Criteria for the extraction of fish oil

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Abstract: The effect of pre-treatment of mackery (Scomber scombrus) on oil recovery and quality was investigated. Fish oil was extracted using the screw expeller press after heat pre-treatment of the fish sample. The effect of cooking temperatures and cooking times was evaluated. It was observed that the oil yield has a positive relationship with cooking temperature. The oil yield increased with increasing cooking temperature at all the cooking times investigated and also increased with increasing cooking time from 5-15 minutes and then decreased for 20 minutes cooking time at all the cooking temperatures investigated. The results revealed that a highest yield of 22.8% was obtained when the fish was treated at 90°C cooking temperature for 15 minutes. The lowest oil yield of 16.5% was obtained for the conditions of 60°C cooking temperature and 5 minutes cooking time. Analysis of oil quality indices (e.g. colour, specific gravity, refractive index, free fatty acid value, saponification value. iodine value, peroxide value, unsaponifiable matter and microbiological analysis) of oil extracted by non pre-treatment and heat pre-treatment indicated that mackery oil from both processes has comparatively similar fatty acids composition. In addition, mackery fish oil that was extracted by heat pre-treatment and mechanical press had lower lipid oxidation compared to several standards.

Keywords: criteria, fish oil, pre-treatment, extraction, oil yield

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1 Introduction

Fish oil is the lipid fraction extracted from fish and fish by products. Generally, fish oils are more complex than land-animal oils or vegetable oils due to long-chain unsaturated fatty acids (Abdulkadir et al., 2010). Fish oils are unique in the variety of fatty acids of which they composed and their degree of un-saturation (Adeniyi, 2006). Refined fish oils are rich in polyunsaturated fatty acids of the linolenic acid family (Hee et al., 2008). Current medical research suggests that these fatty acids might have a unique role to play in prevention of coronary artery disease and the growth of different types of cancers (Kris et al., 2002; Correa et al., 2008). Fish oil is important not only for their application in food, but also for industrial applications, such as leather tanning, production of pharmaceuticals, cosmetics, paints, soap, glycerol and other products (Hee et al., 2008).

The consumption of omega-3 fatty acids derived from fish oil, either in fish or as encapsulated fish oil has been shown to help maintain health, especially cardiovascular health (Zhong et al., 2007). Benefits have been shown in ameliorating inflammatory disorders, kidney-related problems, diabetes, cirrhosis, mental impairment, cataracts, glaucoma, stomach ulcers, pancreatitis, asthma, dyslexia, eczema, obesity, weak bones, rheumatoid arthritis, cystic fibrosis, sickle cell disease and preventing weight loss caused by some cancer drugs (Fournier et al., 2007). But perhaps the most important effects in the future will be in neurological development and mental health, including cognitive function (Valeria et al., 2010). With the important function of EPA and DHA as a component of brain and nervous tissue, and in particular in the development of these organs, dietary omega-3 fatty acids inclusion has an important role to play in the last trimester of pregnancy and in infant nutrition (Zuta et al., 2003; Ruxton et al., 2004; Lee et al., 2012).

Each of the oil extraction methods such as the screw

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press, hydraulic press and the solvent extraction has its advantages, design variations, operation size, technical implication, cost, personnel safety and through put reliability. Fish can be reduced to meal and oil in a number of ways. Common to all methods of practical importance are the following processing steps:

i. Heating, which coagulates the protein, ruptures the fat deposits and liberates oil and physico-chemically bound water;

ii. Pressing (or occasional centrifugation), which removes a large fraction of the liquids from the mass;

iii. Separation of the liquid into oil and water (stick water). This step may be omitted if the oil content of the fish is less than 3%;

iv. Evaporation of the stick water into a concentrate (fish solubles);

v. Drying of the solid material (press cake) plus added solubles, which removes sufficient water from the wet material to form a stable meal,

vi. Grinding the dried material to the desired particle size.

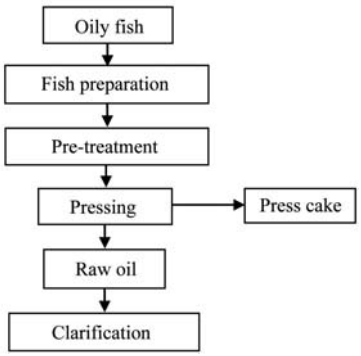


Figure 1 The basic steps involved in processing fish oil by mechanical screw pressing (Adapted from Anna et al., 2012)

Numerous different standards with variable acceptable levels for fish oil quality have been established (Ceriani et al., 2008). The objectives for all those standards are to control the quality of fish oil properties from potential biological and chemical hazards related to oxidation (Farhoosh et al., 2008). High level of PUFA causes fish oil easily to hydrolytic spoilage especially oxidative deterioration (Boran et al., 2006). Oxidation of lipids lead to some disadvantage including rancid odours and flavours, reducing nutritional quality and safety, which may cause health hazards (Suja et al., 2004).

