International Journal of Information, Engineering & Technology

Volume 11, Issue 4, PP 41-48, ISSN: 2360-9194, February, 2021
Double Blind Peer Reviewed International Research Journal
garcjournalssubmit@gmail.com
Journal Series: Global Academic Research Consortium (garc)

Characterization of Fat Content of Different Types of Dried Fishes

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Abstract: *Characterization of fish oil were carried out using Soxhlet apparatus and n-Hexane as the solvent for extraction. The extraction was carried out at the boiling point of the solvent. Three different species of fishes were used for the experiment. From the result of the extraction, it was observed that the cat fish, tilapia and lung fish have a good percentage of oil content of dry mass, as 6.72, 14.52 and 17.92, respectively. The iodine and refractive indexes values of these study is in agreement with those obtained, and also falls within the specification of the standard value. These values, therefore, show that fish oil is a non-drying oil, a good lubricant and a cure for many diseases e.g., goiter; while saponification and acid values were found to be higher than the standard value. However, the boiling points of the oil were close to the boiling point of water.*

Key words: *Fat Content of Different Types of Dried Fishes*

INTRODUCTION

Fish oil is the lipid fraction extracted from fish and fish by-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 oils. Apart from its various uses as consumable oils, it is also appreciable in both pharmaceuticals and industries.

However, the most frequently used technique in fish oil extraction is fractionation by high-speed configurations, low temperature solvent extraction, superficial fluid extraction etc. In this study, solvent extraction was employed during this research. This is because solvent extraction is one of the most efficient methods of oil extraction from oil bearing materials based on the fact solvent can easily be recovered and recycled and it reduces the residual oil in the oil-bearing substance to less than 1%. In view of this, care must be taken when selecting the right solvent for the extraction process. Practically, all fish species as well as other marine animals may be converted into fish oil and meal. The

composition and quality of these fish species are predominant factors in determining the properties and yield of the products [7]. The quality and freshness of the raw material in preparation of premium quality fish oil and fish meal (Sarjent J. R., 1997). Enzymatic and bacteriology activity in the fish and fish products can rapidly increase, which in turn can substantially decrease the content and quality of the protein and oil as protein decomposes to amines and ammonia, and both reduce the protein value and recovery.

Fish oil is different from other oils mainly because of the unique variety of fatty acids it contains including high level unsaturated fatty acid (Oega-3FFA and Omega-6FFA) which are essential to the body. This is known as the eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA). Fishes are creatures that live and breathe in water and there are over 25000 different types of fishes in the world and numerous others yet to be discovered (Adeyemo; F. A., 2004). The production of fish oil started long ago since the 19th century in Northern Europe and North American, where they utilized the non-edible fishes and other fish by-products to produce oil used in leather tanning and in the production of soap and glycerol (Alfred and Patrick 1985).

Fish oils have well documented beneficial health effects. They are readily available sources of long-chain polyunsaturated fatty acid (PUFA), especially the n-3 series consisting mainly of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and the n-6 series including arachidonic acid (AA) and γ-linolenic acid (GLA). These PUFAs have many physiological functions, and are used for various purposes. For example, EPA has been used for the treatment of arteriosclerosis and hyperlipemia since 1990 in Japan. DHA plays a role in the prevention of a number of diseases in humans, including cardiovascular disease. From previous studies, fishes are known to be excellent sources of high-quality protein, and contain sufficient amounts of most of the essential amino acids (Charles *et al*, 2005). Fishes are also high in PUFA, EPA, and DHA, and the effects of these fatty acids are well documented in numerous investigations.

Fish oil is very similar to one another in their physical nature. A whole fish consists of protein, fat, Ash and water irrespective of the species however; these compositions are greatly influenced by seasonal changes due to the nature cycle, maturity stage, geographical location, feeding habit etc. Because the more a fish eats, the greater the oil and other chemical composition will be produced. 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 odour is due to the unsaturated fatty acids, since hydrogenation causes the oil to lose their colour but fish caught in colder water have a higher degree of instauration than that caught in warm water. The lipids are the edible part of fish and is important to the food scientist in two respects, firstly any oil deposit 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.

Some very recent researches carried out at the University of Minnesota found out that emulsified fish oil is much better absorbed than the straight oil in gelatin capsules (Kulli *et al*, 2007). Physical properties of fish oil comprise of melting point, the refractive index and the specific gravity, whilst iodine value, saponification value, and acid value the

chemical properties are iodine value, saponification, and acid value. The world demand for vegetable oil is constantly increasing due to increase in the world population. The production of vegetable oils and fats, which is around 30 metric tons, is not enough to meet the needs of people, since fats and oil are required industrially for the manufacturing of soap and other industrial purposes (Weiss E. A., 2000). Traditionally, the main sources of fish feeds were fish meal and oils from these pelagic fisheries species: anchovy (Engraulis anchoita), sprat (Sprattus sprattus), herring (Clupea harringus), sand eel (Ammodytes spp.), and capelin (Mallotus villosus). These species are fatty fishes (with a considerable content of fat in their flesh and viscera), but hardly any of them are consumed by humans because their size is very small and they are difficult to keep fresh. Also, the trimmings (processing by-products) of fish from fisheries as well as from aquaculture are often processed to produce fish meals and oils. This part of the by-product utilization from fish, to fish oil and meal is undergoing a rapid development. The crude fish oils are produced mostly by cold-pressing and cooking and pressing.

