were used to package and store the oils and kept in a dark box to prevent rancidity.

### 2.8 Evaluation of Oil Yield

The oil yield was calculated by expressing the oil extracted as a percentage of the total oil content of the fish samples. From the values that was obtained, oil yield was calculated according to the formula of Olaniyan and Oje (2011) as:

$$\text{Oil Yield} = \frac{WOE}{WFH + WRC} \qquad \text{…………………………..(1)}$$

where,

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WOE = Weight of oil extracted;
WFH = Weight of fish
WRC = Weight of residual cake

**2.9 Fatty Acid Composition Analysis**

The fatty acid composition analysis was done according to the method of (17) with method number 969.33. Ten grams of fish oil was weighed and put in a flask, methanolic NaOH was added, and the flask was then heated in a water bath for 20 minutes. BF3 reagent and internal standard was added to the mixture, and the mixture was heated again for 20 minutes. The mixture was allowed to cool and then added by saturated NaCl and isooctane, subsequently the mixture was shaken. Isooctane layer formed was transferred with the aid of pipette into a tube containing anhydrous $Na_2SO_4$ to remove $H_2O$, and then awaited for 15 minutes. Liquid phase formed was separated, while oil phase was injected. Previously, injection of FAME standard mixture was performed. Retention time and peak of each component was measured and compared with the standard retention time to get information about the types and fatty acid components in the sample. Determination of fatty acid content in the samples was calculated by using the formula as follows:

Component content of samples = Ax/As x Cstandard x Vsample/100 x 100 %......(10)
(AOAC, 2005)

Ax : Sample area
As : Standard area
Cstandard : Standard concentration
Vsample : Sample volume

**2.10 Statistical Analysis**

The data obtained from the study was collated and analyzed using Statistical Package for Social Sciences (SPSS 22.0). A one way analysis of variance (ANOVA) was employed to reveal significant differences in measured variables. When a difference was detected (*P*= 0.05), Tukey's multiple comparison test was applied to identify differences between the means

## 3. RESULTS AND DISCUSSION

### 3.1 Oil Yield from *Trachurus trachurus* Using Different Extraction Methods

The oil yields from *Trachurus trachurus* using the different extraction methods are presented in Table 1. The wet and dry weights of the experimental fish were within the same range for the gas and charcoal powered kiln but smaller for the soxhlet extraction method due to its carrying capacity of the equipment. The highest weight (290.00± 7.99g) of fish oil obtained after extraction was obtained in the soxhlet extraction method. The oil yields from the different extraction methods were significantly different from each other (*P*=0.05).

**Table 1: Oil Yield from *Trachurus trachurus* Obtained Using different Extraction Method**

| Parameters | Extraction Methods | | |
|---|---|---|---|
| | **Gas** | **Charcoal** | **Soxhlet** |
| Wet Weight (g) | 13000.21 ± 7.88$^{a}$ | 13000.80 ± 4.09$^{a}$ | 2070.00 ± 7.02$^{b}$ |
| Dry Weight (g) | 8000.80 ± 9.03$^{a}$ | 8000.80 ± 9.11$^{a}$ | 480.00 ± 8.87$^{b}$ |
| Oil Weight (g) | 220.79 ± 0.62$^{b}$ | 120.64 ± 0.88$^{a}$ | 290.00 ± 7.99$^{c}$ |
| Yield (%) | 1.69 ± 0.08$^{b}$ | 0.93 ± 0.02$^{a}$ | 14.00 ± 0.79$^{v}$ |

**Means with the different superscript within the same rows are significantly different (*P*=0.05).**

**3.2 Saturated Fatty Acid Profiles of *Trachurus trachurus* Oil Obtained Using Different Extraction Methods**

The saturated fatty acid profiles of oil obtained from the sampled marine fishes using different extraction methods are shown in Table 2. The results revealed that the values of saturated fatty acid profiles such as myristic, palmitic, stearic and lignoceric acid varied significantly (*P*=0.05) among the three different extraction method under consideration, while behenic acid values were within the same range for kiln powered by gas and charcoal hence no significant different (*P*=0.05). However, bethenic acid value for oil obtained using the soxhlet method is signifantly different from the other extraction methods (*P*=0.05).

