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**Research Article**


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# Proximate and physicochemical analysis of oil obtained from two fish species (fresh and frozen)

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## Abstract

The proximate composition and physicochemical analysis of oil obtained from fresh and frozen species of the African Catfish (*Clarias gariepinus*) and *Scomber scrombus* (also known as mackerel) were investigated. Oil was extracted from both fishes by solvent extraction using n-Hexane and the oil was evaluated. Mean values for the proximate composition were; moisture content 86.80 and 80.66%, crude protein 20.32 and 20.81% crude lipid 4.77 and 4.67 % ash 2.33 and 1.73% and carbohydrate 1.77 and 1.57% respectively for fresh and frozen *Clarias gariepinus*, while the mean values for fresh and frozen *Scomber scrombus* were moisture content 65.01% and 52.44%, crude protein 22.68 and 20.55% lipid content 9.43 and 9.88%, Ash 4.00 and 7.47%, carbohydrate 4.00 and 7.47% respectively. There were significant difference increase ($p<0.05$) in the moisture content, lipid, carbohydrate and protein content of fresh and frozen *Clarias gariepinus* but no significant difference was observed in the ash content. There were no significant differences in the moisture, ash and carbohydrate content of the fresh and frozen *Scomber scrombus.* However, there were significant difference increase ($p<0.05$) in protein and lipid content. Results for the physicochemical properties show that there were significant differences ($p<0.05$) in the free fatty acid (2.23mg and 1.97mg), melting point ($3^0$ C and $2.67^0$C) and peroxide value (26.83mil/eq and 26.40 mil/eq) for fresh and frozen *Clarias gariepinus* respectively. However, no significant differences were observed in the saponification value, relative density, and refractive index of fresh and frozen *Clarias gariepinus.* There were significant differences ($p<0.05$) in the saponification value (105.1mg and 98.7mg) and refractive index (0.38 and 0.40) of fresh and frozen *Scomber scrombus.* There were however, no significant differences in the free fatty acid, peroxide value, melting point and iodine value. Based on these findings, the fish oil is suitable for human consumption without any detrimental effect, it is also suitable for application in the industries.

**Keywords:** *Clarias gariepinus*, *Scomber scrombus*, proximate composition, saponification value, relative density, and refractive index.

## Introduction

Fishes are generally defined as aquatic vertebrate that are typically cold blooded animals covered with scales and equipped with two paired fins and several unpaired fins (Helfman , 1997) that use gills to obtain oxygen using gills put together and thus various kinds of fish vary greatly in shape, size and colour (Ayres, 2005; Obeagu, 2018). The African catfish *Clarias gariepinus*, is easily cultured in Nigeria and is of great economic interest. It is generally considered to be of great economic interest. It is considered to be one of the most important tropical catfish species for aquaculture. It has an almost PAN African distribution ranging from the Nile to the West Africa and from Algeria to South Africa (Osibona *et al.,* 2006). Titus fish *(Scomber scrombrus*) on the other hand are long bodied rather thick appearing fish known as high sea fish. In December 1973, a conclusion was reached by Food and Agriculture Organisation (FAO) Technical conference held in Tokyo that conversion of fish is necessary for prevention of waste (FAO, 1986). This among many other things necessitated various researches into the production of fish oil. Fish oil is currently under intensive scientific research due to its numerous health benefits. This fish oil is receiving a lot of attention because of its health benefits associated with the high levels of the long chain omega-3 polyunsaturated fatty acid (PUFA) especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Wu and Peter, 2008). Many studies have shown that fish oil can lower the coronary heart disease, stroke, hypertension, cardiac arrhythmias, diabetes, rheumatoid arthritis, photoreception (vision) and reproductive system, depression, autoimmune disorders (Douglas *et al.,* 1992; Russell *et al.,* 2005; Guy *et al.,* 2009) the most important health benefit of taking fish oil is that it is good for the functional development of infant's nervous system (nerve and brain) as well as the retina particularly in premature infants (Alexander *et al*., 2005; Dexuan *et al*., 2010). Moreover, many studies have shown that fish oil supplementation increase the DHA content of blood components (Connor, 2000). The nutritional benefits of fish oil consumption are due to the presence of protein of high biological value, unsaturated essential fatty acid, minerals and vitamins namely vitamin $B_3$ (niacin), vitamin $B_6$ (pyridoxine), vitamin $B_{12}$ (cobalamine), vitamin E (tocopherol) and vitamin D (cholecalciferol) in fish tissues (Sidhu, 2003). Fish generally encompasses all seafood including crustaceans with chitinous exoskeleton such as lobsters, crabs and shrimps such as muscle cockles and oyster (Adam and Moses, 1995). Fish is one of the most highly perishable food products, during handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product (Sallam, 2007). Marketing of fish in Nigeria is mostly carried out by local fish sellers at ambient temperature therefore the knowledge of spoilage patterns of tropical fish and their shelf life under ambient conditions is very important (Okoro *et al.,* 2010).

