

EXTRACTION PURIFICATION AND ENCAPSULATION OF ESSENTIAL FATTY ACID FROM MARINE FISHES

Submitted in partial fulfilment of the requirements for the award of
Master of Technology Degree in Biotechnology

By

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**PROJECT PHASE II-REPORT
DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Lovely Mathew** (Reg. No. **41810009**) who carried out the project entitled "**Extraction Purification and Encapsulation of essential fatty acid from marine fishes**" under my supervision from **JAN 2023 to MAY 2023**.

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ABSTRACT

Fish oil is made from the tissues of oily fish. It is advised as part of a healthy diet because it includes omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are precursors to eicosanoids, which decrease inflammation throughout the body. Fish oils must be supplied in trace amounts for optimal health benefits, cellular metabolism, and normal physiological activities. Seafood has recently gained popularity due to its high omega-3 fatty acid content. Many studies have been conducted to demonstrate the health advantages of omega-3 fatty acids as a dietary supplement against a variety of life-threatening disorders such as cardiovascular disease, cancer, skin disease, and numerous inflammatory diseases, among others. They are also important for the fetus's healthy growth and development. National and international health organisations have established daily fish oil consumption recommendations owing to the vast health potential it contains, and it is vital to raise societal awareness of its significance, since the modern world has become a hotspot for numerous lifestyle disorders. We addressed some of the most significant, such as fish oil and its uses, in this study.

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LIST OF ABBRIVATIONS

ALA	Alpha-Linolenic Acid
FFAs	Free Fatty Acids
PUFA	Polyunsaturated Fatty Acids
SFAs	Saturated Fatty Acids
EPA	Eicosapentaenoic Acid
DHA	Docosahexaenoic Acid
GC	Gas Chromatography
ECD	Electron Capture Detector
TCD	Thermal Conductivity Detector

1. INTRODUCTION

Several processes, including physical fractionation, are used to create fish oil. [Hirata et al.,1993] low temperature solvent fractionation [Moffat et al.,1993] and supercritical fluid extraction [Dunford et al.,1997], However, the standard technique of heating, pressing, and centrifuging to collect the oil from miscella is the most prevalent method used to manufacture fish oil [Bimbo,1990; Pigott and Tucker,1990]. So far, the traditional extraction technique has been regarded as the best extraction procedure due to its advantageous characteristics such as yield, economic viability, and time consumption. The extraction of lipids from other fish components is involved in the manufacturing of fish oil. In general, the production requires a number of stages for product purification. [Firestone,1989] According to American Oil Chemist's Society, (AOCS), Degumming, neutralizing, bleaching, and deodorizing are the primary processes in fish oil refining. The degumming process eliminates both soluble and insoluble contaminants, followed by neutralization of crude oil with sodium hydroxide, which removes free fatty acids (FFAs). Bleaching assists in the elimination of soap, trace metals, sulphurous compounds, and a portion of the more stable pigments and pigment-breakdown products, aldehydes and ketones. The final stage is deodorization, which eliminates any remaining FFA. aldehydes and ketones, which are responsible for an unpleasant oil aroma and flavor [Young,1978], [Gavin,1978]. Aside from fatty acids, fish oil contains amino acids, which contribute to its positive effects. Fish has high levels of all necessary amino acids, notably lysine, which is deficient in grains [FAO,2005]. In fish, free amino acids serve an important function in metabolism, such as adjusting osmotic pressure and acting as an energy source. Even though amino acids are found in fish oil, they have not been well investigated, and there is currently no definite information about the amino acid composition of fish oil. The fish oil includes a considerable amount of amino acids, which will be removed alongside the fatty acids during the usual extraction procedure. Marine fish oil is extremely unsaturated and easily oxidized when exposed to air, light, and heat [Lytle et al.,1992]. Fish oil degrades in two ways: oxidative deterioration and hydrolytic spoiling [Cmolik & Pokorny,2000]. Because of its high polyunsaturated fatty acid content, fish oil is especially sensitive to oxidative deterioration [Huss,1988] Moreover, the rate of oxidation of fish oil is

substantially higher than that of other oils. The oxidation profiles of derived fish oils and lipids in fish tissues are frequently considerably different [Liston et al.,1963]. The primary cause of fish oil quality decline is autoxidation [Fujimoto et al.,1968]. During the induction period, undesirable tastes and smells occur even at extremely low peroxide levels (early stage of oxidation). Many marine fish species have been examined for fish oil production, but little attention has been dedicated to fish oil production from low-value fishes. In order to better understand the nutraceutical qualities of fish oil and its wide range of applications, the current study attempted to extract fish oil from low-value fish. The goals of this study were to manufacture fish body oil from low-value fish, Sardinella and pomfret, using direct steaming and sun drying methods, and to investigate the influence of purification on the composition of fatty acids, amino acids, vitamins, and oil quality.