Fish oils, produced by a cooking process, are pressed and purified in several steps. The proteins are denatured and removed by pressing. Thereafter, the oil is separated. The main step in the purification of fish oils and the removal of impurities is degumming, where phospholipids, proteins, and carbohydrates (as well as trace metals) are removed. The next step is alkali refining, which removes the free fatty acids, pigments, and residues of the above compounds. Sometimes bleaching is also performed to remove pigments, oxidation products, heavy metals, sulfur compounds, and soaps. One can further purify the oil in a carbon-treatment system to remove dioxins, polychlorinated biphenols (PCBs), furans, and polyaromatic hydrocarbons (PAHs). The oils are often stabilized with α-tocopherol. When concentrates of fish oils are processed, the total persistent organic pollutants (POPs) are removed by a “working fluid” process, a new methodology consisting of fatty acid ethyl esters (Breivik & Thorstad, 2005).

The health effects of fish oils are ascribed especially to the fatty acids EPA (20:5n-3) and DHA (22:6n-3). These fatty acids have a number of beneficial properties in humans. A meta-analysis by Mozaffarian and Rimm (2006) demonstrated the advantage of an intake of these fatty acids in relation to cardiovascular disorders. Beneficial effects on neuro-development during gestation and infancy were also demonstrated. The major conclusion from this study was that the benefits of fish intake exceed the potential risks of the possible harmful effect of pollutants (Mozaffarian & Rimm, 2006). Hites et al. (2004) published an important study of many oil-rich fishes regarding the contents of pollutants. The amount of DHA recommended for daily intake varies between studies. Recommendations on the intake of DHA and EPA are given in several countries, for example, in the United Kingdom (Anonymous, 1994) as well as in the United States (Simopoulos et al., 1999). The need for DHA and EPA is caused by the limited and variable ability of mammals to elongate and desaturate 18-carbon fatty acid chains, both n-6 and n-3. A study showing the desaturases involved in this conversion was published by Voss et al. (1991). Today, the importance of DHA as a nutrient that is vital in gene expression and thereby a factor in lipid metabolism is recognized (Massaro et al., 2008). Recommended dosages of fish oils as foods and as additives or supplements in different foods are being developed. The measured levels of omega-3 in foods are often used in the marketing and labeling of functional food products rich in omega-3 fatty acids. Health claims can relate to the contents of nutritionally beneficial compounds or disease-risk reduction claims. The omega-3 health claims can be

divided into two different claims: omega-3 PUFAs (ALA-rich PUFA oils) and long-chain omega-3 PU- 522 J. Fatty Acid containing oils (LC omega-3-rich PUFA oils, e.g., fish oils), the latter containing mostly EPA 20:5n-3 and DHA 22:6n-3. The legislation related to the omega-3 oil's fatty acid content and composition is still under revision, and will likely continue to undergo changes as new research studies are published and scrutinized.

Characterization of fish oil

In the area of fish oil production, the terms "fish oils" or "marine oils" are used broadly to include all lipids that are derived from organisms that occur in aquatic environments (fish, other aquatic animals, and plants) (Kulli *et al*, 2007). However, fish oil supplements are generally derived from fish – usually tuna, sardine mackerel, herring, anchovy, menhaden, cod, or salmon – because these species contain high concentrations of EPA and DHA.

There are many types of fish oils sold in the marketplace, such as pure fish oil; fish oil enriched with n-3 fatty acids; fish oil supplemented with plant seed oils such as olive oil or flaxseed oil; and fish oil supplemented with an essential element (e.g., calcium). Although there have been several studies on fish oils or fish oil supplements, most of the work has focused on the benefits to human health, the development of production processes to increase the levels of EPA and DHA, or analytical methods for the quantitative determination of fatty acids.

The evaluation of the oil involves the analysis and testing needed for the assessment of the quality, purity and as well as the identification of the oil. A number of physical and chemical "constants" 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 is then related to the composition and therefore the identity of the fats being examined (Weiss E. A., 2000). Fish salting or brining, drying or smoking, are the traditional techniques for the improvement and storage of fish. About 8 million tons of fish (25-30%) of the world catch for human consumption are dried, salted, smoked or treated by some combination of these processes each year (Kamruzzaman, 1992). Drying is regarded as a traditional and primitive method of preservation of fish. It is of vital importance in the developing countries like Bangladesh. Fish usually takes 5 to 7 days to dry during which it gets heavily contaminated (Doe et al., 1977). Quality of dried fish includes organoleptic, microbiological and biochemical quality (Azam et al., 2003). Less work has been done on the quality assessment of dried fish.