Some quality assessment of the oil was done; this includes peroxide value, saponification value, melting point, iodine value, free fatty acid. This is important in order to determine the stability and quality of fish oil. One of the most widely used tests for oxidative rancidity is the peroxide value, which is a measure that expresses in milliequivalents of active oxygen the quantity of Peroxide contained in 1000gram of the substance (European Pharmacopoeia, 2005).The numbers of Peroxide present in the oil is the index of their oxidative level and their tendency to go rancid. Peroxide value could influence lipase activity. Primary oxidation processes in oil mainly form hydroperoxide measured by Peroxide value. Oxidation takes place if contact occurs between oxygen and oil. The peroxide value could increase with increase in temperature (Rabiei *et al.,* 2011).The lower the peroxide value the better the quality of oil and its state of preservation (CDR, 2008).

Saponification is the process of breaking down a neutral fat into glycerol and fatty acid by alkali condition. Saponification value may be contributed by unsaponifiable matter present in leaching material such as impurities, sterols, glyceryl, ethers, hydrocarbons and fatty alcohol. Refining of the fish oil will help in removing the impurities.

According to Aubourg and Medina (1999), the free fatty acid (FFA) value has been produced during frozen storage of fish as a result of enzyme catalyst. This effect was increased with time and temperature of the leaching and with the FFA content (Miyashita and Takagi, 1986) leading to a high proportion of polyunsaturated free fatty acid (Aubourg *et al.,* 1996). Oil is in good quality if it has low free fatty acid content.

The iodine value or iodine number or index measures the number of reactive double bonds present in oil. A higher iodine value number indicates more double bonds in the sample and therefore greater care will be needed to slow down oxidation. Iodine value is an indicator of oil composition.

## Objectives

1. To extract fish oil from two fish species, fresh and frozen.
2. To determine the proximate analysis of the fishes.
3. To determine the physicochemical properties of oil obtained from two fishes; fresh and frozen.

## Materials and Methods

### Sample Collection

All fishes were purchased from the local market in Abraka, Delta state. Two fish species were used for this study. *Clarias gariepinus* (fresh and frozen ) and *Scomber scrombrus* (fresh and frozen). Both fishes are excellent food-fish marketed in fresh or frozen.

### The Fish Oil Extraction Process

The fish oil was extracted using soxhlet extractor and n-hexane as the solvent.

### Pre-treatment of Raw Material

In order to enhance a successful extraction of the oil, the fish underwent some treatment prior to extraction: These include:

**Refrigeration:** The fish when brought was refrigerated in order to preserve it since the extraction did not commence immediately.

**Washing:** The fish was thoroughly washed in order to remove dirt that might get stuck to the body after undergoing a de-freezing process.

**Size reduction:** The fishes were cut into sizes in order to enhance a speedy oven drying because of their size while removing the gills and intestine which were not needed.

