XTRACTION AND EVALUATION OF OIL FROM DIFFERENT SPECIES OF FISHES

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FEDERAL UNIVERSITY OF TECHNOLOGY

MINNA NIGER STATE, NIGERIA

BOSSO CAMPUS

NOVEMBER 2005

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A PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMICAL ENGINEERING IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE BACHELOR OF ENGINEERING (B. ENG)

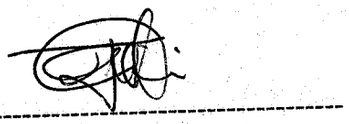
FEDERAL UNIVERSITY OF TECHNOLOGY

MINNA, NIGER STATE, NIGERIA

NOVEMBER 2005

DECLARATION

I hereby declare that this project work was wholly carried out by **Godwin Janet** ider the supervision of **Engr. M Alhassan** owning to the fact that this work is original id has never been submitted anywhere. All other sources of information from published orks and past projects are duly acknowledged as a means of reference.



SIGNATURE OF STUDENT

09/12/05

DATE

CERTIFICATION

This is to certify that, this project titled – Extraction and Evaluation of Fish Oil form Five Species of Fish has been supervised, read and approved as meeting the requirements of the Department of Chemical Engineering School of Engineering and Engineering Technology, Federal University of Technology, Minna for the Award of Bachelor Degree in chemical Engineering (B.ENG).

PROJECT SUPERVISOR
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EXTERNAL SUPERVISOR

DATE

DEDICATION

This project work is highly dedicated to the Almighty God my maker who gave me life and saw me through the rigors of the discipline. To Him be all Praise and Honor.

ACKNOWLEDGEMENT

May all unending praise be given to the "I am that I am" The Lion of the tribe of dah, who gave me life to see to this stage of writing my own project however it was, r not all that started with us, saw this stage.

My sincere and unreserved appreciation to my darling parents, Mr.& Mrs. Godwin nagor who sacrificed a lot to send me to school despite all odds, Nne m na Nna m dalu u oooo!!!!

would like to specially acknowledge and appreciate my able supervisor Engr M Alhassan who tolerated my excesses and never failed to give his brotherly encouragement and constructive criticisms. I remain loyal Sir.

My heartily regards go to my lovely sisters and brother: Ifeoma, Chichi, Njide, Ndidi, Uche and Nnenne. My profound gratitude goes to all the staff of Chemical Engineering Department, Dr. Salihu and Mr. Ndama of Fishery Department (FUT Minna) and also to the staff of Chemistry Department, who helped me in the analysis.

I will not forget to appreciate my caring and indomitable comrades; Ochege Josua(a.k.a Prof,), Chimere Ndukuba,Bashir Jinad and Isikaku Innocent who provided a shoulder to learn on during the trial times, I will always appreciate.

Finally, an unequivocal respects to all my friends out there; Blessing Onu, Ben K.C, Michael C.A, McDonald Ehiwe and Ogu Martin Diala (a.k.a Tiko) and the host of others too numerous to mention for their support during the course of this project. God's blessings on you all. Amen.

ABSTRACT

The production/extraction, purification and the evaluation of fish oil were carried out ng soxhlet apparatus and n-Hexane as the solvent of extraction. The extraction was ne at constant temperature. Five different species of fishes were used for the periment. From the result of the extraction, it was observed that the fish A Mormyrups deliciousus) and fish E (shawa) have a good percentage of oil content after ktraction which is 30.22% and 24.02% of its dry mass respectively, when compared to ne percentage oil content in the other fish samples used in the experiment. Also the odine value falls within the specification of the standard value showing that fish oil is a good cure for goiter and other ailments which were enumerated in the literature review. All other physical and chemical analysis carried out on the oil samples were within specification, except for the saponification value.

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CHAPTER FIVE

CHAPTER ONE

INTRODUCTION

Fish oil is the lipid fraction extracted from fish and fish by-products. Presently, the luction of fish oil is becoming more demanding as there is a sizeable and growing world ket demand for high quality fish oils. Apart from its various uses as consumable oils, it is appreciable in both pharmaceuticals and industries.

However, the most frequently used technique in fish oil extraction are fractionation by speed configurations, low temperature solvent extraction, superficial fluid extraction etc. nwhile solvent extraction was employed during this research. This is because solvent action is one of the most efficient method of extraction from all oil bearing materials ause the solvent can be easily recovered and recycled and it reduces the residual oil in the bearing substance to less than 1% that is in a case, where the right solvent is chosen for the action process so care must be taken in this selection process not to select a solvent which be poisonous to the oil extracted.

Practically, all fish species as well as other marine animals may be converted into fish oil meal. The composition and quality of these fish species are predominant factors in ermining the properties and yield of the products (FAO, 1986). The quality and freshness of raw material is the factor of great importance in preparation of premium quality fish oil fish meal (Isabel, 2002). Enzymatic and bacteriology activity in the fish and fish products rapidly increase, which in turn can substantially decreases the content and quality of the tein and oil as protein decomposes to amines and ammonia, and both reduce the protein ue and recovery.

Fish oil is different from other oils mainly because of the unique variety of fatty acids it ntains including high level unsaturated fatty acid which is essential to the body. This is own as the eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA). The market liquid fish oil for human consumption can be applied in area of pharmaceutical, healthy od compounds and commodity for the food industry.