According to Mousavi et al. (2012) different physical and chemical parameters of edible oil were used to monitor the compositional quality of oils. These physicochemical parameters include iodine value (IV), saponification value (SV), viscosity, density and peroxide value (PV). Edible oils are one of the main constituents of the diet used for cooking purposes. Several researchers studied the impact of temperature on the stability, viscosity, peroxide value, and iodine value to assess the quality and functionality of the oil (Li et al., 2010). Heat pre-treatment improves oil quality. The exposure of material to mild temperatures preserves most thermolabile compounds from degradation reactions (Jinfeng et al., 2011). In order to determine the stability and quality of fish oil extracts, some quality assessment was conducted.

Fish and fish tissues possess relatively high autolytic activities and high polyunsaturated fatty acid content which are prone to both lipolysis and oxidation (Bimbo, 1998). Therefore, the oils extracted from fish have a high free fatty acid content which is problematic during omega-3 extraction or biodiesel production. The allowable limit of free fatty acids value for crude fish oil set by the International Association of Fish Meal and Oil Manufacturers (IFOMA) according to Bimbo (1998) is in the range of 1%-7% but usually 2%-5%. Primary oxidation of oil and the measurement of hydroperoxides are determined by peroxide value analysis (Dave et al., 2014). The allowable limit of peroxide value set by the Global Organization for EPA and DHA (GOED) and Food and Agricultural Organization of the United Nations (FAO) for quality and acceptability of fish oils for human consumption is <10 meq kg^{-1} (FAO/WHO, 2013).

The allowable limit of colour (Gadner), specific gravity, refractive index, saponification value, iodine value, peroxide value and unsaponifiable matter value set by the American Society for Testing and Materials (ASTM, 1952) are 2-3, 0.907-0.915, 1.400-1.473, 175-201 mg KOH g^{-1}, 82-88 Iodine 100 g^{-1}, <10 $mEqO_2$ kg^{-1} and 0.3%-0.7% respectively. According to IFOS (2011), PV should not be exceeding 3.75 meq kg^{-1}.

The allowable limit of bacterial counts for pre-cooked breaded fish products set by the International Commission on Microbiological Specifications for Foods

(ICMSF, 1986) is $<5\times10^5$.

1.1 Justification of the study

Fish oil is usually obtained by various extraction techniques. Several studied on methods and conditions for fish oil extraction have been conducted in the past including solvent extraction, wet rendering, dry (steam) rendering and wet pressing methods. Extraction and purification of the lipids by conventional methods, such as hexane extraction, vacuum distillation, or conventional crystallization have the disadvantages of requiring high temperature processing which results in decomposition or degradation of the thermally labile compounds and/or employing toxic solvents having adverse health effects (Maqsood et al., 2012). Therefore, various research efforts are currently focusing on developments in the field of oil extraction and purification technologies. The demands on these processing technologies for extracting and purifying the fish oil are that they are eco-friendly and able to provide high oil yields and to minimize the loss of nutrients and provide a high quality oil (Maqsood et al., 2012).

Although the emphasis has been on the marketability of the free fatty acid related health benefits of fish oil, it is known that fish body oil and fish liver oil contain other interesting components, such as vitamin A and D. With improved separation techniques and more gentle processing methods, these oils might play an even more important role in the pharmaceutical and food industry in the near future (Adeniyi, 2006).

Although work has been done on fish oil production but literature has shown that little or no work has been done in terms mechanical process of fish oil extraction, characterizing and comparing oil products with standards.

1.2 Objectives of the study

(1) Main objective

The main objective of the study is to evaluate the mechanical process of fish oil extraction using a single screw oil expeller.

(2) Specific objectives

The specific objective of the study is to:

(i) Extract the fish oil from a marine fish,

(ii) Investigate the effect of pre-treatment on extraction yield,

(iii) Analyze the quality of the extracted oil and compare the test results with internationally accepted standards.

2 Materials and Methods

2.1 Experimental materials

For this research work, oily fish species, Atlantic mackery (Scomber scombrus) were obtained fresh from the market in Makurdi.

2.2 Experimental design

The experimental design for the statistical analysis follows a two-treatment effect (cooking temperature and cooking time) in a split-plot factorial design with Completely Randomized Design (CRD) involving a two-way classification with three observations (replications) per experimental unit. The experimental unit comprises two factors; four cooking temperatures (60°C, 70°C, 80°C and 90°C) in each of the four levels of the cooking times (5 minutes, 10 minutes, 15 minutes and 20 minutes) giving a sixteen (16) treatment combinations and forty eight (48) observations for the experiment as cooking temperature versus cooking time. The cooking temperature in the combination forms the levels of factor ‘A’ while cooking time forms the levels of factor ‘B’.

