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Comparative Physico-Chemical Properties of Fish Oil from *Hyperopisus bebe*, *Marcusenius macrophthalmus* and *Mormyrus rume*, Family Mormyridae

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Target Audience: Fish Farmers, Fish food scientists, Nutritionists/Dieticians, Standard Food Regulatory Bodies

Abstract

A study was carried out to investigate the physico-chemical characteristics of fish oil. The experimental fish species studied were Hyperopisus bebe, Marcusenius macrophthalmus and Mormyrus rume belonging to the freshwater fish family Mormyridae. Extracted fish oil was evaluated for saponification, acid, iodine and peroxide values following standard procedures of the Association of Analytical Chemists. Mormyrus rume had the highest acidic and iodine values of 15.99 mg KOH/g and 13.01 gI_2/100g, respectively, it therefore wields a greater comparative advantage compared to the 12.28 mg KOH/g, 11.74 gI_2/100g and 15.99 mg KOH/g, 13.01 gI_2/100g obtained for M. macrophthalmus and M. rume, respectively. The three fish species were generally high in fatty acids; therefore possess great oxidative storage stability as indicated by the low iodine values. The mormyrids' oil is therefore considered safe for human consumption (peroxide value <5.0 meq/Kg). However, M. rume oil is recommended over the other two species within the family Mormyridae.

Keywords: acid value; iodine value; Mormyridae; peroxide value; saponification value.

Description of Problem

Fish is a very important part of a healthy diet. Fish and other seafood are the major sources of healthful long-chain omega-3 fats and are also rich in other nutrients such as vitamin D and selenium, high in protein, and low in saturated fat (1). Eating fish fights heart disease in several ways. The omega-3 fats in fish protect the heart against the development of erratic and potentially deadly cardiac rhythm disturbances. The strong and consistent evidence for benefits of fish oil intake is such that the Dietary Guidelines for Americans and the American Heart Association suggest that everyone eat fish twice a week (2).

There is growing trend to utilize cheap byproducts of industries to prepare valuable products for commercial purpose and value addition (3). Fish oil accounts for about 2 % of world consumption of fats and oils and is traditionally obtained as a by-product of the fish meal industry (4). It is considered as an easily available and invaluable source of long chain omega-3 polyunsaturated fatty acids, which are mainly eicosapentaenoic and docosahexaenoic acids. Fish oil obtained from oily fish has vast health benefits, which includes reduction of risk of heart attacks, strokes and autoimmune diseases like Type 1 diabetes, increase of grey matter in the brain

and prevention of asthma in children (5). Fish oils have been used as edible oils.

Mormyrid fishes (elephant snout fishes and trunk fishes) have long served humans as an important food source along Africa's inland waterways. Mormyrid fishes are characterized as demersal animals of freshwaters and prefer water with pH of 5.0. Mormyrids are increasingly becoming important in the world aquarium business and aquaculture (6).

Omega-3 polyunsaturated fatty acid is one of the essential fatty acids that human bodies cannot produce. The other one being omega-6 fatty acids (7), thus a dependence on dietary source is incumbent. The numerous health benefits associated with the consumption of fish oil is linked to its physico-chemical profile. Mormyrids have been described as oily fish; therefore an investigation into the quality of its oil is imperative. It is against this background that this study aimed at providing baseline information on the physico-chemical profile of fish oil obtained from *H. bebe*, *M. macrophthalmus* and *M. rume*.

Materials and Methods

Experimental Fish Species

Mature samples of the experimental fish species were selected from the catch of fisher folks along the dammed area of River Kubanni, Zaria, Kaduna State; estimated terrain elevation above sea level is 613 metres, latitude 11°4’46.6” longitude 7°44’15.22” (8). The species selected for this research were ray-finned freshwater demersal fishes; *Hyperopisus bebe* (Lacepède, 1803), *Marcusenius macrophthalmus* (Pellegrin, 1924) and *Mormyrus rume* Valenciennes, 1847. The fish samples were conveyed to the Fisheries Laboratory, Department of Biology, Ahmadu Bello University, Zaria. The fish samples were identified using pictorial chart and key (9, 10), then thoroughly washed, degutted, filleted and oven-dried to reduce moisture. The samples were then conveyed to the National Research Institute of Chemical Technology, Zaria for oil extraction and characterization.