OBJECTIVE

Although work has been done on fish oil production but literature has shown that little or work has been done in terms of characterizing and comparing of oil products from different ies of fish. It is therefore the aim of the research to:

Extract the fish oil from a number of fresh water fishes and a marine fish.

Evaluate, refine and characterize the extracted oil.

Recommending types of fish with high nutritional and medicinal value.

CHAPTER TWO

LITERAURE REVIEW

Brief History of Fishes Used

ishes are creatures that live and breathe in water. They use their gills to breathe; have nd a streamlined body suitable for swimming, some have scales for protection.

ever, fishes are vertebrates (i .e. animals with a backbone).

here are over 25000 different types of fishes in the world and numerous others yet to ver. Five different types of fishes were used for this project of which four are fresh water s and one marine fish. The fish samples are number A to E and their distinctive features also enumerated.

Sample A:

Species: Mormyrops deliciousus (Leach, 1818)

Synonyms: Mormyrops angulloides (Linnaeus, 1758)

Common name: Mormyrids or Trunk fish.

Distinctive Features

Dorsal Fin rays	27 – 28
Anal fin rays	37 – 45
Lateral line scales	84 – 98

There are more than 24 teeth occur on each jaw

Colour its yellowish grey generally

Size can grow up to 3 meters in length with a weight of about 6kg.

ECOLOGICAL NOTE

Inhabits swamps, rivers and lakes

It is a bottom dweller.

It has electric organs, therefore it can discharge electric current to Paralyze its prey and Protect it from enemies.

Commonly encountered at night

Utilization

a nice tasty flesh and is eaten by many people.

Sample B:

Species: Bagrus docmac niger Daget (1954)

Synonyms: Bagrus Doemak

Common name: Silver cat fish

Distinctive features

Head short and wide (1.1 to 1.3) longer in length than in width.

Dorsal fin with g branched rays short or non filamentous

Pelvic fins inserted under the last dorsal ray

Upper and lower loses of caudal fin prolonged into filaments.

Color: Grey on the back and white on the belly

Size: A standard length of 60cm with a weight of 4kg. This fish can grow up to a th of 1m with weight of 12.5kg

2 Utilization

(i) The flesh is of high quality, greatly prized and consumed by many people.

(ii) It can be used to control tilapia population when cultured together

(iii) It lives in ponds and its growth rate is satisfactory and therefore good for culture in ponds.

0 Sample C:

Species: Tilapia dagati (thys van de Audenande, 1971)

Synonyms: Tilapia melanopleura thys van de (Dumeril, 1859)

Common name: Chichilid (Tilapia)

3.1 Distinctive features

(i) Gills rakers with 8 to 12 lower part of first gill arch

(ii) Pelvis fin reduces the original of and fin

(iii) Pectoral fin almost reaching the original and anal fin.

(iv) Caudal fin with a straight margin.

(i) Lateral line scales, upper lateral line with 18 to 22 scales which the 1cm lateral line 11 to 16.

2.3.2 Ecological notes

(i) Inhabits streams, river, and lakes with sandy bottom

ii) It prefers deep water always avoiding shallow water and green vegetation.

iii) It is common during the dry season.

2.3.3 Utilization

(i) Human food. The flesh taste very good and is eaten by many people.

(ii) It has low sodium content and therefore a good diet for patients suffering from heart failures.

(iii) It is good aquaculture.

2.4.0 Sample D:

Species: Clarias anguilloris (Linnaeus, 1758)

Synonymes: Clarias Senegatensis (Valenciennes, 1840)

Common name: Catfish (mud fish)

2.4.1 Distinctive features

1. Body roughly cylindrical.
2. Head highly depressed and long
3. Pectoral Spine semated out only
4. Secondary lateral lines bones regular in arrangement.
5. Colour: Black – brown to greenish, belly (dirty-white).
6. Caudal fish may be marked with few numbers of black sports.

7. **Size:** A specimen with a standard length of 52.3 cm and a weight of 1.33kg has been recorded.

4.2 Utilization: The flesh is very tasty and nice.

5.0 Sample: Titus, A marine or frozen type of fish.

5.1 Distinctive features

1. It is usually straight with strip of line, on the body.
2. It has a grey belly.
3. Colour: Grey – black

5.2 Ecological notes

1. It is usually found in marine water.
2. It is abundance because of its high rate of reproduction

5.3 Utilization

Very tasty fish eaten by many people and rapidly available and affordable.

Fish can be found in almost every type of under water environment, for example the Antarctic ice fish can survive in water below the freezing point (32^0F) because their blood contains special anti-freezing chemicals to prevent their body from freezing. Sharks, Salmons, electric eels and seahorses are other examples of fish. Fish is a source of protein and proteinous food, are body building foods. Fish oil is the lipid fraction extracted from fish as fish by-products. Apart from the benefit derived from the consumption of fishes, there are numerous other benefit to be derived from the consumption of fishes due to its high nutritional content.

The production of fish oil started long ago since the 19^{th} century in Northern Europe and North American, where they utilized the non-edible fishes and other fish by-products to produce oil use in leather tanning and in the production of soap and glycerol (Windstor, 1971).

Lately, the production of fish oil is becoming more demanding as there is a sizeable and growing world market demands for fish oils.

