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Extraction and Characterization of Lipid from Pangus Fish *(P. Pangasius)* Available in Bangladesh by Solvent Extraction Method

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Abstract: The aim of this work was to characterize the lipids extracted from pangus fish *(P. pangasius)* available in the local market in Bangladesh. The pangus fish *(P. pangasius)* was collected from the local market of Dhaka. Lipids were isolated from both the fresh as well as cooked fish by solvent extraction method. The physical characteristics of lipid of *(P. pangasius)* were analyzed and also found that the percent of lipid content of fresh pangus fish *(pangasius pangasius)* was 9.126% and lipid content of cooked panagus fish *(pangasius pangasius)* was 6.334% and specific gravity of the lipid was 0.87 at 25°C. Furthermore, the chemical characteristics of lipid of *(pangasius pangasius)* were analyzed and also found that the saponification value, the saponification equivalent, the ester value, the iodine value, the peroxide value and the acid value of pangus fish were 179.88, 311.87, 177.95, 53.93, 43.63 and 1.93 respectively. Besides this, the percent of free fatty acid was 0.97%. However, The Pangus fishes are also played an important role in our daily dietary routine. The lipids from pangus contain polyunsaturated fatty acids (PUFA), which play important roles in cardiovascular system or reduce the risk of heart attract. The daily consumption of fish oils (omega-3 polyunsaturated fatty acids) can significantly lower blood pressure in people suffering from hypertension. The benefit of the fish oils is comparable to that obtainable by sodium reduction and weight loss. Fish consumption also helps to prevent prostate cancer. So, its very important to isolate and characterize the lipid of pangus fish *(P. pangasius)*.

Keywords: Lipids, Pangus Fish, Solvent Extraction, Saponification Value, Ester Value, Iodine Value, Peroxide Value, Acid Value

1. Introduction

The geological position of Bangladesh, in general, is very favorable for fresh water aquaculture. The country enjoys a congenial environment throughout the year to support aquaculture [1]. For this reason she has a very rich fishery resources both marine and fresh water. Favourable geographical condition, sea, canals, beels, haours, baours, tank, pond, etc. give and unique opportunity for development and expansion of this pangus fish *(P. pangasius)*. Generally found in fresh water and brackish water, same common habitats are big rivers, floodplains, estuaries, canal etc. Usually inhibits lower portion of large rivers and estuaries. Mature fish can reach a maximum standard total length of 130 cm and up to 44 kg in weight. Where the ranges of p^H 6.5 - 7.5 and 22 – 26°C. The photographic arrangement of pangus fish is given below:

Fats, oils and fat-like substances because of similar solubility's, are classified as lipids. Chemically they are ester of fatty acids or are capable of forming ester [2]. They are insoluble in water and soluble in one or more, the so-called fat solvents, ether, chloroform, benzene and acetone [3]. Like carbohydrates, fats are composed of carbon hydrozen and oxyzen but in properties that greatly

increase their energy value. The lipids are either esters of fatty acids or substance capable of forming such esters. They are very wide spread in nature, being found in all vegetables and animal matters. Some member of this group. Such as the phosphatides arc sterols found in all living cells where, with the protein and carbohydrates, they form an essential part of the colloidal complex of cytoplasm. Complex lipids are also found in large quantities in brain and nervous tissues, thus indicating the important role, these substance must play in the living organism [4]. Other lipids such as the fats and oils represent the chief form in which excess nutrients are stored in the animal body. They arise from ingested lipids and from the metabolism of carbohydrates and proteins and are stored in fat deposits, such as in the substance connective tissue, in the intramuscular connective tissue, in the momentum, in per renal fat'depots and in the genital fat; lipids act as heat insulators and as reserve supplies of energy.

