



Fatty Acid Profile of Atlantic Horse Mackerel (*Trachurus trachurus*) oil Obtained using Different Extraction Methods

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJFAR/2022/v16i730392

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/85787>

Original Research Article

Received 22 January 2022

Accepted 02 April 2022

Published 02 April 2022

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. Chemical analyses of the oil was carried out to determine its quantity and quality in terms of fatty acid composition by 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. Based on the fatty acid profile of the oils, there are sixteen fatty acids in all the extraction methods with stearic acid (53.62%) as the highest 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 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.*

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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].

Fish consumption has increased significantly as a result of its nutritional value as well as increased consumers' knowledge of its significance in human health and illness prevention [4,5]. Fish processing and preservation are very importance because fish gets bad easily after harvest. 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.

Due to its high quality and numerous health benefits [7] fish oil is still the most valuable oil, and it is widely employed in the pharmaceutical and food industries [8]. Fish oil includes polyunsaturated fatty acids and necessary fatty acids that the body cannot generate but may be received through diet, which lower serum triglycerides, protect cardiovascular illnesses, increase learning ability, and control blood coagulation, according to [9]. They also emit eicosanoid, which is involved in a number of human metabolic pathways [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. MATERIALS 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 $100\pm 5^{\circ}\text{C}$ and then maintained at $55\pm 5^{\circ}\text{C}$ 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 when 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°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 oil was collected and then stored at room temperature for 2 days to prevent oxidation and rancidity.

2.6 Oil Clarification

To extract the oil from its entrained contaminants, it was clarified. Fish oil, water, cell debris, and non-oily particles make up the fluid collected from the press. The mixture was left undisturbed to settle by gravity, allowing the oil to separate and climb to the top because it is lighter than water. The clear oil was sieved after being decanted into a reception container.

2.7 Packaging and Storage:

To avoid rancidity, the oils were packaged and stored in clean, dry, sealed plastic bottles that were stored in a dark box.

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:

$$\text{Oil Yield} = \text{WOE}/\text{WFH} + \text{WRC} \times 100 \dots \dots \dots (1)$$

where,
 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 Na₂SO₄ to remove H₂O, and then awaited for 15 minutes. The liquid phase was separated from the oil phase, which was injected. Previously, a FAME standard mixture injection was conducted. To determine the kinds of fatty acid components in the sample, the retention time and peak of each component were measured and compared to the standard retention time. The following formula was used to calculate the amount of fatty acids in the samples:

$$\text{Component content of samples} = \frac{\text{Ax}/\text{As} \times \text{Cstandard} \times \text{Vsample}}{100} \times 100\% \dots \dots \dots (2)$$

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

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 ± 7.99 g) 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 ($P=0.05$).

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, behenic acid value for oil obtained using the soxhlet method is significantly different from the other extraction methods ($P=0.05$).

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 myristoleic, 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 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 ($P=0.05$)

Table 2. Saturated Fatty Acid Profiles of *Trachurus trachurus* Oil Obtained Using different Extraction Method (Mean \pm 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 ($P=0.05$).

Table 3. Monounsaturated fatty acid profiles of *Trachurus trachurus* Oil obtained using different extraction method (Mean \pm 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 ($P=0.05$).

Table 4. Polyunsaturated fatty acid Profile of *Trachurus trachurus* oil using different extraction method (Mean \pm SD)

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

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

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 ($P=0.05$).

4. DISCUSSIONS

Fish oil has just been approved for human consumption as a food supplement and as a food ingredient. In conventional meal factories and other commercial operations where fish oil is a by-product, several hygienic and scientific procedures were used to increase the quality of fish oil [18]. According to [19], the oil yield obtained from fish extraction depends on temperature, pressure, sample type, sample size, texture and 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 [19,20]. 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 but decreased gradually over a period of time. The rate of extraction slows due to the higher internal mass transfer resistance after the extraction of most accessible oil [19].

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 eicosatrienoic 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 (C18:1 ω -9) and myristoleic acid (C15:1 ω -5), in

which the highest levels of oleic acid (C18:1 ω -9) was 7.05%, obtained from soxhlet method.. MUFA seems to be proficiently extracted by methods other than direct heating and steaming method. The abundance of oleic acid (C18:1 ω -9) and myristoleic acid (C15:1 ω -5) in the fish oil samples were equally reported by [30] in Atlantic Mackerel (*Scomber scombrus*).

Essential fatty acids (EFA) are polyunsaturated fatty acids (PUFA) that must be consumed in order to maintain health. There are two families of EFA, omega-3 (ω -3) and omega-6 (ω -6). The omega-3 and omega-6 polyunsaturated fatty acids, linoleic (-6), linolenic (-3), and arachidonic (-6) fatty acids, are found in the oil samples obtained using the three extraction procedures. The poly unsaturated fatty acids (linoleic and linolenic) from fish oils are rich sources of Eicosapentaenoic (EPA), Decapentaenoic (DPA) and Decosahexaenoic (DHA) (30). Fish and fish oil are the richest sources of this fatty acid with concentrations ranging from 39 % to 50 % for both fresh and salt water fish. Studies have indicated that omega-3 (-3) and omega-6 (-6) fatty acids provide health benefits, including lowering blood lipids, lowering cancer risk, and preventing toxic shock syndrome and cardiomyopathy [31,32].

Furthermore, [33] 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 [34]. 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 [35] 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 [36]. The oil obtained from this study had this recommended ratio.

5. CONCLUSION

The soxhlet extraction method using chloroform and ethanol 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. The three extraction procedures yielded oil samples with high unsaturation and high levels of omega-3 (-3) and omega-6 (-6) polyunsaturated fatty acids, both of which are beneficial to human health.

6. RECOMMENDATION

Although, soxhlet methods consistently recorded the highest oil yield, it is recommended for industrial use only due to the solvents used during extraction. While the Kilns especially the gas powered kiln can be used to extract oil For consumption purposes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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