The quality and freshness of the raw materials are factors of great importance in preparation of quality fish oil and fish meal. The best oil are those that contain the essential fatty acids and

hey include polyunsaturated fatty acid which is divided into two families; the omega-6 EFAs nd the Omega 3 EFAs however, food that contains Omega 3s has more benefits to man.

ish oil finds many application in the food and technical industries as it has good economic mportant to both the producer and the consumers.

Separation or extraction of lipid/oil from fish is a unit operation, which is concerned with hose separation processes that depend upon differences in physical properties, rather than chemical behavior, such processes depend either upon the difference in composition of phases at equilibrium or upon difference in the rate of mass transfer of constituents of a mixture.

However, when faced with problem of separation of components out of a homogenous mixture, the engineer utilizes differences in the properties of the constituents of the mixture to effect separation. The various chemical and physical properties of the constituents of the mixture are examined to determine which properties offer the greatest difference in property will generally permit an easier and more economical separation.

When two phases of different composition are brought into contact, a transfer of component may occur from one phase to the other, and vice versa. This is the physical case of non transfer operation and if the 2-phase is allowed to remain in contact for a sufficient time, they will reach an equilibrium condition where there is no further net transfer of components between phases. In this case, separation is said to have been completed and this is what is observed. The lipid is separated from the sample which is the fish using the Soxhlet apparatus or the same continuous solvent extractor. In Soxhlet method, a sample is oven dried, grind into small particles and placed in a porous thimble. The thimble is placed in an extraction chamber which is suspended above a flask containing the solvent and below a condenser.

The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the samples.

Eventually, the solvent build up in the extraction chamber and completely surrounds the sample. The extraction chamber is designed such that when the solvent surrounding the sample exceeds a certain level it overflow and trickles back down into the boiling flask.

s the solvent passes through the sample, it extends the lipids and carries them back into the ask. The lipids then remain in the flask because of this low volatility, at the end of extraction rocess which typically lasts for an hour, the flask containing the solvent and the lipid is emoved, the solvent is evaporated and the mass of the lipid remaining is measured. The oxhlet method of extraction is batch operation method of extraction because a bitch operation lmost the entire cycle is a start-up transient and a shutdown transient.

.6 Fish Oil Composition (Lipids)

Fish oil is very similar to one another in their physical nature. A whole fish consists of rotein, fat, Ash and water irrespective of the species however, those composition are greatly nfluenced by seasonal changes due to the nature cycle, maturity stage, geographical location, eeding habit etc. because the more a fish eats the greater the oil and other chemical composition. Most fish oil in general is more complex than land animal oils or vegetable oils due to the long chain unsaturated fatty acids. It is generally believed that fish oil odor is due to he unsaturated fatty acids, since hydrogenation causes the oil to lose their color but fish caught in colder water have a higher degree of instauration than that caught in warm water.

The lipids is the edible part of fish and is important to the food scientist in two respects firstly any oil deposits noticeably influence the sensation of the cooked flesh and secondly has some medical applications. Fish oil deteriorates very rapidly due to the natural lipase and bacterial in the fat. Both of these hydrolyze fat to free fatty acids. The condition of the fish at the time of processing affects the oil physically, chemically, and nutritionally. Fish of poor quality yields malodorous oil with high contents of free fatty acids and sulphur. These undesirable properties affect the economic values and the application of the oil.

2.6.1 Processing and packaging.

The processing and packaging of the fish oil are crucial in determining its quality. Low quality oils may be quite unstable and contain significant amounts of mercury, pesticides and undesirable oxidation products. High quality oils are stabilized with adequate amounts of Vitamin E and are packaged in industrial foil pouches or other packaging resistive to light and

oxygen. Some very recent researches carried out at the University of Minnesota find that emulsified fish oil is much better absorbed than the straight oil in gelatin capsules.

2.6.2 Solvent Extraction as the Extraction Method Employed

Solvent extraction, which is also referred to as leaching is a process where by soluble constituents present either as a solid or liquid is removed from a sold or from a liquid by the use of solvents (Richard and Coulson 1993).

In fact, solvent extraction technique is one of the most commonly used method of separating the lipid components in food sample from water soluble components such as protein, carbohydrate and mineral. For a successful extraction of oil, the sample needs to undergo specific preparation prior to solvent extraction.

Drying Samples: Samples are expected to be dried so as to enhance easy penetration of the solvent for effective separation.

Sample Size Reduction: In addition to drying the sample, it is expected to be finely divided by grinding for high rate of extraction.

2.6.3 Solvent selection

Another important factor to be considered is the solvent selection. The ideal solvent for lipid selection would completely extract all the lipid components from oil bearing food samples, while leaving all the other compounds behind.

Generally, the solvent should be inexpensive and readily available with a relatively low boiling point so that it can be recovered or removed by evaporation. It should also be non-toxic to the oil produced and non-flammable (for safety reasons).

The extraction process involves contacting and extraction, solvent recovery and waste disposal.

2.6.4 Factors to be considered in solvent selection

In making any choice of solvent for extraction, the following factors are to be considered:

(i) **Density:** There should be a wide difference in the density of the solvent and oil to be extracted for proper continuous extraction process.

ii) **High purity**: The solvent selected should be highly pure so as not to contaminate the il produced, also it should be non-toxic.

iii) **Chemical reactivity:** There should be stable chemical reaction between the solvent nd the oil extracted and non reactive neither with the containing vessel nor with the extraction ystem.

iv) **Inflammable:** Must be inflammable during handling. Flammability is the measure of he ease with which a solvent catches fire. Generally, all liquid with flash point below 32.2° C s applicable.

v) **Colour:** Solvent colour should not affect the oil produced and should be different from he colour of the oil.

vi) **Recoverability**: There should be a high recovery of the solvent used for recycling if ossible and evaporation.

vii) **Viscosity**: Viscosity is defined as the resistance of flow as it decreases with vaporation and vapor pressure solvent chosen should have sufficiently low viscosity and low reezing point as order to circulate freely.