Figurer 1. *Photos of pangus fish (P. pangasius).*

Fish oils, in general, consist predominantly of triglyceryl ester of fatty acids and minor proportion of free fatty acids vitamins coloring matters hydrocarbons Sterols, phosphotides etc [5-6]. Fish oils are divided into two groups: sea water (marine) fish oils and fresh water fish oils and the two groups differ markedly in their fatty acid composition (Lovern, 1932). In general, sea water fish oils have a relatively complex composition and contain great proportions of C18, C20 and C22 acids [7] ; where as fresh water fish oils contains smaller amounts of C20 and C22 unsaturated acids than sea water fish oils, but great amounts of palmitic acid and C18 unsaturated acids. Cervical cancer heart arrhythmia problems fish oil & statins protect the heart fish oil and propofol treat breast cancer fish oil relieves ulcerative colitis fish oil fights sunburn asthma patient etc [8-9]. HPV, or Human Papilloma Virus, is the most common sexually-transmitted disease, with an estimated 1 in 4 sexually-active young adults having the disease. In women, this infection can eventually lead to cervical cancer, which kills more than 3900 women every year [10]. A recent study found that the taking fish oil inhibited the growth of these HPV cells. Perhaps more remarkable is that it inhibited the growth of already cancerous cells in a benign tumor Asthma is an increasingly common affliction in the Western world. It is estimated that between 20 and 25 percent of all children suffer from one or more symptoms of asthma at some point. Linoleic acid is found in particularly high concentrations in vegetable oils such as safflower, sunflower, and corn oils. The normal dietary intake of n-6 PUFA was determined for all participants at the start of the study and after one month. For the first month participants were given fish oil capsules containing enough EPA and DHA to adjust their intake ratio of n-3 PUFAs (fish oils) to n-6 PUFAs 0.1:1. During seconds the participants had their n-3 PUFAs to n6 PUFAs ratio adjust to 0,5:1. The average fish oil intake required to produce the 0.5:1 ratio was 3.3 gm per day. Extensive testing showed that more more than 40% of the participants experienced a significant improvement in their breathing ability and better resistance to asthma attacks while on the high fish oil die. The researchers conclude that dietary supplementation with fish oils or other enriched sources of n-3 PUFAs may be a viable therapy for asthma [11-12].

In this research it was tried to characterize the lipids extracted from Pangus fish using solvent extraction method.

2. Experimental

2.1. Materials

Pangus fish (P.pangusius) was the raw material for this work which was collected from a food processing landing zone in south-west part of Dhaka division of Bangladesh chloroform 99.4% manufacturer BDH (England), methanol, Ethanol & Absolute alcohol (C2H5-OH) manufacturer BDH (England), Hydro Chloric Acid (HCl) purity 37% manufacturer E. Marck Ltd. Germany, potassium hydroxide (solid) manufacturer BDH (England), phenolpthelein solution (1%) manufacturer E. Marck Ltd. Germany, sodium carbonate and Sodium bicarbonate manufacturer BDH (England), , sodium thiosulphate, Starch solution, potassium iodine solution and potassium dichromate manufacturer E. Marck Ltd. Germany.

2.2. Methods

2.2.1. Extraction of Total Lipid by Bligh & Dyer Method [13-15]

The Pangus *fish (P. pangusius)* was taken for total lipid estimation. According to the method of Bligh and Dyer a mixer of chloroform and methanol (2:1 v/v) was used. About 50 gm of the fish was weighted out and was transferred to a volumetric flask (500 ml capacity). Then 60 ml of chloroform-methanol mixture (2: I v/v) was added and mixed well. For complete extraction, it was kept this over a night at room temperature, preferably in the dark. At the end of this period, a further, addition of 100 m1 chloroform ($CHCI_3$) and 100 ml water was mixed. The resulting solution was subjected to centrifugation, when three layers were seen. A clear lower layer of chloroform contained all

the lipids, a coloured aqueous layer of methanol with all water -soluble material and a thick pasty interface were seen. The methanol layer was discarded and the lower layer was carefully collected free of interphase either sucking out with a tine capillary or by filtration through glass wool. The organic layer from either of the extraction method was taken in a pre-weighted beaker or vial and carefully evaporated.

The method to achieve this was to keep the sample in warm water (Around 50°C) and a slow stream of nitrogen gas was blown on to the surface. It was also noted to keep the sample covered with a dark papers to protect from light. This was because some lipids got polymerized and decomposed in exposure to light heat and oxygen. After evaporation of the chloroform, the weight of the beaker was determined again. The difference in weight gives the amount of the lipids.