Enzymatic Fish Oil Extraction

Extraction of oil from samples of the different fish species was carried out according to the method described by (11). The whole fish was minced in a homogenizer without adding water. A minced fish sample (50 g) was first placed in a 500 ml glass bottle and heated in a water bath at 90°C for 10 min to deactivate the endogenous enzymes. Then, 50 ml of 1 M potassium phosphate buffer (pH 7.5) was added to the fish in the ratio of 1:1 (fish: buffer) and mixed well using a stirrer. The total volume was found to be 100 ml. The pH of the mixture was measured and adjusted to 7.5 with 1 N NaOH. The glass bottle was then placed in a water bath shaker operating at 140 rpm and 55°C, and kept for 30 min. The temperature was measured using a thermometer. The enzymatic hydrolysis was started by adding 0.5% (by weight of raw material) alcalase. After hydrolysis for 1 h, the mixture was taken and placed in another water bath, operating at 90°C for 5 min to inactivate the enzymes.

The mixture was then allowed to cool and centrifuged at 4100 rpm for 40 min. Four layers were formed in the centrifuge tubes: upper oil layer, light-lipid layer, soluble clear protein layer and bottom sludge layer containing the remaining fish tissues, respectively. The upper oil layer was removed using a pipette and stored at -20°C.

Physicochemical Analyses

The physicochemical indices of the fish samples were carried out according to standard recommended methods (12). Clear sediment free liquid was used directly after inverting container several times. Samples were dried by adding anhydrous Sodium sulphate in the

proportion of 1 - 2 gm per 10 gm sample and held in oven at 500°C. It was then stirred vigorously and filtered to obtain clear filtrate.

Determination of Saponification Value

Saponification value is the number of mg of potassium hydroxide required to saponify 1 gram of oil fat. Sample was run through a filter paper to remove any impurities and the last traces of moisture. Sample was mixed thoroughly and about 1.5 to 2.0 g of dry sample was weighed into a 250 ml Erlenmeyer flask. Twenty-five ml of alcoholic potassium hydroxide solution was pipetted into the flask. Blank determination was conducted along with the sample. Sample flasks were connected and the blank flask with air condensers was kept on the water bath, boiled gently but steadily until saponification was complete, as indicated by absence of any oily matter and appearance of clear solution. Clarity was achieved within one hour of boiling. After the flask and condenser were cooled, the inside of the condenser was wash down with about 10 ml of hot ethyl alcohol neutral to phenolphthalein. Excess potassium hydroxide was titrated against 0.5N hydrochloric acid, using about 1.0 ml phenolphthalein indicator.

Calculation:

$$\text{Saponification Value} = \frac{56.1\,(B-S)N}{W}$$

Where, B = Volume in ml of standard hydrochloric acid required for the blank

S = Volume in ml of standard hydrochloric acid required for the sample

N = Normality of the standard hydrochloric acid

W = Weight in gm of the oil/fat taken for the test

Determination of Acid Value

Acid value refers to the number of mg of potassium hydroxide required to neutralize the free fatty acids present in 1 gram of fat. Appropriate amount of the cooled oil sample was weighed in a 250 ml conical flask and 50 ml to 100 ml of freshly neutralised hot ethyl alcohol and about 1 ml of phenolphthalein indicator solution were added. The mixture was boiled for about five minutes and titrated while hot against standard alkali solution and shaken vigorously during the titration. The weight of the oil taken for the estimation and the strength of the alkali used for titration were such that the volume of alkali required for the titration did not exceed 10 ml.