2.7.0 REFINING OF FISH OIL

Refining may defined as the removal of free fatty acids from oil by the action of high temperature, high vacuum and live steam. This can also be called steam refining.

Refining oils involved generally the following processes degumming, neutralization and decolorization. All crude oils and fats which are used for edible purposes contain non-triglyceride substances. These substances detract from the acceptability of the oils or fat as food stuff, because of the flavor or colour which they give to the oil or because they reduce its stability or shelf life. The purpose of refining is to remove the undesirable components so as to achieve a rational specification whilst retaining the desirable features. Fish oils contain significant qualities of polyunsaturated fatty acids having three or more double bonds in the fatty acid chain. These fatty acids are mainly combined with glycerol forming the triglyceride of the natural oil. The polyunsaturated fatty acids are high susceptible to oxidation giving rise to the rapid production of compound, responsible for fresh smell and taste of the oil.

Fish oil refining is achieved through the following steps: degumming, neutralization, bleaching and decolorizing. Phospholipids are removed by degumming, free fatty acids (FFA) are precipitated as soaps and removed during the neutralization process. Bleaching clay absorbs pigments from oil and oxidized compounds can be removed by decolorization. (SFOS) (School of Fisheries and Ocean Sciences). Refined oils are clear, odorless and less harmful than unrefined oils, and are more suitable for high temperature cooking. All polyunsaturated oils should be stored in the refrigerator or the freezer.

2.7.1 Hydrogenation of fish oil

To produce fish oil which are satisfactorily stable for the production of salad oils, margarines and shortenings its necessary to hydrogenate the oil.

Hydrogenation involves the addition of double bonds of unsaturated acids in the molecules of the oil. The addition of this hydrogen changes the properties of the fatty acids and also the properties and physical behavior of the oil. The chemical reaction is carried out be reacting the oil with gaseous hydrogen at elevated temp and pressure in the presence of a catalyst usually nickel. This process is also known as hardening

2.8.0 Oil Constituents

Polyunsaturated fats contains large amounts of polyunsaturated fatty acids (PUFAs) polyunsaturated fatty acids are so named because, due to the presence of two or more double bonds, there are places along the carbon chain where the fatty acids is not "saturated" with hydrogen. Polyunsaturated fats are liquids at room temperature and polyunsaturated are grouped into Omega-3 fats and the Omega-6 fats.

2.8.1 Alpha-linolenic acid (ALA)

ALA is a member of the omega-3 family of fatty acids. It is called an essential fatty acid because the body can not manufacture it. Essential fatty acid must be consumed in the diet. Dietary sources of ALA include flaxed seed, soy bean and pumpkin seed oil. People who diet on ALA rich sources have higher blood levels of omega-3 fatty acid than those consuming lower amount; this may confer some protection against atherosclerosis. ALA has 18 carbon atoms in its backbone and can be converted to EPA in the body (in the liver) by the addition of two carbon atoms.

2.8.2 Eicosapentaenoic acid (EPA): EPA is a member of the omega-3 family of fatty acid, the oil derived from cold-water fish (salmon , tuna, sardines and cod) are concentrated sources of EPA. To a limited extent , human body can make EPA from ALA.

2.8.3 Docosahexaenoic Acid (DHA): DHA is a member of the omega-3 family of fatty acids. This fatty acid is found in cold water fish and in some types of algae.

2.8.4 Linoleic Acid: Linoleic acid is a member of the omega-6 family of fatty acid. It is another essential fatty acid. Dietary source of linoleic acid include sun flower seed and corn oil.

2.8.5 The Differences Between COD liver Oil and Fish Oil

Cod liver oil and fish oil are not the same. Cod liver oil is extracted from Cod liver and is an excellent source of Vitamins A and D. Fish oils are extracted from the tissues (flesh) of fatty fish like salmon ,herry and Trunk and are good sources of EPA and DHA.

Fish oil contains very little vitamin A and D, but Cod liver oil contain EPA and DHA.

2.9 Health Benefits of Fish Oil

There is considerable evidence that fish oils are beneficial to the human health all become of the nutritional and medicinal values of the oil.

The fish oil is said to have "active' components which includes ecosapentaenoic acid (EPA) a polyunsaturated fatty acid with 20 carbon atom on its back bone and docosahesaenoic acid (DHA), a polyunsaturated fatty acid with 22 carbon atom.

Both are members of the omega-3 group of essential fatty acid. The best fats or oils are those that contain the essential fatty acids. Essential fatty acids have 2 families, the omega 3 and 6. EFAs. The main sources of omega 6 fatty acids are vegetable oils such as co oil, soy oil that contain high proportion of linoliec acid. Omega-3 acids are found in flaxseed oil walnut oil and marine plankton and fatty fish. Recognizing the unique benefit of EPA and DHA and the serious consequences of a deficiency the US National institute of Health recently recommended daily intake of fatty acid. They recommend a total intake of 650mg of EPA and DHA. 2.22g/day of alpha linoleic acid and 4.44g/day of linoleic acid. Saturated fat intake should not exceed 8% total calories intake of about 18g/day. Alpha- linoleic acid can be converted to EPA &DHA in the body, but the conversion is quite inefficient in older people.