Estimation of total lipid from Pangus *fish (P. pangusius):* Calculation of Pangus *fish (P. pangusius)*

Percentage of lipid content = (weight of lipid obtained/weight of fish) x 100

Weight of fresh fish = 50 gm

Weight of lipid in fresh fish = 9.162 gm

Percentage of lipid content in fresh fish was = (9.162/100) x 100

Hence, percentage of lipid content in fresh fish was = 9.162%

Weight of cooked fish = 50 gm

Weight of lipid in cooked fish = 6.334 gm

Percentage of lipid content in cooked fish = (6.334/100) x 100

Hence, percentage of lipid content in cooked fish was = 6.334%

2.2.2. Determination of Lipid Content in Fresh & Cooked Pangus Fish (P. Pangasius)

Weight of beaker for fresh fish = 54.108 gm

Weight of beaker for cooked fish = 53.221 gm

Weight of lipid in fresh fish = (58.689-54.108) gm= 4.581 gm

Weight of lipid in cooked fish = (56.388-53.221) gm= 3.167 gm

Final calculation

Percentage of lipid content in fresh fish = (4.581/50) x 100 gm = 9.162%

Percentage of lipid content in cooked fish =(3.167/50)x100 gm= 6.334%

Hence the lipid content of fresh and cooked *Pangasius pungasius* (Pangus fish) were 9.162% and 6.334%.

3. Characterization Methods

3.1. Determination of the Saponification Value

Definition: The saponification value is the number of milligrams of potassium hydroxide needed to neutralize the free fatty acids and to saponify the esters in 1 gm of the fixed oil, fat or wax [16-17]. This value includes the free fatty acids and thus is a measure of both free and combined fatty acids. The saponification value for most edible oils and fats ranges between 179 to 200, with most waxes falling around 100.

Procedure:

The lipid (0.25 gm) was taken in a conical flux and 6.36 m1 of alcoholic 0.5 N potassium hydroxide solution was added to it. The flask was then connected to a reflux condenser and heated on a boiling water bath so that the alcoholic solution boiled gently for 30 minuties. During this time the flask with its content was shaken occasionally to prevent agitation. A blank experiment (with lipid) was performed simultaneously in exactly the same manner as described above. After 30 minutes both the flask were removed from the water bath and their contents, while till hot, were titrated with 0.5 N hydrochloric acid (U.48 N) using phenolphthalein as indicator.

Saponification value was determined, following the procedure as described by kolles Dorfer using the formula,

$$\text{Saponification value} = (56.1 \text{ x-(A-B) x strength of acid}\}\backslash W \quad (1)$$

Where,

B= The no of ml of acid required for the actual experiment.

A=The no of ml of acid required for blank experiment. W=Weight of the lipid taken in gm.

56.1= a constant quantity which represents the gm molecular weight of potassium hydroxide.

3.2. Determination of the Saponificaion Equivalent of Lipid [18-19]

Saponification equivalent was determined using the formula:

$$\text{Saponification equivalent} = \frac{56100}{\text{Saponification value of the lipid}}$$

Calculation:

The HCL was standardized against a standardized solution of Na_2CO_3(0.502 N) and the strength of HCl was found to be 0.48 N.

Weight taken of the lipid = 0.25 gm

Volume of HCl for the actual experiment = 1.83 ml

Volume of HCl for the blank experiment = 3.5 ml

Therefore from equation (1)

Saponification value = { (3.5-1.83)X56.1x0.48}/0.25= 179.88

Hence saponification value of the lipid of Pangus fish *(P. Pangusiu*s) was 179.88

Again from equation (2) we get,

Saponification equivalent = (56100/179.88)

Therefore, the saponification equivalent of the lipid of Pangus fish *(P. Pangusius)* was 311.87

3.3. Determination of Iodine Value of the Lipids (Henus Method)

Definition: The iodine value is a measure of the degree of unstauration of the fatty acids compounding the glycosides of

the fixed oil or fat and is expressed as the grams of iodine absorbed by 100 gm of the oil or fat [20].