**Drying:** The moisture content of the fishes was reduced by oven drying since water is immiscible in oil.

**Further size reduction:** The samples were further reduced in size and later blended into a finer form by pounding in a mortar after undergoing the moisture content elimination in the oven.

**Weight:** The weights of the samples were taken accordingly, noting the difference in weight due to weight loss through evaporation process.

### The Extraction Process

The fish oil was extracted using soxhlet extractor and n-hexane as the solvent .The solid substance or sample was placed in a thimble covered with cotton wool and the weight of the sample taken before it is placed in the inner tube of the apparatus and then fitted to a round bottom flask of appropriate size that contained the solvent. Heat was applied to heat the solvent to its boiling point for 1 hour. As the heating continued, the solvent in the flask started boiling just within 5 minutes of heating and the water begins to drop from the top to the sample in the thimble. When the solvent reached the top of the tube, it siphoned over into the flask and thus removes the portion of the oil which has been extracted in the process of refluxing. It was noticed that 18 minutes later, after boiling had started, there was refluxing and this continued at 2 minutes interval.

The solvent used was later recovered by applying heat and collected above the round bottom flask into the soxhlet apparatus while the oil extracted was collected and measured.

### Proximate Analysis

### Determination of Moisture Content

Moisture was determined by drying the sample at $+105^0$ in an oven. By subtraction the moisture was calculated. For determining moisture content, the weight of the aluminium dish was taken. Differences between two weights, of the sample was ascertained .Then the dish with sample was put in a controlled oven and was dried at $105^0$C until a constant weight was achieved. The percentage moisture content was calculated using the following equation:

$$\text{Moisture (\%)} = W_2 - W_3 / W_2 - W_1 \times 100$$

Where
W1=initial weight of empty crucible
W2= weight of crucible and sample before drying
W3= Final weight of crucible and sample after drying

### Determination of Ash Content

Ash was determined by muffling the sample at 6000-7000$^0$ C to dry ash. First, clean porcelain crucibles were heated in a muffle furnace at 6000$^0$C and the crucibles were then weighed until a constant weight was obtained. The sample with the crucible was weighed and recorded. The sample was ignited at 600$^0$ C for about 6 hours until the residue was uniformly grayish to white. Afterwards crucibles were transferred to a dessicator to cool them at room temperature for few minutes. Final weights of the crucible were recorded .The following equation was used to determine the ash content of the dry fish samples:

Ash(%) = weight of ash /weight of sample ×100

$$W_3\text{-}W_1/W_2\text{-}W_1 \times 100$$

Where:
W1= weight of empty crucible
W2= weight of crucible + sample before ashing
W3= weight of crucible + ash

### Determination of Crude Protein

To a conical flask 0.2g of sample was added, 20ml of $H_2SO_4$ (concentrated) was also added. To the mixture, 5ml of $CUSO_4$ (0.2N) was added .This was allowed to digest for thirty minutes and greenish blue colour was observed. This was diluted by adding 10ml of water, and to the mixture 5ml of 2% boric acid was added, and 2 drops of phenalpthalein indicator was added to the mixture. From the digested material, 10ml was obtained and to that was added 20ml of NaOH (40%) and titrated against (0.1N) HCl.

The following equation was used to obtain amount of protein

N(%) = (Volume of HCl ×normalityHCl×0.014×100)/weight of sample
Protein (%) = %Nitrogen×6.2

### Determination of Lipid

The AOAC (1984) methods were used. To a thimble 5g of sample was placed and placed on into a soxhlet apparatus containing tissue paper. A 500ml round bottom flask was attached to the base of the extractor and clamped to a retort stand. A 300ml volume of n-hexane solvent was poured into the thimble and the assemble unit placed on an electrothermal heater with the top of the extractor connected to the reflux condenser. The source of heat was turned on as well as water source supplied to enable the solvent in the flask to boil and extract the lipid in the sample for about three hours. On completion, the thimble was removed and the solvent reclaimed by distillation.