It is estimated that 85% or more people in the western world are deficient in omega -3 fatty acids and most get far too much of the omega -6 fatty acids because vegetarian diets which they mostly consume is very high in Omega -6.

2.9.1 Good for the Brain

The human brain is one of the largest "consumers" of DHA. A normal adults human brain contains more than 20grams of DHA. Low DHA level have been linked to low brain serotonin levels which again are connected tendency to depression, suicide and violence.

A high intake of fish has been linked to a significant decrease in age-related memory loss and cognitive formation impairment and a lower risk of developing Alzheimeir's disease.

A recent study formed that Alzheimeir patient given an omega 3-rich supplement experienced a significant improvement in their quality of life.

2.9.2 Important during pregnancy and lactation.

An adequate intake of DHA and EPA is particularly important during pregnancy and lactation. During this time the mother must supply all the baby's need for DHA and EPA because it is unable to synthesize these essential fatty acid itself. DHA makes up to 15 to 20 % of the cerebral cortex and 30 to 60% of the retina so it is absolutely necessary for normal development of the fetus and the mother.

There is some evidence that are insufficient intake of omega-3 fatty acids may increase the risk of premature birth and an abnormally low birth weight. There is also an emerging evidence that low levels of omega-3 acids are associated with hyperactivity in children. The content drain on a mother's DHA resources can easily lead to a deficiency and some researchers believed that (Pregnancy-related high blood pressure) and post partum depression could be linked to a DHA deficiency. Experts recommended that women get at least 500-600mg of DHA every day during pregnancy and lactation. The easiest way to ensure this intake is to take a good fish oil supplement daily.

2.9.3 Benefits for Children

Researchers at the University of Sydney have found that Children who regularly eat fresh, oil fish have a four times lower risk of developing asthma that do children who rarely

eat such fish. They speculate that EPA present in the fish may prevent the development of asthma or reduce its severity by reducing airway inflammation and responsiveness.

2.9.4 The heart's best friend

An enormous amount of medical literature testifies to the fact that fish oils prevent and may help to ameliorate or reverse atherosclerosis, heart attack, congestive heart failure, stroke and peripheral vascular disease. Fish oil help maintain the elasticity of artery walls, prevent blood clothing, reduce blood pressure and stabilize heart rhythm.

An adequate daily intake (about 1 gram) of EPA and DHA is essential to maintain a healthy heart. Fish oils are especially important for diabetics who have an increased risk of heart disease.

2.9.5 Reduces pain and help prevent cancer

Fish oils are particularly effective in reducing the inflammation and can be of great benefit to people suffering from rheumatoid arthritis or ulcerative colitis. Daily supplementation with as little as 2.7g of EPA and 1.8g DHA can markedly reduce the number of tender joints and increase the time before fatigue sets in. Patient with ulcerative colitis have abnormally low blood level of EPA.

Clinical trials have shown that supplementation with fish oil (2.7g of EPA and 1.8g of DHA daily) can reduce the severity of the condition by more than 50% and enable many patient to discontinue anti-inflammatory medication and steroids

There is now also considerable evidence that fish oil consumption can delay or reduce tumor development in breast cancer. Studies have shown that a high level or omega -3 fatty acids combined with a low level of omega-6 acids reduces the risk of developing breast cancer.

2.10 PROPERTIES OF FISH OIL.

2.10.1 Physical properties of fish oil.

This comprises of melting point, the refractive index and the specific gravity.

The melting point is the measure of the temperature at which the oil melts after undergoing a solidification process.

The slip point method was employed when determining the melting point where the solidified sample in a capillary tube was heated in a beaker containing paraffin and a thermometer. As the paraffin is being heated, the solidified ore begins to melt until it finally stops melting, at this point, the thermometer is being read and temperature recorded which becomes melting point.

2.10.1.1 Refractive index

This measures the angle through which a beam of light is bent when passing through thin film of melted fat. The index of each fat falls within a narrow range and can be used to determine the purity of the oil. It is also temperature dependant and is usually measured at 104° F (40°C) a temperature at which most fats are liquid.

2.10.1.2 Specific gravity:

The specific gravity of oil is the ratio of mass of a given volume of material at 25°C to that of an equal volume of water at 25°C. It is a useful factor when converting volume to mass and mass to volume.

2.10.2 Chemical Properties of fats and oil

These include iodine value, saponification value, and acid value

2.10.2.1 Acid value

This is a measure of the amount of free fatty acid present in the fish oil. It is defined as the number of potassium hydroxide (KOH) required neutralizing the few fatty acids in 1g of the sample. Hence, acid value gives an indication of the age and quality of the fats (Plummer, 1978), though it is still the most accurate parameter for oil quality and yield assessment.

2.10.2.2 Iodine Value

Iodine value is the number of grams or iodine taken up by 100g of fats or oil. Iodine value is the measure of the proportion of unsaturated fatty acids present in the oil, by an indication of

he amount of iodine which can be absorbed by the unsaturated acid. Also iodine value ndicates the degree of reactivity of the oil which influences the stability of the oil as high ontent of unsaturated fatty acid makes the oil very sensitive to oxidation.