Iodine value was determined according to the method, which was also described by Hanus using the formula,

$$I.V = \frac{S \times (X - Y) \times 0.127 \times 100}{W}$$

Where,

LV =Iodine value.

S = Strength of sodium thiosulphate solution.

X = Volume of ml of sodium thiosulphate solution required for the blank Titration.

Y = Volume of the ml of sodium thiosulphate solution required for the Sample titration.

W = Weight of the lipids taken in gm.

Procedure:

The lipid (0.21 gm) was dissolved in 3.15 ml of chloroform in dry glass stoppered bottle (500 m1) . To the content of the bottle 7.89 m1 of Henus solution was added and the mixture was allowed to stand in the dark for exactly 30 minutes with occasional shaking. 1.5 N Potassium iodide solution (3.15 ml) was mixed to it and the mixture was shaken well. Freshly boiled and cooled distilled water (31.59 ml) was added to the mixture and the content of the bottle was titrated with standardized 0.1 N sodium thiosulphate solution, using starch indicator. A blank titration (with the lipid) was performed exactly in same manner as described above.

Calculation :

Weight of lipid =0.21 gm

The strength of sodium thiosulphate solution (as standardized by 0.1 N $K_2Cr_2O_7$ solution) was found to be 0.098 N.

The volume of sodium thiosulphate required for the blank titration =16 m1

The volume of sodium thiosulphate required for the sample titration =6.9 ml

The iodine value of sample ={(16.9-6.9)x0.098x0.127x100} /0.21

Hence, The iodine value of sample = 53.93

3.4. Determination of Peroxide Value of Lipid

Definition: The peroxide value is the determination of amount of iodine liberated from potassium iodide by the peroxides of the oil or fats in terms of mill equivalents per kilogram[21].

Peroxide value of the lipid calculated by using the formula,

$$\text{P.V} = \frac{(A - B) \times N \times 1000}{weightofsample} \quad (2)$$

Where,

P.V = peroxide value.

A = Volume of nil of sodium thiosulpfate solution required for the sam pie titration.

B = volume of ml of sodium thiosulfate solution required for the blank titration.

N = Normility of sodium thiosulphate solution.

Procedure:

The lipid (1.65 gm) was taken in 250 ml glass stopper bottle and 30 ml of acetic acid- chloroform (1:1) was added to it. To the content of the bottle,0.5 ml of saturated potassium iodide solution was added and the mixture was allowed to stand with occasional shaking . Distilled water (30 m1) was added to the mixture and the content of the bottle was titrated with 0.16 N normal sodium thiosulphate solution using starch solution as indicator. A blank experiment (without the lipid) was performed exactly in the same manner as described above. The peroxide value of the lipid of Pangus fish *(P. pangusius)* was calculated and has been computed in table.

Calculation of Pangus *fish (P. pangusius)*

Sodium thiosulphate solution was standardized against solution of potassium dichromate and the strength of the thiosulfate solution was found to be 0.09.

Weight of lipid =1.65 gm

The volume of sodium thiosulphate required for the blank titration = 7.3 ml

The volume of sodium thiosulphate required for the sample titration =8.1 ml

The peroxide value of sample ={(8.1-7.3)x0.090X1000}/1.65

Hence, The peroxide value of sample = 43.63

3.5. Determination of Acid Value and Percentage of Free Fatty Acid (As Oleic) of The Lipid

Definition: The acid value of fixed fat or oil is defined as the milligram of potassium hydroxide required to be neutralized the fatty acid in one gm of the sample. This value may be converted to give the percentage of free fatty acid such as oleic, palmitic or lauric [22-23].

Acid value of the lipid was calculated using the formula given below:

Acid value ={56.1X(A-B)XN}/W

Where,

N = Strength of alkali in normality.

B = No of ml of aqueous alkali required for sample titration.

A = No of ml of aqueous alkali required for blank titration

V = No of ml of aqueous alkali required for titration.

W = Wight of the sample taken in grams.

Procedure:

A known weight of the lipid (0.61 gm) was taken in a 25 ml conical flask and mixed with 50 ml of 95% neutralized alcohol. The mixture was heated to boiling and the content of the flask was titrated with aqueous potassium hydroxide solution until a faint pink color persisted for at least 10 seconds. The content of the flask was shaken continuously and vigorously during the titration.