2.3 Determination of percentage by weight of oil in fish sample

Total oil content was determined using methanol/ chloroform extraction (Bligh and Dyer, 1959). A representative sample of fish tissue (50 g) was homogenized in a blender for 2 minutes with a mixture of methanol (100 mL) and chloroform (50 mL). Then 50 mL of chloroform was added to the mixture. After blending for additional 30 seconds, distilled water (50 mL) was added. The homogenate was stirred with a glass rod and filtered through a Whatman no.1 filter paper on a Buchner funnel under vacuum suction. 20 mL chloroform was used to rinse the remainder. The filtrate was allowed to settle to separate into the organic and aqueous layers. The chloroform layer containing the lipids was transferred into another beaker and 3 g of anhydrous sodium sulphate was added to remove any remaining water. The mixture was filtered through a Whatman no. 1

filter paper and chloroform was used to rinse the remainder. Finally, a known amount of Butylated hydroxytoluene (BHT) of about 0.02 g was added to the lipid solution as an antioxidant. The solution was then evaporated to a constant weight in a tared 100 mL round-bottom flask with a rotary evaporator at 40°C. Results were expressed as grams of lipid per kilogram of samples.

$$\text{Fat content (\%)} = \frac{\text{Final weight of flask contents in grams}}{\text{Weight of sample}} \times 100\% \quad (1)$$

2.4 Fish oil extraction

2.4.1 Raw material preparation

Atlantic mackery (Scomber scombrus) was used in this experiment. The fish sample was thawed and cut into small pieces using stainless steel knife. Prior to analysis, the internal organs were removed and the fish was washed to remove the residual blood.

2.4.2 Pre-treatment

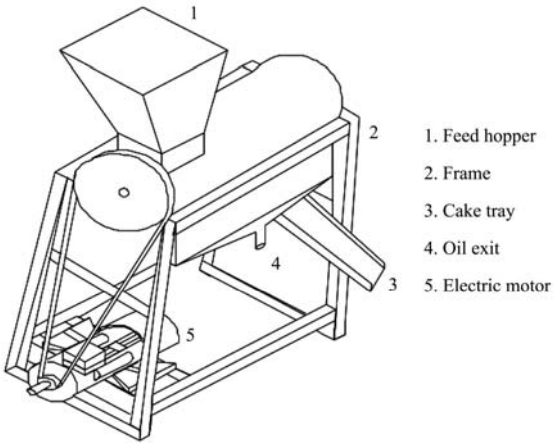
The material was heated to 60°C-90°C for approximately 5-20 minutes. This process coagulates the proteins and disrupts the cell membranes thus allowing the leakage out of bound water and oil.

2.4.3 Oil extraction

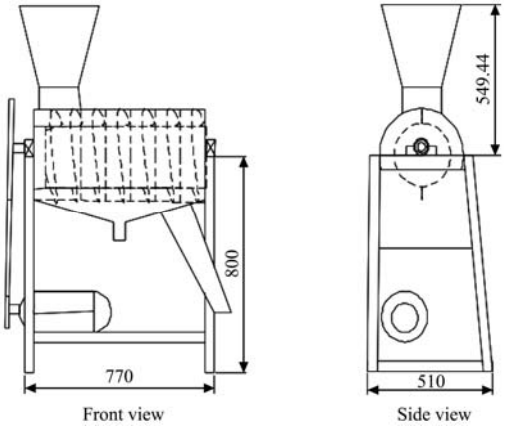
The oil extraction was done mechanically with an oil expeller press. The expeller powered by a 5hp electric motor was set into operation and known weights (4000 g) of each prepared sample were fed into the machine through the feeding hopper. The interrupted helical screw drum conveyed, crushed, squeezed and pressed the fishes in order to extract the oil. The oil and water phases (containing water-soluble proteins as well) are separated from the solid phase (press cake). The fluid extracted and the press cake were collected and weighed separately. Figure 2 shows the isometric and orthographic drawings of the expeller press.

(1) 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, sieved and heated to remove moisture in the oil.

(2) 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. Figure 3 is photograph of the mackery and extracted mackery oil in plastic bottles.



a. Isometric drawing



b. Orthographic drawing

Figure 2 Isometric and orthographic drawings of the expeller press

a. Mackery

b. Mackery oil

Figure 3 Photograph of the mackery and extracted mackery oil in plastic bottles

2.5 Method of evaluation

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

$$O_Y = \frac{100W_{OE}}{W_{FE} + W_{RC}}\% \quad (2)$$

where, W_{OE} = Weight of oil extracted; W_{FE} = Weight of fish extract; W_{RC} = Weight of residual cake.

2.6 Analysis of fish oil quality

The evaluation of the oil involves the analysis and testing needed for the assessment of the quality, purity and as well as the identification of the oil. A number of physical and chemical constants were established for these purposes. Each of the constituents used in examining the oils and fat is chosen to measure one of the characteristics of the glycerol or fatty acids present in the oil.

2.6.1 Physicochemical properties of the fish oil

The physicochemical properties of the oil are determined to assess its quality and purity. A number of physical and chemical constants have been established for these purposes. These include colour, specific gravity, refractive index, free fatty acid content, saponification value, iodine value, peroxide value, unsaponifiable matter, anisidine value, viscosity and moisture content. Eight oil quality indices were used to determine the quality of the extracted oil. They are the colour, specific gravity, refractive index, free fatty acid content, saponification value, iodine value, peroxide value and unsaponifiable matter. The experiments were replicated three times.