Table 1.1: Fishes and its omega 3 content

Type of fish	Omega-3 content (DHA and EPA)	Mercury concern level, per the FDA
Farmed Atlantic salmon (cooked)	1.83 grams	Best choice
Atlantic herring (cooked)	1.71 grams	Best choice
Wild Atlantic salmon (cooked)	1.57 grams	Best choice
Whitefish (cooked)	1.38 grams	Best choice
European anchovies (raw)	1.23 grams	Best choice
Atlantic mackerel (cooked)	1.02 grams	Best choice
Greenland halibut (cooked)	1 gram	Good choice
Pink salmon (canned and drained)	0.91 grams	Best choice
Bluefish (cooked)	0.84 grams	Good choice
Wild rainbow trout (freshwater trout) (cooked)	0.84 grams	Best choice
Atlantic sardines (canned in oil)	0.83 grams	Best choice

2. LITERATURE SURVEY

2.1 Fish oil composition

The physicochemical characteristics of fish oils are fairly similar. Regardless of the species, a fish's entire body is made up of protein, fat, ash, and water, although these compositions are strongly altered by seasonal variations because of the natural cycle, stage of maturation, location, feeding behaviour, etc. Because a fish will create more oil and other chemical composition the more it eats. Due to the presence of long chain unsaturated fatty acids, fish oil generally has a higher level of complexity than oils from terrestrial animals or plants. The unsaturated fatty acids in fish oil are widely thought to be the cause of the fish oil's odour. because hydrogenation makes the oil lose its colour, yet fish caught in cooler water have a higher degree of instauration than fish captured in warm water.

The lipids, which make up the majority of a fish's edible body, are crucial to food scientists for two reasons: first, any oil deposits have a noticeable impact on how cooked meat tastes, and second, they may have some medicinal uses. Due to natural lipase and bacteria present in the fat, fish oil degrades quite quickly. These two hydrolyse fat to produce free fatty acids. Physical, chemical, and nutritional aspects of the oil are impacted by the state of the fish at processing time. Fish of inferior quality produce foul-smelling oil that is rich in free fatty acids and sulphur. The economic worth and use of the oil are impacted by these unwanted qualities.

2.2 Properties and Structure of Fatty Acids

A methyl group is found at the opposite end of a hydrocarbon chain (CH_2) or a chain having an acid or carboxyl group (COOH) at one end. They are often $\text{CH}_3(\text{CH}_2)_n\text{COOH}$ in structure. Depending on how many hydrogen atoms are present, fatty acids are frequently categorized as saturated or unsaturated. PUFAs include two or more double bonds, whereas saturated fats (SFAs) have the greatest amount of hydrogen atoms and so do not. As a chain of carbon atoms with one double bond, monounsaturated fatty acids (MUFAs) are composed. Because the initial double bond is located on the third carbon atom from the methyl- or N-terminal end of the fatty acid chain, poly-unsaturated fatty acids, also known as n-3 fatty acids, are separated from other fatty acids by this feature (Hossain 2001; Querques *et.al.*, 2011) The longer-chain

metabolites of α -linolenic acid, eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (C22:5n-3), and docosahexaenoic acid (DHA, C22:6n-3), make up the n-3 PUFA family (Moussa et al. 2014).

2.3 Importance of n-3 fatty acids in fish for human diet

The significance of n-3 fatty acids in fish for human nutrition Today, we are aware of the positive impact eating fish has on preventing coronary artery disease, diabetes, hypertension, and cancer. Particularly significant in illness prevention are DHA and EPA. (Visentainer *et al.*, 2007; Cengiz *et al.*, 2010; Ljubojevic *et al.*, 2013)

2.3.1 Some benefits of n-3 fatty acids

Since they are not manufactured by the body, n-3 fatty acids are crucial for all living processes. As a result, the food is the only source of these acids. The following are some benefits of n-3 fatty acids:

1. It has been demonstrated that eating fish or n-3 fatty acids helps lower cardiovascular risk factors. A population-based, nested, case-control study found a significant negative correlation between fish consumption and the risk of sudden death (5.5 g of n-3 fatty acids per month, or two fatty fish meals per week, was linked to a 50% lower chance of primary cardiac arrest) (Etherton *et al.*, 2002).
2. As DHA rather than ALA, the n-3 fatty acids required for healthy brain development, oily fish consumption is crucial for the brain and neural system development of children. Since n-3 fatty acids are crucial for the development of nerve cell membranes, they aid in the communication of nerve cells, a crucial component in maintaining mental health. Compared to women who don't consume fish, a recent FAO/WHO expert consultation found that including fish in the diet lowers the chance of women giving birth to infants with less-than-optimal brain and neural system development (Suvarna, 2008; FAO 2014). Thus, n-3 LC PUFAs from marine sources provide numerous advantages for the health of women, including those related to depression, mental health, maternal and poly-cystic ovarian syndrome, dysmenorrhoea and some cancers (Manus & Hunt, 2013).