The flask and extracted lipid was placed in an oven at 70$^0$C for a few minute to completely remove all the solvent residues and then placed in a dessicator to cool.

The percentage of lipid was calculated using the equation below:
Where weight of lipid = Weight of flask and content after extraction – Weight of flask before xtraction

### Determination of Carbohydrate

The percentage of carbohydrate was calculated by subtracting the value obtained for percentage of the protein, fat, moisture and ash from 100.The following equation was used to determine the amount of carbohydrate.
Carbohydrate (%) = 100% - (protein + fat + moisture + ash)

### Physico Chemical Properties

### Physical Properties

### Relative Density

The relative density of oils can be said to be the measure of the ratio of the weight of a measured volume of oil to the weight of equal volume of water at the same temperature.

The bottle type method was used. A density bottle was weighed and 10 millilitres of oil was poured into the bottle. The density bottle was weighed with its present content. This was repeated for equal volume of water .The relative density was then calculated using:

Density = mass/volume

Relative density = density of sample/density of water

### Melting Point

Melting point measures the temperature at which the oil starts melting .This value serves as an indicator of the kinds of fatty acid in triglyceride. Unsaturated and low molecular weight fatty acid give low melting points while saturated and high molecular weight fatty acid give high melting points.

Into a beaker 10 millimetres of oil was poured and was placed in a freezer to freeze before inserting thermometer into the beaker. The oil was heated and the temperature at which the oil started melting was recorded.

## Refractive Index

The refractive index is a physical attribute of triglycerides which is measured by the angle through which a beam of light is bent when it is passed through on thin film of melted fat. Refractive index of an ordinary fat depends upon its glyceride structure as well as the degree of unsaturation. Refractive index increases with unsaturation and decrease with the molecular weight of the fatty acids.

## Procedure

The Abbey refractometer was used. On the polished part of the lower prism of the refractometer 3 drops of oil sample was placed. The upper prism was lowered and the oil was trapped in a thin layer between the prisms. The prism shutter was opened on the upper side. The setting control was adjusted as well as the adjustment knobs until borderline of light was cut across sharply on looking into the telescope. The borderline was adjusted such that it was dead on the intersection of the cross wire .The circular shutter on the left was opened to illuminate the scale and the refractive index was read off. The temperature at which the refractive index was measured was also recorded.

## Iodine Value

The iodine value is expressed as the number of grams of iodine absorbed by 100 grams of fat or oil. The unsaturated glycerides of oil or fats have the ability to absorb a definite amount of iodine and thus form a saturated compound. The quantity of iodine absorbed is therefore a measure of the unsaturation of that oil or fat. Also, the greater the degree of unsaturation (that is the higher the iodine value), the greater the ability of the oil or fat to go rancid by oxidation.

Procedure: Into a 250 millilitres conical flask, 1 gram of oil was weighed and added, 10millitres of carbon tetrachloride was also added. To the mixture, 13 millilitres of Wij's solution was added. The flask was stoppered, shaked and allowed to stand in the dark for one hour after which 10 milliliters of 15% potassium iodide solution together with 50 millimeters of water were added to the flask. The liberated iodide solution was titrated with standard thiosulphate solution and was shaken vigorously. Starch was used as indicator until the blue colour disappears. A blank test was carried out without the oil under the same conditions .The iodine value was calculated using:

$$\text{Iodine value (mg)} = 12.69 \times M \times (V_b - V_a)/W$$

Where

$V_b$ = Volume of standard $Na_2S_2O_3$ solution used for blank test
$V_a$ = Volume of standard $Na_2S_2O_3$ solution used for the test sample
M = Molarity of $Na_2S_2O_3$
W = Weight of oil sample (gram)

## Free Fatty Acid Value

The acid value of an oil fat is defined as the number of milligram of potassium hydroxide required to neutralize the free acid in 1 gram of the sample .The result is often expressed as the percentage of free acidity. The acid value is a measure of the extent to which the glyceride in the oil has been decomposed by lipase action. Since rancidity is usually accompanied by free fatty acid formation, the determination is often used as a general indication of the condition and edibility of the oil which also gives an indication of the age and the quality of fat.