2.6.1.1 Determination of colour

The colour of test oils were measured by AOCS (1992) official methods as absorbance of 50% (v/v) solution of sample with n-hexane (1:1), then filtered through 0.45 μm millipore membrane filters. The filtered samples were placed in vacuum dissector to remove n- hexane and the absorbances were measured at 420 nm with the help of Shimadzu UV-160 Spectrophotometer.

2.6.1.2 Determination of specific gravity

Density bottle was used to determine the density of the oil. A clean and dry bottle of 25 mL capacity was weighed (W_0) and then filled with the oil, stopper inserted and reweighed to give (W_1). The oil was substituted with water after washing and drying the bottle and weighed to give (W_2). The specific gravity ($Sp.gr$) was obtained from the expression (AOCS, 1992):

$$Sp.gr = \frac{W_1 - W_0}{W_1 - W_0} = \frac{\text{Mass of the substance}}{\text{Mass of an equal volume of water}} \quad (3)$$

2.6.1.3 Determination of Refractive Index

Refractometer was used in this determination. Few drops of the sample were transferred into the glass slide of the refractometer. Water at 30°C was circulated round the glass slide to keep its temperature uniform. Through the eyepiece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index (AOCS, 1992).

2.6.1.4 Determination of free fatty acids (FFA) and Acid value

FFA value was determined according to the method describe in AOCS method (AOCS, 1992). An amount of 5 g oil sample was mixed with 75 mL of 95% neutral ethyl alcohol and swirled. Phenolphthalein was added as indicator. The solution was titrated with 0.1 N sodium hydroxide until pinkish colour was observed at end point. Percentage for FFA was expressed as oleic acid. FFA was calculated (Laila et al., 2014) as:

$$\%\ \text{FFA as oleic acid} = \frac{\text{alkali volume (mL)} \times \text{alkali normality} \times 28.2}{\text{Sample weight (g)}} \quad (4)$$

Acid value =% FFA × 1.99 (Laila et al., 2014).

2.6.1.5 Determination of saponification value (SV)

The saponification value (SV) of the fish oil was determined following procedures described in AOCS method (AOCS, 1992). Oil sample (1 g) was dissolved in 15 mL of 1.0 N ethanolic potassium hydroxide. The mixture was refluxed for 30-40 minutes until oil droplets disappeared and was left to cool to room temperature. Phenolphthalein indicator was then added and the hot soap solution was titrated with 0.5 N HCl until a pink endpoint was reached. A blank titration was also carried out in the same manner except no oil was added. Saponification value was calculated using Equation (5)

(Abiona and Shola, 2015).

$$\text{Saponification value } (SV) = \frac{56.1(a-b)\times N}{W} \quad (5)$$

where, a = Volume (mL) of 0.5 mol L^{-1} hydrochloric acid consumed in the blank test; b = Volume (mL) of 0.5 mol L^{-1} hydrochloric acid consumed in the test; N = Normality of hydrochloric acid; W = Weight of oil sample, g.

2.6.1.6 Determination of Iodine Value (IV)

Iodine value was determined following the method of the AOAC as described by AOAC (2002), Othman and Ngassapa (2010). Fish oil sample (0.1 g) was weighed into a conical flask and 20mL of carbon tetra chloride was added to dissolve the oil. Then 25.0mL Hanus solution was added and sealed. It was shook for one minute, kept sealed and left in a dark room (about 20°C) for 30 minutes. 10.0 mL of 15% potassium iodide and 100 mL water were added, sealed and Shook for 30 seconds. The mixture was titrated with 0.1 mol L^{-1} sodium thiosulfate to obtain iodine value. Likewise, blank test was performed to obtain blank level. The iodine value was obtained using Equation (6):

$$\text{Iodine value } (IV) = \frac{127(a-b)\times N}{10W} \quad (6)$$

where, a = Volume (mL) of 0.1 mol L^{-1} sodium thiosulfate consumed in the blank test; b = Volume (mL) of 0.5 mol L^{-1} sodium thiosulfate consumed in the test; N=Normality of sodium thiosulfate; W=Weight of sample.

2.6.1.7 Determination of Peroxide value (PV)

The Peroxide values (PV) of fish oil were determined according to AOAC method (AOAC, 2005). Oil sample (5 g) was weighed into a 200 mL conical flask and mixed with 30 mL of glacial acetic acid and chloroform (3:1) and mixed thoroughly by swirling the flask. Saturated potassium iodide (0.5 mL) was then added and the mixture was left in the dark for 1 min with occasional swirling, followed with further addition of 30 mL distilled water. The mixture was titrated with 0.1 N sodium thiosulphate solution with 1 mL of 1.0% soluble starch as indicator until the blue colour disappeared. A blank sample titration was also carried out in the same manner but with no oil added. The peroxide value (milliequivalents peroxide/1000 g sample) was calculated as (Laila et al., 2014):

$$\text{Peroxide value} = \frac{(a-b)\times N\times 1000}{\text{Weight (g) of the sample}} \quad (7)$$

where, a = Volume (mL) of 0.1 mol L^{-1} sodium thiosulfate consumed in the blank test; b = Volume (mL) of 0.1 mol L^{-1} sodium thiosulfate consumed in the test; N = normality of sodium thiosulfate solution per 1000 g of sample.