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**Table 2: Saturated Fatty Acid Profiles of *Trachurus trachurus* Oil Obtained Using different Extraction Method (Mean ± SD)**

| Fatty acid (%) | Extraction Methods | | |
|---|---|---|---|
| | **Gas** | **charcoal** | **Soxhlet** |
| Myristic acid | 7.19 ± 0.02[b] | 5.09 ± 0.04[a] | 8.35 ± 0.04[c] |
| Palmitic acid | 5.19 ± 0.03[c] | 0.22 ± 0.03[a] | 2.60 ± 0.03[b] |
| Stearic acid | 45.05 ± 0.05[b] | 53.62 ± 0.03[c] | 23.36 ± 0.03[a] |
| Arachidic acid | 1.98 ± 0.03[c] | 1.91 ± 0.02[b] | 0.00 ± 0.00[a] |
| Behenic acid | 0.00 ± 0.00[a] | 0.00 ± 0.00[a] | 7.31 ± 0.02[b] |
| Lignoceric acid | 0.79 ± 0.03[c] | 3.23 ± 0.03[b] | 0.00 ± 0.00[a] |

**Means with the different superscript within the same rows are significantly different (*P*=0.05).**

### 3.3 Monounsaturated Fatty Acid Profiles of *Trachurus trachurus* Oil Obtained Using Different Extraction Methods

The values of monounsaturated fatty acid profiles of oil in *Trachurus trachurus* obtained using different extraction methods are presented in Table 3. The results obtained indicated that the values of monounsaturated fatty acid profiles which include myritoleic, palmitoleic, oleic, and eicosenoic acid varied significantly (*P*=0.05) among the three different extraction methods being investigated.. However, zero values were recorded in erucic acid in all the three extraction methods under assessment (Tables 3).

**Table 3: Monounsaturated Fatty Acid Profiles Of *Trachurus trachurus* Oil Obtained Using different Extraction Method (Mean ± SD)**

| Fatty acid (%) | Extraction Methods | | |
|---|---|---|---|
| | **Gas** | **Charcoal** | **Soxhlet** |
| Myristoleic acid | 0.67 ± 0.02[a] | 2.06 ± 0.02[b] | 3.79 ± 0.19[c] |
| Palmitoleic acid | 0.41 ± 0.02[b] | 0.00 ± 0.00[a] | 0.00 ± 0.00[a] |
| Oleic acid | 5.26 ± 0.02[b] | 0.90 ± 0.03[a] | 7.05 ± 0.04[c] |
| Eicosenoic acid | 6.47 ± 0.04[c] | 0.27 ± 0.02[b] | 0.11 ± 0.03[a] |
| Erucic acid | 0.00 ± 0.00[a] | 0.00 ± 0.00[a] | 0.00 ± 0.00[a] |

**Means with the different superscript within the same rows are significantly different (*P*=0.05).**

### 3.4 Polyunsaturated Fatty Acid Profiles of *Trachurus trachurus* Oil Obtained Using Different Extraction Methods

The values of polyunsaturated fatty acid profiles of oil in *Trachurus trachurus* obtained using different extraction methods are shown in Table 4. The results revealed that the amount of polyunsaturated fatty acid such as Linoleic, Linolenic, Eicosadienoic, Eicosatrienoic and Arachidonic acid, in all the three extraction methods were significantly different (*P*=0.05).

**Table 4: Polyunsaturated fatty acid Profile of *Trachurus trachurus* oil Using different extraction method (Mean ± SD)**

| Fatty acid (%) | Extraction Methods | | |
|---|---|---|---|
| | **Gas** | **Charcoal** | **Soxhlet** |
| Linoleic acid | 0.26 ± 0.06[b] | 0.45 ± 0.08[c] | 0.00 ± 0.00[a] |
| Linolenic acid | 20.09 ± 0.08[b] | 19.94 ± 0.56[a] | 23.31 ± 0.03[c] |
| Eicosadienoic acid | 0.26 ± 0.04[b] | 0.00 ± 0.00[a] | 0.72 ± 0.02[c] |
| Eicosatrienoic acid | 6.45 ± 0.03[b] | 6.17 ± 0.03[a] | 21.18 ± 0.04[c] |
| Arachidonic acid | 0.00 ± 0.00[a] | 6.81 ± 0.05[c] | 2.34 ± 0.04[b] |

**Means with the different superscript within the same rows are significantly (*P*=0.05).**

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## 4. DISCUSSIONS

Recently, fish oil has been approved for human consumption as food supplement and as an ingredient in food. Several hygienic and scientific measures were employed, so as to improve the quality of fish oil, in conventional meal plants and in other commercial processes, where fish oil is a by-product (18). The study analysed the proficiency in yield between the three extractions methods used. According to (19), the oil yield obtained from fish extraction depends on temperature, pressure, type of sample, size of sample and, texture moisture content in the sample. The result from the extraction methods comparison, it was revealed that the soxhlet method of oil extraction gave the highest yield of 14% compared with the kiln method. Research has proven Soxhlet method as the best method for polar lipid extraction. This method can be used for the extraction of lipids and body oil triglycerides (20). (19) stated that oil extraction by soxhlet method is easier, cheaper and quicker to perform.