## Procedure

Into a 250 milliliters conical flask, 2 grams of oil was weighed and added, 100 milliliters of 95% ethanol previously neutralized with 2 millimeters of phenolphthalein indicator was also added. The conical flask was placed on a hot water bath until the oil was completely dissolved in the solvent. The hot solution was titrated with 0.1 mole KOH until a pink colour appeared which persisted for about 10 seconds .The acid value was calculated using:

$$\text{Acid value (milligram)} = 56.1 \times M \times V/W$$

Where
V= titre value
M= molarity of KOH used
W= weight in grams, of sample

## Peroxide value

The peroxide value is a measure of the peroxides contained in the oil due to their uptake of oxygen. Peroxides are not directly responsible for taste and odour of rancid fats but their concentration as represented by the peroxide value is often useful for assessing the extent of spoilage in early stages .When oxidation is well advanced in a fat system, peroxide value ceases to be reliable since their decomposition into shorter chained products has started.

## Procedure

Into a clean dry boiling tube, 1 gram of oil sample was weighed and while still liquid, 1 gram of powdered potassium iodide (KI) and 20 millimeters of solvent mixture (2 volume glacial acetic acid +1 volume chloroform) were added, the tube was placed in a boiling water such that the liquid boiled within 30 seconds and was allowed to boil vigorously. The content was poured into a flask containing 20 milliliters of 5% KI solution. This was then washed out twice with 25milliliters of water and then this was titrated with 0.002 Mole sodium thiosulphate solution using starch as the indicator .A blank test was also carried out simultaneously using the same condition.

Peroxide value is often reported as the number of milliliter of 0.0002mole sodium thiosulphate per gram of sample. If the value, so obtained is multiplied by 2,the figure then equals milliequivalent of peroxide oxygen per kilogram of sample (meq/Kg) which has greater International recognition.

Peroxide value is given by:

Peroxide value (millieq/kg) = T×M×100/Weight of sample

Where
T = titre value
M = molarity of $Na_2S_2O_3$

## Saponification Value

This is defined as the number of milligram of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1 gram of sample. Soap is formed during the saponification process. The esters of fatty of the fatty acid of low molecular weight require more alkali for saponification value is inversely proportional to the mean of the weights of the fatty acids in the glycerides present. It is therefore an index of the average molecular size of the fatty acids present.

## Procedure

Into a 250milliliter round bottom flask, 1 gram of oil sample was placed. To the sample, 25 milliliters of 0.5 mole ethanoic Potassium hydroxide solution and some boiling chips were added in the flask. The flask was connected to a reflux condenser properly fitted with a reflex tube and then connected to a source of water. Heat was applied with a heating mantle and it was refluxed for 30 minutes before the source of heat was switched off. To the mixture,2 milliliter of 1% phenalpthalein solution (indicator) was added and titrated hot with 0.5 mole HCl. A blank test was carried out simultaneously under the same conditions.

Equation for the Reaction

$HCl + KOH \ldots\ldots\ldots KCl + H_20$
Mole ratio of acid to base = 1:1
Saponification value is given by:
Saponification value (mg) = $56.1\times M\times(V_a-V_b)/W$
$= 56.1 \times 0.5\times(V_b-V_a)/1.0$
Where

56.1 = molecular weight of KOH
M = molarity of the standardized HCl solution
$V_b$ = volume of standardized HCl solution used for the blank
$V_a$ = Volume of standardized HCl solution used for the test sample
W = Weight of sample, in gram.

## Statistical Analysis

All experiments were conducted in triplicates and the statistical significance differences of mean were calculated using student's T-test. Results are expressed as means± SD. A probability value at ($p<0.05$) was considered to denote the statistically significant difference.