3. The growth of children can be aided by n-3 fatty acids. They also have an impact on kids' learning, problem-solving, and cognitive abilities, which helps lower their chance of developing asthma, as well as their IQ and eyesight (Visentainer *et.al.*, 2007).
4. There are signs that eating fish or n-3 fatty acids may help lower blood pressure, hyperlipidemia, hyperglycemia, and inflammation. Eating fish may also reduce your risk of developing some malignant illnesses (Fisger & Gleib, 2015).
5. According to reports, eating fatty fish cuts the incidence of dementia by 28%, whereas eating lean, fried fish had no protective impact (Fotuhi *et.al.* 2009).
6. The researchers state that fish oil and n-3 fatty acids in particular may be useful in preventing diabetes illness (Soltan, 2012).

2.4 Recommendations for EPA and DHA intake

The recommended daily EPA and DHA intake amounts vary widely between nations, with France's recommendation being 500 mg/day and Norway's being 1-2 g/day. The appropriate intakes for adults in the USA have been determined at 17 g of n-6 fatty acids (LA) per day for males (19–50 years old) and 12 g of n-3 fatty acids (ALAs) per day for women (19–50 years old), respectively (Candela *et.al.*, 2011; www.nutrifacts.org). In terms of international organisations, the World Health Organization suggests consuming 0.3–0.5 g daily, While the NATO suggests 800 mg/day and the International Society for the Study of Fatty Acids and Lipids (ISSFAL) suggests 500 mg/day. Two 4-ounce servings of fish per week, preferably fatty fish, are advised by the American Heart Association and Academy of Nutrition and Dietetics (especially, n-3 fatty acids can be found in fatty fish, such as salmon, tuna, anchovies and sardines, other marine life such as algae). This amount of fish consumption corresponds to around 500 mg of EPA and DHA each day (Candela *et.al.*, 2011; Watters *et.al.*, 2012). Maintaining a proper balance of n-3 and n-6 PUFAs in the diet is important since these two nutrients work together to prevent coronary heart disease and perhaps other chronic diseases. Most n-6 fatty acids have a tendency to increase inflammation, whereas n-3 fatty acids help minimise it. A poor balance of these critical fatty acids promotes the growth of disease, whereas a healthy balance promotes the maintenance

and even improvement of health (Simopoulos, 2010; Mirajkar *et.al.*, 2011). The consumption of omega-3 fatty acids is currently significantly lower due to the decline in fish consumption and the industrial manufacture of animal feeds high in grains containing omega-6 fatty acids, which results in the creation of meat rich in omega-6 and low in omega-3 fatty acids (Simopoulos, 2004). The diets of Western nations therefore often include 11 to 30 times more omega-6 than omega-3 fatty acids. Contrarily, the modern Mediterranean diet has a healthier and more suitable combination of n-3 and n-6 fatty acids (Dhikav *et.al.*, 2004).

2.5 Fish Species containing n-3 Fatty Acids

Fish species are divided into four categories based on their fat content: lean (less than 2% fat), medium fat (2-7% fat), fat (7-15% fat), and extremely fat (more than 15% fat) (Tang *et.al.*, 2009). A fish's fat content fluctuates depending on the season, whether it is sexually mature and spawning, and what it consumes. The fat level of farmed fish is highly correlated with the type of feed that it consumes. The proportion of n-3 fatty acids in a species is often correlated with its overall fat content. In general, fish with darker flesh—including herring, salmon, mackerel, and bluefish—contain more total fat than fish with leaner, lighter-colored flesh, such cod, flounder, and pollock. In reality, all fish species contain EPA and DHA, although the levels of these compounds vary widely across and within species based on environmental factors including temperature, salinity, season, life stage, nutrition, and habitat, as well as whether the fish are herbivorous, omnivore, or carnivorous (Cengiz *et.al.*, 2010; Querques *et.al.*, 2011). As a result, the species of fish and shellfish ingested has a significant impact on the amount of EPA and DHA received through food (Mahaffey *et.al.*, 2011). Five commercially significant fish species from the Persian Gulf—*Scomberomorus commersoni*, *Thunnus tonggol*, *Euthynnus affinis*, *Scomberomorus guttatus*, and *Dussumieria acuta*—had their fatty acid compositions in various parts examined. These included the liver, viscera, muscle tissue, and the head, tail, fins, and skin (HTFS)—all excellent sources of n-3 PUFAs the liver, whole body, and livers of *D. acuta*, *E. affinis*, and *T. tonggol* were the highest sources of n-3, followed by the viscera of *E. affinis*. The richest sources of n-3 were HTFS in *S. guttatus* and *S. commersoni*, liver in *S. guttatus*, and liver in *S. guttatus* (Sahari *et.al.*, 2013) Fish from seawater contain greater