Free fatty acids (F. F. A):

Percentage of free fatty acid (as oleic) was calculated using the formula:

%F. F. A. (as oleic) = (A.V x M) /561

Where,

%F. F. A = Percentage of free fatty acid.

A. V = Acid value.

M = Molecular weight of the oleic acid.

Calculation of Pangus *fish(P. pangusius)*

The potassium hydroxide solution was standardized against a standard solution of hydrochloric acid and the strength of alkali was found to be 0.01 N.

Acid value ={(4.5-2.4)x0.0ix56.1}/0.61=1.93

Here, A= 4.5 ml

N = 0.01

N1= 0.61 gm.

The acid value of the lipid of Pangus fish (P. pangusius) was 1.93

Free fatty acid:

Percentage of free fatty acid (as oleic) ={(Acid valuexMolecular weight of oleic acid)-561} = (1.93x282)=561= 0.97%

∴ The percentage of free fatty acid (as oleic) of Pangus *fish(P. pangusius)* was 0.97%

3.6. Determination of Ester Value

Definition: The ester value is the number of mg of potassium hydroxide required to saponify the ester contented in 1 gm of the oil or fat [24].

Procedure:

For the oil or lipid to be analyzed, the acid value or the saponification value was determined which had been described before. The ester value was calculated using the formula,

Ester value = Saponification value - Acid value.

Calculation:

Ester value of Pangus *fish(P. pangusius)* = Saponification value -Acid value= 179.88-1.93=177.95

The Ester value of the lipid of Pangus *fish(P. pangusius)* was 177.95

4. Results and Discussion

In this research work the lipid in the body muscle of Pangus Fish *(Pangasius Pangasius)* recorded. The fatty acid composition of the lipid of Pangus Fish *(Pangasius Pangasius)* with its physical, chemical characteristics are recorded and discussed in the present work.

4.1. Total Lipid Content of Fresh and Cooked Fish

Lipid are structural material, reserve fuels, barriers to the environment, vitamins, emulsifiers, flavor and aroma compounds solvents.

The lipids were extracted from the Pangus Fish *(Pangasius Pangasius)* by Bligh and Dyer method, a mixture of Chloroform and methanol (2:1/V/V) was used. Total lipids were determined gravimetrically from these lipid extracts.

The amount of total lipid content of fresh Pangus Fish *(Pangasius Pangasius)* was 9.126%.

The amount of total lipid content of cooked Pangus Fish *(Pangasius Pangasius*) was 6.334%.

4.2. Characterization of the Lipid From

A number of physical and chemical characters were employed to determine the nature and sometimes for the identification of fats and oils. These characters may also help to evaluate the suitability of given oil or fat for a given purpose. Mixture of oils and fats possesses properties of the individual oils and fats comprising the mixture. The physical and chemical characteristics of an oil or fat vary *between* certain limits and due to the comparatively small variations, they are considered to be constants. Although the chemical constant are more important to characterize oil, but the physical constant are also often capable of expressing valuable information's. Some of the more important chemical constants are the iodine value, the saponification value and saponification equivalent, the acid value and the percentage of free fatty acid, the ester value, the percentage of un-saponifiable matter. The refractive index, specific gravity and co-efficient of viscosity are the important physical constants. Some of the physical and chemical constants of the lipid under investigation were determined.

4.3. Specific Gravity

The specific gravity (sp.gr.) of fats or oil does not vary as a general rile to an extent, which make this property useful in discriminating between one fat and another. The specific gravity of practically of all fats or oils lies between 0.90 and 0.95 the specific gravity of the lipid of Pangus Fish *(Pangasius Pangasius*) was found to be 0.87 at 25°C. The value obtained in the present studies has a close similarity of that of olive oil (0.915-0.919) by Williams and Maslov.