2.6.1.8 Determination of unsaponifiable matter content

A mixture of 5 g of the fish oils and 50 mL 1.0 N ethanolic KOH was saponified in a capped flask in an oven for 1 hour at 95°C. After cooling, 100 mL of distilled water was added and mixed. The resulting solution was extracted 2 times with 100 mL diethyl ether. The upper organic layers were combined and washed twice with 75 mL distilled water, once with 100mL 0.5 N ethanolic KOH, and then 100 mL distilled water until neutrality. The organic layer was then separated and dried over Na_2SO_4. After filtration of this solution, the solvent was evaporated to dryness under vacuum at 45°C. To purify more effectively, the dry unsaponifiable matters was dissolved with chloroform and, after filtration, was evaporated to dryness under vacuum at 45°C. The residue was dissolved in 50 mL of warm ethanol which has been neutralised to a phenolpthalien end point and was titrated with 0.02 N NaOH. Unsaponifiable matter was obtained from Equation (8) (Lozano et al., 1993);

$$\text{Unsaponifiable matter} = \frac{100(A-B)}{W} \quad (8)$$

where, A = Weight in g of the residue; B = Weight in g of the free fatty acids in the extract; W = Weight in g of the sample.

Weight in g of the free fatty acids in the extract as oleic acid = 0.282 VN. Where, V = Volume in mL of standard sodium hydroxide solution, N = Normality of standard sodium hydroxide solution.

2.6.2 Microbiological analysis of the fish oil

2.6.2.1 Determination of total viable count (TVC) using the pour-plate method

Iron agar (IA) containing sodium thiosulfate and L-cysteine was used for determination of total psychrotrophic bacteria and H_2S – producing organisms. The fish oil sample (1 mL) was first dissolved in 9 mL of

distilled water resulting in 1/10 dilution and diluted further using three tubes with 9 mL buffer from 1/100, 1/1000 and 1/10000 dilution. One plate was used for each dilution. Of each dilution used, 0.1 mL was transferred with pipettes to the plates. Melted 45°C iron agar was poured on the plate and the content was mixed and allowed to solidify. After solidification the plates were covered with overlay of iron agar and incubated at 37°C for 24-48 hours. Count was done using hand lens. The colony forming unit (CFU mL^{-1}) was calculated according to the Methods of American Public Health Association (1992) from Equation (9):

$$Cfu = \frac{N}{VD} \tag{9}$$

where, N = number of colonies; V = volume of diluents; D = dilution factor.

2.6.2.2 Determination of total coliforms and faecal coliforms counts

Lauryl sulphate tryptose (LST) broth was used as a pre-enrichment media and brilliant green lactose bile (BGLB) broth was used for total coliforms and Escherichia coli (EC) broth for faecal coliforms. Three tubes of LST media was used for each dilution, and each dilution was transferred with pipettes to tubes, for first three tubes using 10 mL from 1/10 dilution and then from other six tubes using 1 mL from 1/100 and 1/1000 dilution and were incubated at 35°C for 48 hours. After primary incubation one loopful of positive tubes (gas formation tubes) were transferred to BGLB media for total coliforms (incubation at 35°C for 48 hours) and EC broth for faecal coliforms (incubated at 44.5°C for 24 hours). Results were expressed as CFU mL^{-1} (colony forming units mL^{-1}).

2.7 Data analysis

All experiments were carried out in triplicates. Data collected were subjected to analysis of variance (ANOVA) to test for significant effects at 95% confidence limit using the procedure recommended by Steel and Torrie (1980). When significant difference was observed, treatment means were separated using the F-LSD.

3 Results and Discussion

The effect of heat pre-treatment on oil extraction yield by mechanical screw press was carried out using fish with heat pre-treatment and no pre-treatment or untreated samples. Experimental results of the effects of cooking temperature and cooking time on fish oil yield (%) using mechanical expeller press is shown in Table 1, Table 2 shows the analysis of variance (ANOVA) of the effects of cooking temperature and cooking time on oil yield and results of the quality analysis of fish oil is shown in Table 3.