Calculation:

$$\text{Acid value} = \frac{56.1VN}{W}$$

Where, V = Volume in ml of standard potassium hydroxide or sodium hydroxide used

N = Normality of the potassium hydroxide solution or Sodium hydroxide solution

W = Weight in g of the sample

Determination of Iodine Value

An appropriate quantity of the dry oil was weighed into a 500 ml conical flask with glass stopper, to which 25 ml of carbon tetrachloride had been added and mixed well. The weight of the sample was such that there was an excess of 50 to 60 percent of Wij's (iodine monochloride) solution over that actually needed. Twenty-five ml of Wij's solution was pipetted and the glass stopper replaced after wetting with potassium iodine solution. The mixture was swirled for proper mixing and the flask kept in dark for half an hour. A blank was carried out simultaneously. After standing, 15 ml of potassium iodide solution was added, followed by 100 ml of recently boiled and cooled water, rinsing in the stopper also. Liberated iodine was titrated with standardized sodium thiosulphate solution, using starch as indicator at the end until the blue colour formed disappears after thorough shaking with

the stopper on. Blank determinations were conducted in the same manner as test sample but without oil.

Calculation:

$$Iodine\ value = \frac{12.69\ (B - S)\ N}{W}$$

Where, B = volume in ml of standard sodium thiosulphate solution required for the blank

S = volume in ml of standard sodium thiosulphate solution required for the sample

N = normality of the standard sodium thiosulphate solution

W = weight in g of the sample.

Determination of Peroxide value

Five grams (±50 mg) sample of each fish species was weighed into a 250 ml stoppered conical flask. 30 ml acetic acid chloroform solvent mixture was added and swirled to dissolve. 0.5 ml saturated potassium iodide solution was added with a mohr pipette and allowed to stand for 1 min in the dark with occasional shaking, then about 30 ml of water was added. The liberated iodine was titrated slowly with 0.1 N sodium thiosulphate solution, with vigorous shaking until yellow colour was nearly gone. About 0.5 ml starch solution was added as indicator and titration with vigorous shaking was continued to release all I_2 from $CHCl_3$ layer until blue colour disappeared. Blank determination was conducted using 0.1 ml 0.1 N $Na_2S_2O_3$.

Calculation: Peroxide value expressed as milli equivalent of peroxide oxygen per kg sample (meq/kg):

$$Peroxide\ value = \frac{Titre\ x\ N\ x\ 100}{Weight\ of\ the\ sample}$$

Where, *Titre* = ml of Sodium thiosulphate used (blank corrected)

N = Normality of sodium thiosulphate solution.

Data Analysis

SPSS version 21 statistical software package was used to run all statistical analysis (13). Descriptive statistics was used to summarize data obtained. One-way ANOVA was adopted to determine if there is significant difference ($p \leq 0.05$) in the means of physicochemical indices among the three fish species. Pearson correlation was used to test for the relationship between physicochemical parameters of the fish species (14).

Results and Discussion

The physicochemical parameters of mormyrid fishes are presented in Table 1. All physicochemical parameters varied significantly ($p<0.05$) except for iodine value. ***M.*** *macrophthalamus* had the highest saponification value of 38.85 mg KOH/g, while *M. rume* had the least, which was 9.96 mg KOH/g. The saponification value is an index of mean molecular weight of the fatty acids of glycerides comprising a fat. Lower saponification values imply larger molecular weight of fatty acids in the glycerides and vice-versa (15). *Marcusenius macrophthalmus* had the highest saponification value, which suggests that the fatty acids in the glycerides of ***M.*** *macrophthalmus* have lower molecular weights.

Acid value for the fish family ranged from 8.70 - 15.99 mg KOH/g. Acid value is a relative measure of rancidity as free fatty acids normally formed during decomposition of triglycerides. Acid value determination is often used as a general indication of the condition and edibility of the oil (16). *Mormyrus rume* oil had the highest acid value (15.99 mg KOH/g) and this value is a measure of the amount of fatty acids which have been liberated by hydrolysis from the glycerides due to the action of moisture, temperature and lypolytic enzyme lipase. The permissible level of acid value for all edible oils should be below 4.0 mg KOH/g (17).

Iodine value for the fish family ranged from 8.57 – 13.01 gI_2/100g. The low iodine values may contribute to the oils greater oxidative storage stability (18). The oxidative and chemical changes in oils during storage are characterized by an increase in free fatty acid contents and a decrease in the total unsaturation of oils (19). *Mormyrus rume* had the highest iodine values 13.01 gI_2/100g. However, the values obtained in this research are lower than that reported by (3) which ranged from 38.49–63.10 gI_2/100g. All the values for iodine values obtained in this study are lower than most edible oils of plant source except for coconut oil, which range from 8-10 gI_2/100g (20).