4.4. Saponification Value and Saponification Equivalent

Hydrolysis of a fat or oil by alkali into glycerol and alkali salt is known as saponification and the number of mg of potassium hydroxide required to saponify one gram of fat or oil is called saponification value. It is inversely proportional to the average molecular weight of chains length of the fatty acids present in the fat or oil. 29 For example butterfat (sap. val. 210-230) contained fatty acids of smaller molecular weight (in average) in its glycet ides that of castor oil (sap. val. 175-180)

The saponification value of the lipid of Pangus Fish *(Pangasius Pangasius)* was found to be 179.88. The value has close similarities to that of Cotton seed oil (175-198). The saponification equivalent of the lipid was found to be 311.87 respectively, which are calculated from saponification value as, described in the materials and methods. It is directly proportional to the average chain length of fatty acid present. Fats or oils consisting largely of C 18 fatty acids along with some myristic, palmitic acids, a little unsaponiftable matter and a low free acidity generally have a saponification equivalent around 290.80; higher value indicates the presence of appreciable quantity of

higher acids. Findings of the results clearly indicated that the lipid of Pangas fish contained mainly fatty acids of C^{18} molecular weight along with some palmitic acid.

4.5. Iodine Value

Iodine value is defined as gm of iodine absorbed but 100 gm of fat or oil. It gives an estimate of the degree of unsaturated fatty acids and so, of the relative amounts of unsaturated fatty acids in the triglyceride molecules of the fat or oil. For example coconut oil (Iodine value 6-10) contained much less unsaturated fatty acids than cod liver oil (IM. 145-170). The iodine value (I. V) of Pangus Fish (Pagsius Pangasius) lipid of was found to be 53.93.

It may be concluded from the results that the lipid under examination contains unsaturated fatty acids at a satisfied concentration. Again, since the lipid has iodine value of about 53.93 which is below 90 and does not appreciably change its consistency on exposure to air, hence the oil is of nondrying type.

4.6. Peroxide Value

Fixed oils and fats absorb oxygen from the air in the auto-oxidation of the double bonds present in the component fatty acids. In the reaction, the oxygen adds to the double bonds of the fatty acids forming unstable hydroperoxides. It is a measure of content of reactive oxygen, in terms of moles of peroxide or mini-equivalents oxygen per 1000 gm of oil.

The peroxide value of the lipid Pangus Fish (Pangasius Pangasius) was found to be 43.63.

4.7. Acid Value and Percentage of Fatty Acid (As Oleic)

Acid value (A. V.) is the amount of potassium hydroxide (in mg) required to neutralize the free fatty acid in one gram of fat or oil. The formation of free fatty acids in a natural fat samples due to hydrolysis by lipase, may be an important contributory factor for rancidity. A high value may indicate a higher tendency to become rancid.

The acid value of the lipid of Pangus Fish *(Pangasius Pangasius) was* found to be 1.93. The percentage of free fatty acid (F. F. A.), which calculated from acid value, was found to be 0.97.

A low percentage of free fatty acid (below 1.15%) is an indication of suitability of the lipids for edible purpose.

The ester value of an oil or fat is of value at times, and is obtained by deducting the acid value from the saponification value; it thus represents the saponification value of the neutral glycerides in 1 gm o the substance.

The ester value of the lipid of Pangus Fish *(Pangasius Pangasius)* was calculated and found to be 177.95

5. Conclusions

The Pangus Fish *(Pangasius Pangasius)* may be played an important role to maintain human body function due to its high fish oil value, which is not commonly available in other food. Fish oil is oil which is usually derived from the tissues of oily fish. This oil naturally contains the Omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Because the oil is so high in Onieea-3, fish oil is now recocrunended fur a healthy diet. Finally, Pangus Fish *(Pangasius Pangasius)* were taken for the estimation of the physical and chemical characteristics of its lipid. This fish is used as a source of lipid. This lipid has also an anti carcenogenic effect. The extracted lipid showed a positive and standard saponification value, saponification equivalent, the iodine value, the acid value, the percent of free fatty acid, the peroxide value, the ester value as well as specific gravity. The selective food sample was Pangus Fish. All the experiment is basis on the official methods of analysis of AOAC international. So, in a nut shell, it can be said that Pangus Fish is a good source for oil or lipid which is good for health and growth of human body. Besides this it is also cheaper than any other fish available in Bangladeshi local markets.

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Declaration

There is no conflict of interest regarding this research work.

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