## Results

**Table 1: Proximate composition of fresh and frozen *Clarias gariepinus* and *Scomber scombrus***

| Sample | Moisture content(%) | Crude protein(%) | Crude lipid (%) | Ash content(%) | Carbohydrate(%) |
|---|---|---|---|---|---|
| *Clarias gariepinus* (fresh) | 86.80±0.44[a] | 20.32±0.62[a] | 4.77±0.25[a] | 2.33±0.76[b] | 1.77±0.25[a] |
| *Clarias gariepinus* (frozen) | 80.66±0.56[b] | 20.18±0.79[b] | 4.67±0.80[b] | 1.73±0.21[b] | 1.57±0.67[b] |
| *Scomber scrombus* (Fresh) | 65.01±4.37[c] | 22.68±2.12[c] | 9.43±0.81[c] | 4.00±0.20[c] | 4.00±0.20[c] |
| *Scomber scrombus* (Frozen) | 52.44±2.86[c] | 20.55±1.49[d] | 9.88±0.18[d] | 7.47±0.45[c] | 7.47±0.45[c] |

Values are means ± SD of triplicate determination
Mean values in a column that do not share the same superscript differ significantly at $p<0.05$
The superscripts a, b, c, d represent significance difference between both fish species.

Table 1 above shows the moisture content of the fish samples. The moisture content for the fish samples was 86.80% for fresh *Clarias gariepinus*, 80.66% for frozen, 65.01% for fresh *Scomber scrombus* and 52.44% for frozen. Fresh *Clarias gariepinus* had the highest value. There was significant difference ($p< 0.05$) between fresh and frozen samples of *Clarias gariepinus*. The moisture content of the frozen catfish is within the range for fish 66-81%, while that for fresh is slightly above the normal.

**Table 2: Physicochemical analysis of fish oil results**

| Sample | Free fatty acid(mg) | Peroxide value(mil/eq) | Saponification value(mg) | Melting point($^0$c) | Relative density | Refractive index | Iodine value(mg) |
|---|---|---|---|---|---|---|---|
| *Clarias gariepinus* (fresh) | 2.23±0.75[a] | 1.00±0.10[a] | 98.46±38.32[a] | 3.00±1.00[a] | 2.00±1.00[a] | 0.56±0.38[a] | 126.83±1.91[a] |
| *Claris gariepinus* (Frozen) | 1.97±0.2[b] | 1.33±1.26[a] | 120±45.21[a] | 2.67±1.53[b] | 2.33±1.53[a] | 0.44±0.09[a] | 126.40±3.99[b] |
| *Scomber scrombrus* (fresh) | 2.03±0.42[c] | 1.40±1.22[b] | 105.1±38.44[b] | 3.33±0.58[c] | 0.90±0.10[b] | 0.38±0.04[b] | 121.60±0.92[c] |
| *Scomber scrombus* (frozen) | 2.4±0.95[c] | 2.52±2.14[b] | 98.7±36.43[c] | 2.67±1.15[c] | 1.57±0.67[b] | 0.40±0.04[c] | 127.33±8.74[c] |

Values are means ± SD of triplicate determinations
Mean values in a column that do not share the same superscript differ significantly at $p<0.05$
Super scripts a, b, c, d represent significance difference between both fish species.

Table 2 shows the physicochemical properties of both fishes *Clarias gariepinus* and *Scomber scrombus*. The free fatty acid value ranges from 1.97mg for frozen *Clarias gariepinus* to 2.4mg as observed in frozen *Scomber scrombus*. There is significant difference increase at ($p<0.05$) between fresh and frozen *Clarias gariepinus*. For fresh and frozen Scomber scrombus however, there is no significant difference in their free fatty acid value.