Table 1 Experimental results of the effects of cooking temperature and cooking time on fish oil yield (%) using mechanical expeller press

Cooking temperature, °C	Cooking time, mins	Oil yield,%					
		Replications			Total	Mean	Standard deviation
		1	2	3			
0	0	12.5	13.6	14.2	40.3	13.4	0.86
60	5	16.0	16.5	17.1	49.6	16.5	0.55
	10	16.8	17.2	17.4	51.4	17.1	0.31
	15	19.4	19.3	19.5	58.2	19.4	0.10
	20	18.8	18.4	18.6	55.8	18.6	0.20
70	5	17.3	16.9	17.6	51.8	17.3	0.35
	10	17.8	18.2	17.9	53.9	18.0	0.21
	15	20.8	20.6	20.9	62.3	20.8	0.15
	20	19.3	19.4	19.7	58.4	19.5	0.21
80	5	18.5	18.1	18.3	54.9	18.3	0.20
	10	18.8	19.2	18.9	56.9	19.0	0.21
	15	21.9	22.0	21.9	65.8	21.9	0.06
	20	21.3	20.5	21.4	63.2	21.1	0.49
90	5	18.8	20.1	19.3	58.2	19.4	0.66
	10	20.7	21.2	21.0	62.9	21.0	0.25
	15	22.9	22.6	22.8	68.3	22.8	0.15
	20	22.3	21.8	22.0	66.1	22.0	0.25

Note: FSLD: 0.523 at $P \leq 0.05$.

Table 2 ANOVA of the effects of cooking temperature and cooking time on oil yield (%)

Sources of Variation	Df	SS	MS	F- Statistic	F- Tabulated (5%)
Cooking Temperature (A)	3	77.21	25.74	257.4*	2.92
Cooking Time (B)	3	6106.51	2035.50	20355.0*	2.92
Interaction (A×B)	9	6106.51	678.50	6785.0*	2.21
Error	32	3.19	0.10		
Total	47	163.67			

Note: * - significant

3.1 Determination of percentage by weight of oil in fish sample

The oil content was found to be 27.2% in this study. According to Ackman (1989), fish can be grouped into four categories according to their fat content as lean fish (<2%), low (2%-4%), medium (4%-8%) and high fat (>8%). In terms of the oil content, the fish species

examined can be considered to be in the high fat fish category.

3.2 Effect of cooking temperature and cooking time on the oil yield

The experimental results (Table 1) showed that the mean oil yield obtained from the untreated sample was 13.4±0.86% while mean oil yield of treated sample falls between 16.5±0.55%-22.8±0.15%. From treated samples, the best oil yield of 22.8% was obtained for 90^{o}C cooking temperature and 15 minutes cooking time while the lowest oil yield of 16.5% was obtained for the conditions of 60^{o}C cooking temperature and 5 minutes cooking time. It was also observed from Table 1 that the oil yield has a positive relationship with cooking temperature. The oil yield increased with increasing cooking temperature at all the cooking times investigated and also increased with increasing cooking time from 5-15 minutes and then decreased for 20 minutes cooking time at all the cooking temperatures investigated.

This temperature trend is in agreement with previous works which attribute this behaviour of oilseed to the fact that heat coagulates the protein and reduces the viscosity of the oil thereby facilitating oil expression process as moisture reduction takes place simultaneously. At higher temperature, prolonged heat treatment causes a substantial moisture loss leading to hardening of oil seed sample which best explains the reason behind the reduction in yield at higher temperature (Alonge et al., 2003; Bamgboye and Adejumo, 2011). This observation conforms to findings on previous works carried out on dika nut (Abidakun et al., 2012), groundnut (Olajide et al., 2014), and shea kernel (Olaniyan and Oje, 2007).

From the ANOVA (Table 2) there was a significant difference in the cooking temperature, cooking time and their interaction. From the means separation (Table 1), there was significant difference in the oil yield at all the cooking temperatures investigated for the entire cooking times.

3.3 Analysis of fish oil quality

3.3.1 Physicochemical properties of the fish oil

Table 3 shows the results of the quality analysis of the fish oil. The appearance of the untreated fish oil was reddish brown (colour gadner 10) and the appearance of the treated fish oil was dark brown (colour gadner 13) due to the prolonged heating period, which often oxidizes the product (i.e. the oil) and produces a dark colour. The cake produced was of good palatable quality with good appearance and rancid free flavour and therefore suitable for feed formulation for livestock. Standard colour gadner value in fish oil according to the American Society for Testing and Materials (ASTM, 1952) is in the range of 2-3. The colour gadner values for all fish oil obtained in this study were above the ASTM standard. This indicates that in its natural form, the fish oil is only suitable in applications where bright colour is not the major consideration e.g. pigmented coatings. However, it is possible to enhance the colour of the oil by bleaching.

The specific gravity of edible oil is lower than that of water and the difference in specific gravity of edible oils are quite small, particularly amongst the common edible oils. Generally, the specific gravity of oil decreases with increase of molecular weight and unsaturation level. From the experiment conducted the specific gravity of untreated and treated fish oils are 0.91 and 0.90 respectively which are within the range of standard values of 0.907 to 0.915 for fish oil, stipulated by the American Society for Testing and Materials (ASTM, 1952).