Peroxide value (PV) is used as a measure of the extent to which rancidity reactions have occurred during storage. It could be used as an indication of the quality and stability of fats and oils and to determine the extent to which the oil has undergone rancid (21). Peroxide value ranged from 0.15 – 0.57 meq/Kg. *Hyperopisus bebe* had the highest peroxide value, 0.57 meq/Kg. The significantly high ($p<0.05$) peroxide value for *H. bebe* indicates the extent of oxidation suffered by the oil of the fish, and this means *H. bebe* oil suffered more oxidation than the rest. Fresh oils usually have peroxide values well below 10 meq/Kg. A rancid taste often begins to be noticeable when the peroxide value is above 20 meq/Kg. The result for peroxide value of mormyrid oils indicates that all the oils are within the range that is safe for consumption. The values for peroxide value all fall within the range (5.0 meq/kg) recommended for consumption (17). High peroxide values have been reported to interfere with growth (22).

Table 2 presents the results of correlation between all physicochemical parameters. Saponification value correlated negatively with acidic and iodine values, and positively with peroxide value. Saponification value did not correlate significantly with any physicochemical parameter. Correlation of acidic value was significant ($p<0.05$) with iodine and peroxide values, which were positive and negative, respectively. Iodine value also correlated significantly ($p<0.05$) with acidic ($r = 0.823$) and peroxide ($r = -0.848$) values, and correlations were positive and negative, respectively. The correlation between peroxide value with acidic ($r = -0.887$) and iodine ($r = -0.848$) values, and were both negative. The correlation between saponification value and other physicochemical parameters indicates that only peroxide value increased as saponification value increased. The increase and decrease was however not significant. Acidic and iodine values decreased with increase in peroxide value. The only significant increase and decrease of physicochemical parameter was exhibited between peroxide, acidic and iodine values.

Conclusion and Application

It was concluded that:

1. The oil from *Hyperopisus bebe* has the least acidic and iodine value compared to the oil of *Marcusenius macrophthalmus* and *Mormyrus rume*; which suggests that the oil is of high quality and its peroxide value falls within the range termed safe for consumption by the Standard Organisation of Nigeria.
2. The oil of *Hyperopisus bebe* has a relatively high saponification value compared to those of *Marcusenius macrophthalmus* and *Mormyrus rume*, suggesting the former contains low molecular weight, short chain fatty acids hence suitable for human nutrition and soap making.

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Table 1: Physicochemical Indices of *Hyperopisus bebe, Marcusenius macrophthalmus* and *Mormyrus rume* oil

	Parameters			
	Saponification Value (mg KOH/g)	Acid Value (mg KOH/g)	Iodine Value (gI_2/100g)	Peroxide Value (meq/kg)
H. bebe	25.53 ± 0.56^{b}	8.70 ± 0.28^{c}	8.57 ± 0.95^{a}	0.57 ± 0.06^{a}
M. macrophthalmus	38.85 ± 0.42^{a}	12.28 ± 1.06^{b}	11.74 ± 0.32^{a}	0.22 ± 0.04^{b}
M. rume	9.96 ± 0.60^{c}	15.99 ± 0.28^{a}	13.01 ± 0.95^{a}	0.15 ± 0.02^{b}
P-value	0.000	0.010	0.061	0.010

Mean values with same superscripts along columns do not vary significantly ($p>0.05$).

Table 2: Pearson Correlation between Physicochemical Parameters of *Hyperopisus bebe, Marcusenius macrophthalmus* and *Mormyrus rume* oil

		SV	AV	IV	PV
SV	Correlation (r)	-	-0.608	-0.290	0.203
	P-value	-	0.201	0.577	0.700
AV	Correlation (r)	-0.608	-	0.823*	-0.887*
	P-value	0.201	-	0.044	0.018
IV	Correlation (r)	-0.290	0.823*	-	-0.848*
	P-value	0.577	0.044	-	0.033
PV	Correlation (r)	0.203	-0.887*	-0.848*	-
	P-value	0.700	0.018	0.033	-

***** Correlation is significant at $P \leq 0.05$.
Note: SV – saponification value, AV – acid value, IV – iodine value, PV – peroxide value