.10.2.3 Saponification Value

This is the number of milligrams of potassium hydroxide (KOH) required to saponify the fatty cid resulting from the complete hydrolysis of 1g of oil. Saponification value is also a measure f the mean molecular weight of the free fatty acid present in the fat.

.10.3 Advantages of Solvent Extraction

. solvent extraction is one of the most efficient method of extraction from all earing materials.

. The solvent used can be easily recovered and reused so here it safe money. t reduces the residual oil in the oil bearing substance to a very negligible point.

.10.4 Disadvantages of Solvent Extraction

. Solvent extract present high degree of fire explosiveness due to it easy flammability, xcept in a case where a non-flammable solvent is employed.

. The equipment and solvent need for solvent extraction are relatively very xpensive.

. Choice of a good solvent also prove a problem because the choice of a wrong solvent vill yield many disadvantages such as contaminating the odor of the fish oil produced and ven poisoning it.

CHAPTER THREE

3.0 EXPERIMENTAL WORKS

3.1 Experimental apparatus and reagents

3.1.1 Apparatus

The following equipments/apparatus were for the research.

1. Soxhlet extractor
2. Electric weighing balance
3. measuring cylinder
4. Thimble
5. Gallenkamp electric oven
6. Thermometer
7. Burette (50ml)
8. Pipette (25ml)
9. Round bottom flask
10. Pestle and mortal
11. Electric Blender
12. Petri dish
13. Boiling water bath
14. Reflux condenser
15. Conical flask 250ml
16. heating mantle
17. Sample bottles
18. Abbey Refractometer
19. Test tubes
20. Cotton wool
21. Refrigerator

3.1.2 Experimental Reagents used

1. n – Hexane
2. Starch indicator

3. Sodium thiosulphate
4. Phenolphthalein
5. Potassium iodide
6. Distilled water
7. Potassium hydroxide
8. Iodine
9. Potassium Hydroxide
10. Carbon tetrachloride
11. Hydrogen Chloride
12 Fat Samples

3.2 Methodology

The principle employed involve, pretreatment of sample extraction of the oil from the fishes, characterization of the extracted oil and subsequent comparison of the oil extracted for the different species used for the experiment.

3.2.1 Raw materials

The raw materials used are the 5 species of fishes earlier named. They include Mormirops deliciosus (Trunk fish) Bagrus docmac (Silver cat fish), Tilapia, Clarias (Catfish or mud fish) and Titus (shawa).

3.2.2 Pre-treatment of raw material

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

Refrigeration: The fish when bought was freezed in order to preserve it since the extraction did not commence immediately.

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

Size reduction: the fishes were then cut into sizes in order to enhance a speedy oven drying because of their size while removing the gills and intestine which were unwanted.

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

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

Weight: The weight of the sample were taken accordingly noting the difference in weight due to weight lost through evaporation

3.3 The fish oil Extraction process

The fish oil was extracted using soxhlet extraction apparatus and n-Hexane as the solvent. The solid substance or sample was placed in a porous thimble covered with cotton wool and the weight of the sample taken, before it was placed in the inner tube of the apparatus and then fitted to a round bottom flash of appropriate size that contain the solvent (Hexane) were heating commence at 60^{o} C for 1 hour.

As the heating continued, the solvent in the flask started boiling just within 5 minute of heating and the water begins to drop from the top to the sample in the thimble.

The water droplet was a function of the temperature of the heater and the rate of boiling because the high the temperature, the faster the droplets.

When the solvent reaches the top of the tube, it siphons over into the flask and thus remove the portion of the oil which has been extracted in the process of refluxing. It was noticed that 18minutes later, after boiling has started, there was refluxing and it continued like that every 2 minute until the lipid reduced and the refluxing time reaches since the higher the lipids content the slower the refluxing.

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

3.4 Comparison of Solvent extraction method with other methods of oil extraction

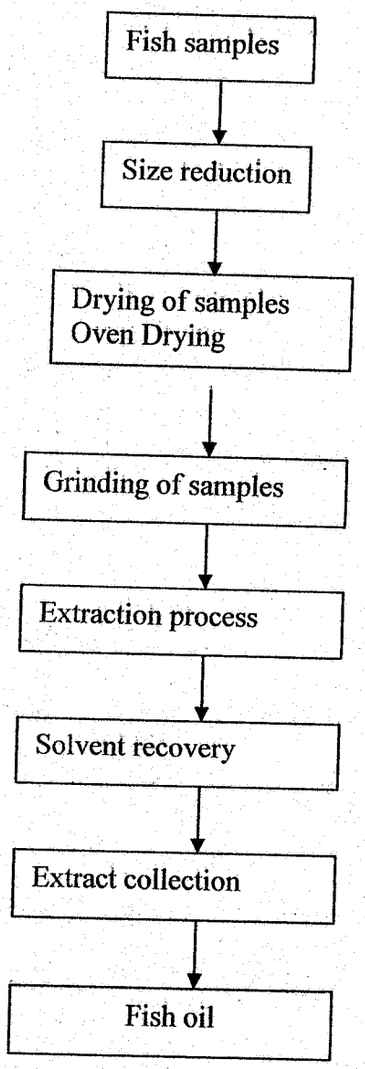
Soxhlet extraction is one of the most commonly used methods for determination of total lipids in dried samples. This is mainly because it is fairly simple to use and is the officially recognized method for a wide range of fat content determination. The main disadvantages is that a relatively dry sample is needed to allow the easily extraction how ever, it is time consuming and destructive for high moisture content food samples, it is often better to use non-solvent techniques or batch solvent techniques. Consequently, many instrument methods are simple to operate rapid require simple preparation and are not destructive.