Refractive index analysis showed that there was little difference between the value obtained for untreated oil, 1.460 and that of the treated oil, 1.415 at 30^{o}C. Comparing this result with the standard values of the American Society for Testing and Materials (ASTM, 1952), that ranges from 1.400-1.473 for fishes, the values fall within the specification. According to Chantachum et al. (2000), the refraction index is dependent on the analysis temperature and unsaturation contents of the fatty acids. They establish that high analysis temperatures showed lower refraction index values, and high unsaturation content is related to high refraction index values.

Free Fatty Acids (FFA) measures the extent of decomposition of lipase action and decomposition is accelerated by heat and light. The lower the FFA value, the better the oil as the lipase content of the oil is low (Aryee and Simpson, 2009). Free Fatty Acids are a key of hydrolytic degradation associated with off flavor and

textural changes. FFA content obtained from the untreated and treated samples are 2.43% and 2.82%, respectively. Standard FFA value in fish oil according to the International Association of Fish Meal and Oil Manufacturers (IFOMA, 1981) is in the range of 1%-7% oleic acid.

Table 3 Analysis of fish oil quality

Parameter	Unit	Experimental value		Standard value			
		Non pre-treatment	Heat pre-treatment	ASTM, 1952	IFOS, 2011	IFOMA, 1981	ICMSF, 1986
Physicochemical properties of the oil							
Colour (Gadner)		10 (Reddish Brown)	13 (Dark Brown)	2-3	-	-	-
Specific gravity		0.91	0.90	0.907-0.915	-	-	-
Refractive index		1.460	1.415	1.400-1.473	-	-	-
Free fatty acid value	%	2.43	2.82	-	-	1-7	-
Saponification value	mg KOH g^{-1}	189.21	197.37	175-201	-	-	-
Iodine value	Iodine 100 g^{-1}	95.67	110.51	82-88	-	-	-
Peroxide value	$mEqO_2$ kg^{-1}	8.05	8.35	<10	<3.75	-	-
Unsaponifiable Matter	%	0.85	0.47	0.3-0.7	-	-	-
Microbiological analysis of the oil							
Total viable count (TVC)	CFU mL^{-1}	42.0×10^1	3.0×10^1	-	-	-	$<5\times10^5$
Total coliforms count	CFU mL^{-1}	12×10^1	0.5×10^1	-	-	-	$<5\times10^5$
Faecal coliforms count	CFU mL^{-1}	0.98	0.25	-	-	-	$<5\times10^5$

FFA values for all fish oil obtained in this study were within the IFOMA standard. Acceptable levels of FFA content in fish oil ranges were between 1.8% to 3.5% (Sathivel et al., 2009), and 2% to 5% (Crexi et al., 2010) with maximum acceptability at 4% (Aryee and Simpson, 2009). This study showed that FFA value was 2.43% and 2.82% for untreated fish sample and heat pre-treated fish sample respectively. The Free Fatty Acids (FFA) values are within the acceptable range and close to the one found by Chantachum et al. (2000). The authors studied the separation and quality of fish oil from precooked and non-precooked tuna heads, obtaining FFA of approximately 3% for oils separated from non-precooked heads heated at 95^{o}C for 30 minutes. A higher percentage of free fatty acid (above 3.5%) is an indication of unsuitability of the oil for edible purpose. So the fish oil obtained might be suitable for edible purposes as it contains free fatty acid less than 3.5%.

Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by alkali treatment. Saponification value (SV) is an index of average molecular mass of fatty acid in the oil sample. It is the number of milligrams of potassium hydroxide required to neutralize the fatty acid resulting from the complete hydrolysis of 1g of the samples. Fish oil having low saponification value is less prone to rancidity. The SV obtained for the oil samples showed 189.21 mg KOH g^{-1} for untreated fish oil and 197.37 mg KOH g^{-1} for heat treated fish oil. Standard saponification value in fish oil according to the American Society for Testing and Materials (ASTM, 1952) is in the range of 175-201 mg KOH g^{-1}. Saponification values for all fish oil obtained in this study were within the ASTM standard. The values also fall in the expected range of 195–205 mg KOH g^{-1} of edible oils as specified by SON (2000). The lower value of saponification values suggests that the mean molecular weight of fatty acids is lower or that the number of ester bonds is less. This might imply that the fat molecules did not interact with each other.

Iodine value (IV) measures the degree of unsaturation in a fat or oil (Thitiphan and Waranya, 2015). It determines the stability of oils to oxidation, and allows the overall unsaturation of the fat to be determined qualitatively (Asuquo et al., 2012). The iodine value shows the number of iodine absorbed by 100 g of the oil. Based on this study, iodine value for oil extraction of fish oil was 95.67 Iodine 100 g^{-1} and 110.51 Iodine 100 g^{-1} for untreated fish and heat treated fish, respectively. The iodine values for all fish oil obtained in this study were above the ASTM standard set at 82-88 Iodine 100 g^{-1}.