## Discussion

The high moisture content is as a result of catfish belonging to category of fish with high water or moisture content (Gallagher *et al.*, 1991). The high moisture content of *Clarias gariepinus* shows that it is highly perishable and hence an urgent step must be taken for its protection against destructive agents like microorganisms. There was no significance difference ($p< 0.05$) between the fresh and frozen *Scomber scrombus*. The moisture content for *Scomber scrombus* was below the standard. Water is usually the component of highest value in fish as much as 80% in lean fish and 70% in fatty fish (Pearson, 1981). The lipid content in fishes rises as the moisture content decreases and vice versa as noted with *Scomber scrombus* with lower moisture content and high lipid content ranging from 9.43 to 9.88% thus falls within the fatty fish category. There is significance difference ($p< 0.05$) between the fresh and frozen *Scomber scrombrus* in their lipid content. *Clarias garipienus* on the other hand belong to the low fat fish category with lipid value (< 5%).There is significance difference ($p< 0.05$) between the fresh and frozen *Clarias gariepinus* in their lipid content. Fishes with lipid content below 5% are considered lean (Stansby 1982 and Ackman 1989) and hence *Clarias gariepinus*. The lipid content also falls within the range previously detected in fish (Mendez *et al.*, 1996). These observed values for all the samples studied suggest that the oils may be edible and utilized for industrial purposes. The data obtained for moisture and lipid content of both fishes; *Clarias gariepinus* and *Scomber scrombus* compares favorably in general with the data of Thurston *et al.* (1959).

Both fishes belong to the high protein category (15-20%) with the values ranging from 20.18 to 22.68%.The highest value was observed in fresh *Scomber scrombrus*. There were significant ($p< 0.05$) differences between the fresh and frozen Clarias gariepinus as well as the fresh and frozen *Scomber scrombrus* .The high protein content agreed with other analysis carried out by earlier researchers such as Effiong and Mohammed (2008),Mumba and Jose (2005) and Abdullahi (2001).The range obtained for protein in *Scomber scrombus* is far higher than the recommended safe level of 8% (Beaten and Swiss 1974).

The ash content for the fishes ranged from 1.73% to 7.47% with the highest value observed in frozen *Scomber scrombus*. There were no significant difference ($p< 0.05$) between the fresh and the frozen species of both fishes. The observed range of ash content indicate that both fish species are good source of minerals such as calcium, potassium, zinc, iron and magnesium (Andrew, 2011).The percentage ash content is also similar to that reported by other authors including Ssali (1988), Osibona *et al*.(2006), Oyebamiji *et al*.(2008) and Egbal (2010) were it was reported that the observed range of ash content showed that the fish species were good sources of minerals.

Magnesium is an important mineral element in connection with circulatory diseases such as ischaemic heart disease and calcium metabolism in bone (Ishida *et al.*, 2000).Potassium and Sodium are required to maintain osmotic balance of body fluid and the pH of the body; regulate muscles and nerve irritability, control glucose adsorption and enhance normal retention of protein during growth (NRC, 1989).

These values are comparable with other values obtained (Abiona *et al.*, 2015). A low free fatty acid value means that the oil is in good quality. The lower the free fatty acid value, the better the oil as the lipase content of the oil is low. One of the most widely used tests for oxidative rancidity, is the peroxide value. The number of peroxides present in oil is an index of their primary oxidation and their tendency to go rancid. The peroxide value for *Clarias gariepinus* and *Scomber scrombrus* was below the standard value of 3-20meq/Kg. The Peroxide value was 1.00 and 1.33 meq/Kg for fresh and frozen *Clarias gariepinus* and 1.40 and 2.52meq/Kg for fresh and frozen *Scomber scrombus* this value is similar to that reported by other authors(Adeniyi and Bawa 2006) indicating that the fish oil is in good quality. In general, the lower the peroxide value, the better the quality of oil and its state of preservation (CDR, 2008). However, peroxide value could decrease as secondary oxidation products appear. Most people require a value of less than 10 in marine oil but a peroxide value may need to be close to 2, depending on the market. Peroxide values above 10-20meq/Kg develop rancid taste and smells (Connell, 1995).

