EXTRACTION OF OIL AND ITS PHYSICO-CHEMICAL PROPERTIES FROM *ETROPLUS SURATENSIS* (PEARL SPOT) AND *CATLA CATLA* (CATLA) FISHES FROM MARATHWADA REGION

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Abstract: Consumption of fish is very beneficial to the health and development of the human body. They provide essential nutrients to the human. Fish oil contains higher amount of polyunsaturated fatty acids which have significant effect in maintaining a healthy cardiac life. This study has been undertaken to investigate the extraction of oil and their physico-chemical properties from two fishes of Marathwada region. The extracted oil yields are 12.10% and 7.56% respectively. The physico-chemical parameter of oil has analyzed. The peroxide value, free fatty acid value, acid value and saponification value, 140.275 and 137.46 mg KOH/g, 5.35% and 3.66%, 10.64 and 7.28 mg KOH/g and 8.0 and 6.0 meq/kg respectively.

***Index Terms*- fish oil, peroxide value, acid value and saponification value.**

I. INTRODUCTION

Consumption of fish is very beneficial to health and development of the human body. They provide essential nutrients to the human being. Fish oil is the lipid fraction extracted from fish and fish by-products. Fish oil is a virtually unique source of natural long-chain omega-3 fatty acids, comprising eicosapentaenoic acid and docosahexaenoic acid. Fish oils account for about 2% of the world's consumption of fats and oils and are a by-product of the fish meal industry (Rizliya and Eresha Mendis, 2014). Fish oil contains higher amount of polyunsaturated fatty acids which have significant effect in maintaining a healthy cardiac life. It plays a crucial role in the prevention of atherosclerosis, heart attack, depression, stroke, diabetes, obesity, premature ageing, hypertension, cancer, improve the vision power and memory (Chin and Dart, 1995). Generally, fish oils are more complex than land animals due to long chain unsaturated fatty acids (Hall, 1992). Fish oil is considered as liquid oil, but in fact contains triglycerides of intermediated melting points for the oils to be partially solids at 20 ºC. Fish oils are unique in the variety of fatty acids of which they composed and their degree of unsaturation (Ackman et al., 1982). Current medical research suggests that these fatty acids might have a unique role to play in prevention of coronary artery diseases and the growth of different type of cancers. The oil is industrially used in leather tanning, production of soap, glycerol and other products. Presently, the production of fish oil is becoming more demanding as there is a sizeable and growing world market demand for high quality fish oil.

II. MATERIALS AND METHODS

A) Fishes collection

The freshwater fishes selected for oil extraction were obtained from local fish market. The fishes were transported to the laboratory in frozen condition (Bako et al., 2014).

Etroplus suratensis

Catla catla

B) Sample preparation

The fish was thoroughly washed in order to remove dirt that might get stuck on the body after taking from the market and for cleaning the fish body. After that internal organs of the fish were removed and the fish was washed to remove the residual blood. The fish fleshes were separated from bone and were sun dried for about 72 hours. The sun-dried fleshes were then grinded by mechanical grinder and stored in refrigerator until further analysis (Bako et al., 2014).

C) Oil Extraction and yield

The oil was extracted from fish of two different species. In this method, Chloroform: Methanol: Water is used in the ratio 4:2:1 ratio respectively. In this extraction procedure, 200 gm of muscle tissue powder sample of each species was taken. The sample was vortexed thoroughly in the presence of solvent mixture for 24 hrs in refrigerator. The homogenized mixture was then filtered by using whatman Filter paper No. 2 and the filtrate was collected. The tissue residue was re-extracted with the same amount of chloroform in order to get high recovery of oil. The combined filtrate solutions were poured into a separation funnel to allow separation into two phases and the organic layer containing the lipids was collected in a flask. The solvent was evaporated until all chloroform and methanol were removed (Bligh and Dyer, 1959).

The yield of recovered oil was calculated as:

$$\%\ \text{Yield} = \frac{\text{W (Oil)} - \text{W (Sample)}}{\text{W (Sample)}} \text{X } 100$$

The weight of oil in the initial sample was determined using the oil content as measured by the proximate analysis.

D) Physico-chemical analyses on extracted fish oil

i) Determination of Peroxide value of oil (PV)

The peroxide value is defined as the amount of peroxide oxygen per 1kg of fat or oil. Traditionally, this was expressed in units of milli equivalents, although if we are using SI units then the appropriate option would be in millimoles per kg. The Peroxide Value (PV) of fish oil was determined according to AOAC method. Oil sample (5 g) was weighed into a 200 conical flask and mixed with 40 ml glacial acetic acid and chloroform (3:1) and mixed thoroughly by swirling the flask. 0.5 ml of saturated Potassium Iodide was added and then mixture was placed 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 (Bako et al., 2014).

$$\textbf{Peroxide Value} = \frac{\text{(S} - \text{B)x N of sodium thiosulphate x 1000}}{\text{weight of sample}}$$

ii) Saponification Value

Saponification value is a measure of the free acid and saponifiable ester group. It is expressed as the number of milligrams of Potassium Hydroxide required to neutralize the free acids and saponify the esters contained in one gram of the fish muscle. Ester value is a measure of the saponifiable esters in the material. It is calculated as the difference between the saponification value and the acid value. Saponification of the fish oil was determined following procedures described in AOCS method. Oil sample (1 gm) was dissolved in 12.5 ml of 0.5 N Ethanolic KOH. The mixture was refluxed for 30 min. until oil droplets disappeared and was left to cool to room temperature. Phenolphthalein indicator was added and then titrated with 0.5 N HCL until the pink colour disappeared. A blank titration was also carried out in the same manner except no oil was added. The following formula is used for calculation of saponification value (Bako et al., 2014).