Direct smoking method is considered as a good old traditional and economic technique for extraction of oil. (21) emphasised that kiln method is a simple and economical technique that ensures viable results. Among the kilns, gas powered kiln had the highest oil yield. The higher extraction yield using gas powered kiln might be due to extraction at high and consistent temperature for a longer period of time, which facilitates higher extraction of oil (21). In gas powered kiln, oil extraction rate was higher at initial stage that was decreased gradually with the time elapse.

One of the major criteria to assess the quality of the oil produced is the estimation of the fatty acid composition. There are three categories of fatty acid profile consider i.e. saturated, monosaturated and polysaturated fatty acid. The fatty acid profiles showed that saturated fatty acid of the crude fish oil varied in respect of extraction methods. Similar result was reported by (22) who explained that the level of saturated fatty acid varies depending on the extraction method adopted. The saturated fatty acid was higher in gas powered kiln extraction method as observed in (23). The predominant saturated fatty acids in all the samples were stearic acid (C18:0); myristic acid (C14:0), palmitic acid (C16:0) followed by and arachidic acid (C23:0). The results of the present investigation about the predominant saturated fatty acids in the oil samples were supported by the works of (24) in the oil extracted from migratory fish (*Thunnus tonggol*) using different extraction methods. The fatty acid composition of fish lipids is also dependent on a number of factors, among which the diets of the fish play a substantial role in health benefits. (25, 26, 27).

The monounsaturated fatty acid (MUFAs) levels in the extracted oils from the marine fishes varied between the three extraction methods. MUFAs such as myristoleic and oleic were higher in the soxhlet extracted oil, except eicosarienoic acid that was very high in gas powered kiln methods. Similar levels of monounsaturated fatty acids were reported in sardine fish oil by (28), and oil extracted from Indian Mackerel (29). The dominant MUFAs in all samples were oleic acid (C18:1ω-9) and myristoleic acid (C15:1ω-5), in which the highest levels of oleic acid (C18:1ω-9) was 7.05%, obtained from sohxlet method.. MUFA seems to be proficiently extracted by methods other than direct heating and steaming method. The abundance of oleic acid (C18:1ω-9) and myristoleic acid (C15:1ω-5) in the fish oil samples were equally reported by (30) in Atlantic Mackerel (*Scomber scombrus*).

The term essential fatty acids (EFA) refer to those polyunsaturated fatty acids (PUFA) that must be provided in the food and necessary for health. There are two families of EFA, omega-3 (ω-3) and omega-6 (ω-6). The oil samples from the three extraction methods consist of the omega-3 and omega-6 polyunsaturated fatty acids namely: linoleic (ω-6), linolenic (ω-3) and Arachidonic (ω-6) fatty acids. The poly unsaturated fatty acids (linoleic and linolenic) from fish oils are rich sources of Eicosapentaenoic (EPA), Decopentaenoic (DPA) and Decosahexaenoic (DHA) (30). Fish and fish oil are the richest sources of this fatty acid with contents ranging from 39 % to 50 % for both fresh and salt water fish. Studies have shown that the omega-3 (ω-3) and omega-6 (ω-6) fatty acids have benefits on human health such as prevention of coronary heart diseases by reducing the plasma lipids, reducing cancer risk as well as prevention of toxic shock syndrome and cardiomyopathy (31).

Furthermore, (32) reported that in assessment of oil extracted from marine fishes, polyunsaturated fatty acid was dominated. In the present study, five different polyunsaturated fatty were observed in the fish oil samples by all three extraction methods. (33) reported higher levels of polyunsaturated fatty acids in Atlantic mackerel. Among Polyunsaturated fatty acids, the highest value was in linolenic acid content (23.31%) of oil obtained in soxhlet method. The composition of linolenic acid (omega 3) content in the present study was comparatively higher than that of linoleic (omega 6) which is in line with works of (34) in oil from Atlantic salmon using different extraction methods. . The linolenic:linoleic acid ratio has been suggested as a useful indicator for comparing relative nutritional values of fish oils. It was suggested that a ratio of linoleic: linolenic acid is 1:12–20 would constitute better for healthy human diet (35). The oil obtained from this study had this recommended ratio.

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## 5. CONCLUSION

**T**he soxhlet extraction method using chloroform and methanol recorded the best oil yield hence regarded as the best polar lipid extraction. Direct smoking method using gas powered kiln is considered as a good old traditional and economic technique for extraction of oil for consumption. Result has shown that the three extraction methods produced oil samples with high unsaturation and rich in omega-3 (ω-3) and omega-6 (ω-6) polyunsaturated fatty acids beneficial to human health.

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