The change in peroxide value is an indicator of lipid oxidation (oxidation increases the peroxide value) (Ahmed and Mahendrakar, 1996). As oil goes rancid, triacylglycerides is converted to fatty acid and glycerol which increases acid number (Memon *et al*., 2010).Rancidity in oil causes undesirable chemical changes in flavour, odour and nutritional value of oil. The low acid and peroxide values of the oil is indicator of the oils ability to resist lipolytic hydrolysis and oxidative deterioration (Akanni *et al*., 2005)

Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by alkali condition. The saponification values obtained from this study were 98.46mgKOH/g and 120mgKOH/g for fresh and frozen *Clarias gariepinus*, 105.1mgKOH/g and 98.7mgKOH/g for fresh and frozen *Scomber scrombrus* respectively. The highest value was observed in frozen *Clarias gariepinus* .These values are however below the standard value for fish oil (180-200mgKOH/g). (AOCS, 1992).The values are however comparable with other authors such as (Adeniyi and Bawa 2006). Saponification value may be contributed by unsaponifiable matter present in leaching material such as impurities, sterols, glyceryl, ethers, hydrocarbons, fatty alcohol. There is no significant difference ($p< 0.05$) between the value for fresh and frozen *Clarias gariepinus*. However, there is significant difference ($p< 0.05$) between fresh and frozen *Scomber scrombrus*.

The melting point ranges from 2.67 $^0$C for frozen *Clarias gariepinus* to 3 $^0$C for fresh *Clarias gariepinus* which had the highest melting point, these values were far below the melting point of water. The relative density values ranges from 0.90 for fresh *Scomber scrombrus* to 2.00 for fresh *Clarias gariepinus* which had the highest value. There is no significant difference ($p< 0.05$) between the relative densities of both fishes.

The refractive index of the oil obtained from the fishes was 0.56 and 0.44 for fresh and frozen *Clarias gariepinus* and 0.38 and 0.40 for fresh and frozen *Scomber scrombrus*. This value is below the standard for oils which is 1.4-1.473 as compared with other works (Adeniyi and Bawa 2006; Abdulkadir *et al*., 2010). This could mean a less oil. There is no significant difference at ($p<0.05$) for *Clarias gariepinus*, however, significance difference ($p< 0.05$) is observed for the *Scomber scrombrus*.

The iodine value measures the number of reactive double bonds present oil. The iodine value obtained ranged from 121.60 to 127.33$I_2$/100g of sample this is within the standard 120-180$I_2$/100g of sample.

There is significant difference ($p< 0.05$) between fresh and frozen *Clarias gariepinus*. However, there is no significant difference ($p< 0.05$) for fresh and frozen *Scomber scrombrus*. The iodine value of the oil could imply that few of the double bonds present has been saturated giving the oil wider applications. A higher iodine value number indicates more double bonds in the sample and therefore that greater care will be needed to slow down oxidation. Iodine value is not a measure of quality but is an indicator of oil composition. The iodine value obtained is higher than that obtained by (Abiona *et al*., 2015).It is however comparable with that obtained by (Adeniyi and Bawa 2006).

## Conclusion

In general, the proximate analysis of both fishes showed that there were significant differences between the fresh and frozen species of both fishes (*Clarias gariepinus* and *Scomber scrombrus*) this indicates a variation in their nutrient composition. Fresh *Clarias gariepinus* had a higher content of moisture, protein, lipid, ash and carbohydrate than the frozen fish. Hence, fresh *Clarias gariepinuis* recommended for consumption due to its high nutritional content. Nutrient loss in frozen fish (*Clarias gariepinus*) could be due to the method of preservation; freezing. Fresh *Scomber scrombrus* had a higher proportion of moisture and protein than frozen *Scomber scrombus* and is recommended for human consumption. The results of the physical and chemical properties of oil determined were comparable with the standard values, nevertheless, some were outside the normal range. From the experiments it could be concluded that *Scomber scrombrus* were potential useful source of fish oil which could be a source of economically useful omega-3 polyunsaturated fatty acid.

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