$$\textbf{Saponification value} = \frac{\text{(Blank} - \text{sample)x M of KOH x Mol. of KOH}}{\text{weight of sample}}$$

iii) Free fatty acids (FFA) and Acid value (AV)

The acid value is the number that express, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1 gm of the substance. The acid value may be overestimated if other acid components are present in the system. The acid

value is often a good measure of the breakdown of the triacylglycerol's into free fatty acids, which has an adverse effect on the quality of many lipids. Acid value is the measure of hydrolytic rancidity. In general, it gives an indication about edibility of the lipids. Edible oil contains> 1%. FFA was determined according to the method describe in AOCS method. 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 0.1N potassium hydroxide until pinkish colour was observed at end point. FFA values were determined from equation gives in the method. From the FFA value, acid value was also calculated. Free fatty acid and acid value calculations are used as per the formula (Bako et al., 2014).

$$\textbf{Free Fatty Acid} = \frac{(\mathrm{S} - \mathrm{B})\mathrm{x}\mathrm{N\ of\ KOH\ x\ 28.2}}{\mathrm{Weight\ of\ sample}}$$

$$\textbf{Acid value} = \frac{(\mathrm{S} - \mathrm{B})\mathrm{x\ N\ of\ KOH\ x\ 56.1}}{\mathrm{Weight\ of\ sample}}$$

III. RESULTS AND DISCUSSION

A) Yield of fish oil from the muscle tissue

In the table 1 shown yields of oil extracted from fishes *Etroplus suratensis* and *Catla catla* are 12.10% and 7.56% respectively. Bligh and Dyer method has been recognized as the most reliable method currently available for total lipid extraction (Bailey and Wells, 1998.). Bligh and Dyer method uses polar solvent, chloroform and methanol mixture to extract the oil from fish body muscle tissue. The polar solvents can penetrate into the cells and extract the lipid from the cell membrane and muscle fibres including the phospholipids material (Bligh, and Dyer, 1959).

Table 1. Shows the % yield of fishes

Fishes	**% Yield**
Etroplus suratensis	12.10
Catla catla	7.56

B) Physico-chemical properties of fish oil extracts

In order to determine the stability and quality of fish oil extracts, some quality assessments were conducted. These results are shown in table. 2, 3 and 4. Undeland et al. (1998) indicated that unsaturated character of the lipids and the strong pro-oxidative systems naturally present in fish tissue could cause susceptibility of lipids to oxidize during processing and storing especially for the two fish species viz. *Etroplus suratensis* and *Catla catla*.

Table 2. Showing the Calculated Peroxide Values (n – 3) for peroxide in the muscle tissue of the fishes

Fishes	**Tissue**	**PV value** (meq/kg)	**Standard value by AOCS**
Etroplus suratensis	Muscle	8.0	3-20 (meq/kg)
Catla catla	Muscle	6.0	

Young (1993) has reported that peroxide value (PV) of crude fish oil was between 3-20 meq/kg. In this study, the PV was found to be 8.0 meq/kg and 6 meq/kg respectively, which is well below acceptable limit of 20 meq/kg oil. This indicated that the fish oil extracted had low lipid oxidation rate.

Table 3. Showing the Saponification Values of (n – 3) in the muscle tissue of the fishes

Fishes	**Tissue**	**SV**(mg KOH/g)	**Standard values by AOCS**
Etroplus suratensis	Muscle	140.275	180-200 (mg KOH/g)
Catla catla	Muscle	137.46	

Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by alkali treatment. The SV of fish oil obtained in this study was lower (140.27 mg KOH/g and 137.46 mg KOH/g respectively) than standard value for fish oil (180-200 mg KOH/g), as indicated in AOCS.

Table 4. Showing the Fatty Acids and Acid Values of (n – 3) in the muscle tissue of the fishes

Fishes	**Tissue**	**FFA (%)**	**Acid Value** (mg KOH/g)	**Standard value by AOCS**
Etroplus suratensis	Muscle	5.35	10.64	7-8 (mg KOH/g)
Catla catla	Muscle	3.66	7.28	

Koning et al. (1999) has reported that high heating temperature during oil extraction deactivated the enzyme and the release of free fatty acids by the lipase activity thus lowered the FFA value. The values of acid value (AV) and free fatty acids (FFA) in extracted fish oil were found to be 10.64 mg KOH / g and 7.28 mg KOH / g and 5.30 % and 3.66 % respectively. Increase in AV is generally associated with the lipase activity originating from microorganism or biological tissue. The acceptable limit for AV was reported to be 7-8 mg KOH /g.

IV. Conclusions

In the study calculated values and standard values are negligible different. Some values come within range of standard value which is decided by AOAC and AOCS. Saponification value of both fish species becomes lower than standard value due to some impurities and sterile conditions. Free Fatty acid and acid values are increased in analysis of one fish species. An increase in Acid Value is generally associated with the lipase activity originating from microorganism or biological tissue. The hydrolysis of fish was greatly reduced upon sterilization. Since, we did not sterilize the samples nor studies them under aseptic conditions; it is possible that some enzyme or microorganism contamination might have occurred during sample removal.

V. REFERENCES

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