METHODOLOGY

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

The samples were purchase from Tasha Baga fish market in Maiduguri, Borno State. The raw materials used are the 3 species of fishes earlier named. They include Clarias (Catfish or mud fish), Chichilid (Tilapia), and N. blanchardi (Lungfish). In order to enhance a successful extraction of the oil, the fish underwent some treatment prior to the extraction. These include:

Refrigeration: The fish when bought was dried and preserve at room temperature, since the extraction did not commence immediately.

Washing: The fish was thoroughly washed in order to remove dirt that might get stuck to the body during dry preservation 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 was reduced by oven drying since water in oil. Further size reduction: the samples were further reduced in size and later blended into a finer form by pounding in a mortar after undergoing the moisture content elimination in the oven.

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

The fish oil extraction process

The fish oil was extracted using Soxhlet extractor 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. Heat was applied to heat the solvent to its boiling point 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.

When the solvent reached the top of the tube, it siphoned over into the flask and thus removes the portion of the oil which has been extracted in the process of refluxing. It was noticed that 18 minutes later, after boiling has started, there was refluxing and this continued at 2 minutes interval. 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 collected and measured.

Determination of moisture content of the fish

The principle was that a test portion was heated at 105^{0C} until moisture and volatile substances are completely eliminated, and the loss in mass determined.

Procedure: An empty Petri dish was weighed (w1) the wet sample of the fish was then put into the Petri dish. The weight of the fish and Petri dish was taken (w2), this was then transferred into the Gallenkamp oven which was set for 105^{oC} 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 (w3) and the difference calculated. The percentage moisture removed represents the percentage loss in mass of the sample.

Calculation:

$$\text{Moisture content removed } (\%) = \frac{[(w2-w1)-(w3-w1)]}{(w2-w1)} X100$$

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 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.

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 colour was observed and the value noted.

Determination of saponification value

The saponification value is the number of milligrams of KOH required to neutralize the fatty acids present as a result of the complete hydrolysis of 1g fat [1].

Procedure: 1.00g of the samples was 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 30 minutes with constant shaking. Similarly, 2.5 cm^3 of alcohol and 10 cm^3 alcohol 0.5 M
KOH was treated while adding few drops of phenolphthalein to the warm solution and then titrated against 0.5 HCL until the pink colour of the indicator just disappeared. Same procedure was used for the other samples and the blank solution.

Determination of Iodine value

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

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

To all the flask an equal quantity of about 25 cm^3 wigits reagents was added 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 15cm3 of 10 % potassium iodide solution and 100 cm3 of distilled water using starch as an indicator.

RESULTS AND DISCUSSIONS

4.1. RESULTS

Table-1. Moisture and oil content of the five samples.

Sample	Moisture content (%)	Oil extracted (%)
A	4.84	6.72
B	9.54	14.52
C	4.92	17.93

Table-2. Comparison of the properties of the oil samples with the standard.

Sample	R.	Acid value	Iodine	Saponification	Melting

	index	(mg)	value (mg)	(mg)	point (^{0C})
A	1.6769	7.84	182.88	367.46	92
B	1.6240	6.72	187.88	347.82	88
C	1.5990	5.04	187.11	318.48	91
Standard Value	1.400	0.40-4.8 mg/KOH	135-190 I_2/100 of sample	170-195	-

4.2. DISCUSSIONS

Experimental analysis was conducted on the oil extracted from each of the samples of fishes and the results in Table-1 above shows that, the percentage of moisture content of species A, B, and C were, 4.84, 9.54, and 4.92 %, respectively. This signifies that species A, B and C will require different drying time and condition. However, specie A has the least moisture content. While their oil content was 6.72, 14.52, and 17.93 %, respectively signifying that specie A has a least amount of oil while C has the large amount of oil extracted. From the same table, it can be deduced that moisture content of the fishes is a reflection of their oil content, this is because, the species with higher moisture content yield high amount of oil when extracted.

The result of the characterization carried out for the samples of the fishes presented in Table-2 shows that samples A, B, and C has a refractive index of 11.677, 1.624, and 1.599 respectively which are outside the range of standard value of 1.4 - 1.473 for fishes. The significance of the result is that, the oil obtained from the species is denser that water. However, these values fall within the specification. The melting point of samples A, B, and C were 92, 88, and 91^{0C} respectively. The melting point observed seem to be within the range of boiling point of water for specie A and C while that of B is far from the melting point of water. From the same table, species A, B, and C has saponification values of 367.46, 347.82, and 318.48 respectively. These values were far above the standard range of 176-195.

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