Nonetheless they are often expensive to purchase and can only be used on certain types of food samples (Mc Clements, 2003) Extraction technique tend to be more accurate and generally applicable and are therefore the standard methods for official analysis of many food materials. Instrumental methods are more useful in quality assurance laboratories of food factories where many samples must be measured rapidly.

Solvent extraction however oxidizes the product and produces a reddish color and a reduction in vitamin A potency, and this is worsened at high temperatures or with prolonged heating time. (Hall 1992)

3.5 Extraction process of fish oil.

Process flow diagram for the extraction of fish oil using samples of fishes.



3.6 Evaluation of fish oil

The evaluation of the oil involves the analysis and testing needed for the assessment of the quality and purity as well as the identification of the oil. A number of physical and chemical "constants" have been 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. An assessment of all these are then related to the composition and therefore the identity of the fats being examined (ihekoronye and Ngoddy, 1985)

3.6.1 Determination of moisture content of the fish

The method specified by International standard organization (ISO) 1988 was used. The principle was that a test portion was heated at 105°C until moisture and volatile substances are completely eliminated, and the loss in mass determined.

Procedure: An empty Petri dish was weighed (w_1) the wet sample of the fish was then put into the Petri dish. The weight of the fish and Petri dish was taken (w_2), this was then transferred into the Gallenkamp oven which was set for 105°C this allowed the complete evaporation of the moisture content from the sample.

At the end of the drying, the dried sample in the Petri dish was removed and allowed to cool for a while after which the weight was taken (w_3) and the difference calculated. The percentage moisture removed represents the percentage loss in mass of the sample.

Calculation: Moisture content removed (%) $= \frac{[(w_2 - w_1) - (w_3 - w_1)] \times 100}{(w_2 - w_1)}$

3.6.2 Determination of Refractive Index

Refractive index is the ratio of the speed of light at a definite wave length in a vacuum to its speed in the medium and this varies with the wave length of the light and temperature.

Procedure:

Abbey refractometer was used in determining the refractive index of the oil. The measuring prism surface was cleaned with solvent and distilled water, and then wiped with a clean towel after which the mode selector was regulated to the desired mode position.

A drop of oil was dropped on the prism surface using a glass dropper and covered. The illumination arm was then positioned so that the exposed face of the upper prism will be fully illuminated. The refractometer was used through the eyepiece, the dark position viewed was

adjusted to be in line with the cross line. At no parallax error, the pointer to the scale pointed in the refractive index, the reading was then taken. This measurement represents the refractive index of the oil sample.

3.6.3 Determination of Acid value

The acid value is the number of milligrams of KOH required to neutralize the free fatty acid present in 1g of fat. Hence acid value gives an indication of the age and quality of the fat.

Procedure:

An account weight of 1g of fat sample was taken and dissolved in carbon tetrachloride and the solution was titrated with 0.05m Alkali; using phenolphthalein as indicator with constant shaking until a dark color was observed and the value noted.

3.6.4 Determination of Saponification value

The saponification value is the number of milligram of KOH required to neutralize the fatty acids present as a result of the complete hydrolysis of 1g fat (Plummer, 1978)

Procedure:

1.00g of the samples were weighed into 2.5cm^3 of alcohol 10cm^3 of 0.5m alcoholic KOH solution. This was then attached to a reflux condenser; the mixture was allowed to boil for 30mins with constant shaking. Similarly 2.5cm^3 of alcohol and 10cm^3 alcohol 0.5M KOH was treated while adding few drops of phenolphthalein to the warm solution and then titrated against 0.5 HCl until the pink color of the indicator just disappear. Same procedure was used for the other samples and the blank solution.

3.6.5 Determination of Iodine value.

The amount of iodine consumed is determined by titrating the iodine released (after adding KI) with a standard Thiosulphate.

Procedure:

0.3g of fats was weighed into a small weighing dish and placed in a 250cm^3 conical flask 10cm^3 of carbon tetrachloride was added to the samples.

To all the flask an equal quantity of wigits reagents was added about 25cm^3 using a burette, this was mixed well and kept in the dark for an hour, after that it was titrated with standard

0.1M sodium thiosulphate solution while adding 15cm^3 of 10% potassium iodide solution and 100 cm^3 of distilled water using starch as an indicator.