This result is within the typical iodine values for fish oil, 95-118 Iodine 100 g^{-1} (Rai et al., 2010). A low iodine value contributes to its greater oxidative storage stability. The higher the iodine value, the higher the degree of un-saturation and the better the oil.

Peroxide value is the milligram equivalents of peroxide oxygen combined in a kilogram of oil. Peroxide value of oil is used as a measurement of rancidity, which occurs by autoxidation (Othman and Ngassapa, 2010). The lower the peroxide value, the lower the ability of the oil to go rancid. Peroxide values obtained were 8.05 $mEqO_2$ kg^{-1} oil and 8.35 $mEqO_2$ kg^{-1} oil for untreated fish and heat treated fish, respectively. The ASTM and WHO/FAO stipulated a permitted maximum peroxide level of not more than 10 M equivalent of peroxide oxygen kg^{-1} of the oils. Therefore, the oil in this study may be suitable for consumption since it has a peroxide value below 10. The acceleration of oil oxidation during application of heat observed by an increase in peroxide value due to presence of reactive radicals that might be formed by exposure to heat.

Unsaponifiable matter in oils and fats are the substances that are not saponifiable by alkali hydroxides but are soluble in the ordinary fat solvents and to products of saponification that are soluble in such solvents. Hydrocarbons, alcohols and sterols, and non-fatty constituents like mineral oil make up the unsaponified matter. At least 2% of unsaponifiable matter can be found in pure fats and oils. However, high values of unsaponifiable matter may indicate adulteration and contamination (Durmaz and Gökmen, 2011). The highest unsaponifiable matter content was observed in untreated fish sample (0.85%), while the lowest levels were found in preheated fish sample (0.47%). The low content of unsaponifiable matter (0.47%), suggests a low content of organic matter (sterols, hydrocarbons, pigments, phospholipids, vitamins) and, hence, a low impurities quantity. The results fall within the standard of the American Society for Testing and Materials (ASTM, 1952), which is in the range of 0.3% - 0.7%. The results also confirmed a previous report that heating was found to cause an increase in the passage of phenolic compounds to the oil whereas the level of tocopherols, phospholipids, lutein and β-carotene was decreased (Durmaz and Gokmen, 2011). These parameters indicate that the untreated fish oil and the different treatments of fish oils should have good keeping capacity.

3.3.2 Microbiological analysis of the fish oil

According to Middlebrooks et al (1988), mackerel stored at 0°C, 15°C, and 30°C have counts of about 10^6 CFU g^{-1} of flesh. The results of the microbiological analysis of fish oil showed that the total viable counts (TVC) were 42.0×10^1 $CFUmL^{-1}$ and 3.0×10^1 CFU mL^{-1} of oil for untreated and treated fish oil, respectively. The total coliforms count was 12×10^1 CFU mL^{-1} and 0.5×10^1 CFU mL^{-1} of oil for untreated and treated fish oil, respectively. Faecal coliforms count was 0.98 CFU mL^{-1} and 0.25 CFU mL^{-1} of oil for untreated and treated fish oil, respectively. According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1986), bacterial counts for pre-cooked breaded fish products should be less than 5 x 10^5 CFU mL^{-1}. The bacterial counts of all extracted fish oil were below 5×10^5 CFU mL^{-1}. The reduction in the coliforms counts of the treated fish oil is as a result of the use of heat in the oil extraction process. So the fish oil obtained might be suitable for edible purposes as it contains bacterial counts less than 5×10^5 CFU mL^{-1}.

4 Conclusions

Fish oil was extracted using the single screw expeller after heat pre-treatment of the fish sample. The result obtained shows that this process (mechanical extraction) is a suitable method for extracting fish oil because of its high yield and high oil purity, both in large or small quantity. This process also generates little or no waste since the fish cake will be used as animal feeds thereby reducing cost of waste disposal.

The effect of heat pre-treatment on extraction oil yield and oil quality from mackery (scomber scombrus) was investigated. The result revealed that heat pre-treatment could be applied rather successfully to mackery oil extraction prior to using mechanical expeller press. Pre-treatment at 90^oC cooking temperature and 15 minutes cooking time was considered as the most appropriate conditions of heat pre-treatment by giving the

best oil yield of 22.8%. It is suggested that heat pre-treatment can be used to help in oil extraction from whole fish or waste fish processing.

The oil was evaluated and some of its physical, chemicals and microbiological properties were determined. The characterization of oil from different samples of fishes was carried out and the results showed that the fish oil has a very high percentage of iodine value, hence can be recommended for patient suffering from goiter. Mackery oil was extracted by heat pre-treatment and mechanical press had lower lipid oxidation compared to several standards. These values are within the acceptable standards for edible fish oils and much better compared to several fish oil extraction methods.

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Nomenclatures

Abbreviation	Meaning
ASTM	American Society for Testing and Materials,
IFOS	International Fish Oil Standard
IFOMA	International Association of Fish Meal and Oil Manufacturers
ICMSF	International Commission on Microbiological Specifications for Foods