CHAPTER FOUR

4.0 Experimental Result

Moisture content: Initially the samples were oven dried in the electric oven before been blended into the finer form. It was at this form that the moisture content was determined by oven drying the sample for another 24hrs at temp 105°C

Table 4.1 **Moisture content percentage determination**

Sample	Mass of Sample before drying (g)	Mass after drying (g)	Percentage moisture content %
A	50	38.58	22.84
B	50	47.58	4.84
C	50	45.23	9.54
D	50	47.54	4.92
E	50	37.25	25.50

Table 4.2 **Content of oil present in dried fish sample**

Sample	Mass before extraction (g)	Mass after Extraction (g)	Percentage Extracted %
A	27.49	19.18	30.22
B	43.91	43.91	6.72
C	36.39	31.10	14.52
D	37.27	30.57	17.93
E	33.02	25.09	24.02

Table 4.3 **Analysis of oil samples**

Sample	R. index	Acid value (mg)	Iodine value (g)	Saponification (mg)	Melting point °C
A	1.6642	8.40	174.41	549.78	100
B	1.6769	7.84	182.88	367.46	92
C	1.6240	6.72	182.88	347.82	88
D	1.5990	5.04	187.11	318.48	91
E	1.5850	6.44	178.65	398.31	98
Standard value	1.473 - 1400	0.40-4.8 mg/KOH	135 – 190 I_2/100 of sample	176 - 195	-

4.4 DISCUSSION OF RESULT

Experimental analysis was conducted on the oil extracted from each of the samples of fishes and the results in table 4.1 shows that the percentage moisture content of species A, B, C, D ,E were 22.84, 4.84, 9.54, 4.92 & 25.05% respectively. This signifies that species A & E will require higher drying time than species B, C, D when subjected to the same drying condition. However, specie B has the least moisture content.

Table 4.2 show that A, B, C, D & E has 30.22, 6.72, 14.52, 17.93, 24.02% oil contents respectively signifying that specie A has a higher amount of oil while B has the least amount of oil extracted.

Comparing table 4.1 & 4.2, it can be deduce that moisture content of the fish is a reflection of its oil content, because all the species with higher moisture content yield high amount of oil when extracted.

The result of the characterization carried out of the sample of the fishes presented in table 4.3 shows that samples A,B, C, D & E has a refractive index of 1.664, 1.677, 1.624, 1.599 &1.585 respectively which are outside the range of standard value of 1.4 – 1.473 for fishes. The significant of the result is that, the oil obtained from the species is denser that water. However, we could not account for the disparity between the refractive index and the standard value.

The melting point of samples A, B, C, D & E were 100, 92, 88, 91 & 98 ^{o} C respectively. The melting point observed seem with the rang range of the boiling point for specie A & E while B-D were far from the melting point of water.

From the same table, specie A, B, C, D & E has saponification value of 549.78, 367.46, 347.82, 318.48 & 398.31 respectively. These values were far above the standard range of 176 – 195. This higher value could be of industrial importance when employed in soap making though may be very expensive.

PRECAUTIONS TAKEN DURING THE EXPERIMENT WORK

The following precautions were observed during the experimental processes.

1. During the sample handling, adequate care was taken to avoid loss in weight when sealing up the thimble with a cotton wool.
2. All error due to parallax were avoided when taking the weight of the sample with electric balance.
3. The apparatus used for the experiment are thoroughly washed to avoid dirt and handled carefully due to the fragility of some of the apparatus
4. Water continuously flow through the condenser and into the soxhlet apparatus to avoid cracking of the condenser in order words there was continuous inlet and outlet of water during the experiment
5. All flow channels were kept at airtight to avoid atmospheric pressure from interfering with the vapor pressure.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The production and characterization of oil from different samples of fishes was performed and the results showed that fish oil has a very high percentage of iodine value, hence can be recommended for patient suffering from goiter. Also, the moisture content of fish is a reflection of its oil content. From the analysis of the content of the different species it can be concluded that sample A (mormyrups deliciousus) and sample E (titus) has the highest content among the five species analyzed.

5.2 RECOMMENDATION

As a result of the numerous health benefit of fish consumption. Easting fish especially [fatty fish] is recommended at least two times a week. It is also good for those who have any heart infection

Population	**Recommendation**
Patients without documented coronary heat diseases [CHD]	Eat a variety of [preferably fatty fish] atleast twice a week .including oils and food rich in alpha- linoleic acid [flaxseed, walnuts ,soy beans oils].
Patients with documented coronary heart diseases (CHD)	Consume about 1g of EDA. DHA per day preferably from fatty fish. EPA + DHA supplements could considered in consultation with the physician
Patients who needs to lower triglycerides	2 to 4 grams of EPA + DHA per day provided as capsule under a physician care.

Patients taking more than 3 grams of omega 3 fatty acids from supplements should do so under a physician care.

Patients taking more than 3grames of omega-3 fatty acids from supplements should do so under a physical care.

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APPENDIX

Percentage Moisture Calculation

Percentage moisture = $\frac{\text{mass of wet sample -- mass of dry sample}}{\text{Mass of wet sample}} \times 100$

A. $\frac{50 - 38.58}{50} \times 100 = 22.84\%$

B. $\frac{50 - 47.58}{50} \times 100 = 4.84\%$

C. $\frac{50 - 45.23}{50} \times 100 = 9.54\%$

D. $\frac{50 - 47.54}{50} \times 100 = 4.92\%$

E. $\frac{50 - 37.25}{50} \times 100 = 25.50\%$

...of oil Extracted

...ction yield x 100
...ver weight Extracted yields initial – final mass

A. $\frac{27.49 - 19.18}{27.49} \times 100 = 30.22\%$

B. $\frac{43.91 - 40.96}{43.91} \times 100 = 6.72\%$

C. $\frac{36.39 - 31.10}{36.39} \times 100 = 14.54\%$

D. $\frac{37.27 - 30.57}{37.27} \times 100 = 14.54\%$

E. $\frac{33.02 - 25.09}{33.02} \times 100 = 24.02\%$