concentrations of EPA and DHA than fish from freshwater (Tang *et.al.*, 2009). Fish from warm or temperate climates tend to be high in saturated and monounsaturated fatty acids, whereas fish from colder places tend to be higher in PUFAs (Dhaneesh *et.al.*, 2012). The number of fatty acids in fish tissues really corresponds to the number of fatty acids in their diet. Research has indicated that compared to wild fish, farmed fish had higher fat content and lower levels of n-3 fatty acids (Hardy, 2003). However, the farmed fish exhibits more consistent rates of EPA and DHA synthesis, maybe because the diet is monitored and adjusted throughout the farming process (Moussa *et.al.*, 2014). The amount of n-3 PUFAs in commonly sold wild and cultivated freshwater fish was examined by Li and Hu (2009). Crucian carp, mandarin fish, silver fish, and snake headed fish—four kinds of wild and domesticated freshwater fish—were investigated. With the exception of EPA and DHA for mandarin fish, long-chain n-3 fatty acid levels in cultivated samples were greater than those in the wild. Wild fish had higher concentrations of DHA for silver fish and ALAs for snake headed fish.

2.6 Encapsulation technology

Encapsulation is a process whereby active ingredients are entrapped or coated by a polymeric matrix, and then dried to obtain a free-flowing powder (Dziezak, 1988). Typically, the entrapped agent is referred as the “core material”, whereas the outer coating is referred as the “wall material”. Capsules can be classified, according to size, into three broad categories: macro-capsules ($>5000\ \mu\text{m}$), micro-capsules ($0.2\sim5000\ \mu\text{m}$) and nano-capsules ($<0.2\ \mu\text{m}$) (King, 1995). depending on the core material and processing conditions, microcapsules can be spherical, oval or irregular in shape (Madene *et al.*, 2006). A summary of mechanical and chemical encapsulation techniques is presented in Table 2.1. Microcapsules typically have mononuclear or polynuclear structure (Figure 2.1). In the former, the core material is concentrated at the center of the capsule, surrounded (or coated) by the biopolymer wall material. In the latter, the core material exists as phase-separated inclusions, dispersed throughout the biopolymer wall matrix. Regardless of capsule design, both functions to delay degradative reactions (i.e., oxidation) of the entrapped core material. Advantages of using encapsulation technology in the food industry include: a) increasing the stability and bioavailability of sensitive core materials; b) improving miscibility within aqueous-

based food products; c) masking off-flavours and odours; and d) controlling and targeting the release of entrapped core materials at a desired rate to optimize site- and dose-delivery (Desai and Park, 2005).

Table 2.1 Mechanical and chemical microencapsulation techniques.

Type	Technique	Summary of process	Application
Mechanical	a) Spray drying (5-5000 μm) ^a	A core-wall emulsion is spray dried to give wall hardening. ^b	Lipids Flavours
	b) Extrusion (250-2500 μm) ^a	Liquid core is passed through a center orifice, while the wall is passed through the outer annulus of the concentric orifices. The wall material is then hardened. ^b	Flavours
	c) Spinning disk (5-1500 μm) ^a	A core-wall emulsion is loaded onto a spinning disk; the spinning action allows coating of the core component with the wall material; cooling results in wall hardening. ^a	Flavours
Chemical	a) Coacervation (2-1200 μm) ^a	Interactions of polymer(s) to form wall material; coacervate coated droplets are dried to give wall hardening. ^b	Dyes Flavours Lipids
	b) Molecular inclusion (5-50 μm) ^c	Encapsulation of a polar molecules by cyclodextrins, which have a hydrophobic hollow center. ^b	Flavours
	c) Interfacial polymerization (0.5-1000 μm) ^a	A nonpolar polymerizable monomer and the core material are dispersed within a polar phase; a catalyst induces polymerization of monomers to surround the core material. ^b	Lipids

^a Adapted from Ghosh, 2006;

^b Adapted from Jackson and Lee, 1991;

^c Adapted from Madene et al., 2006.

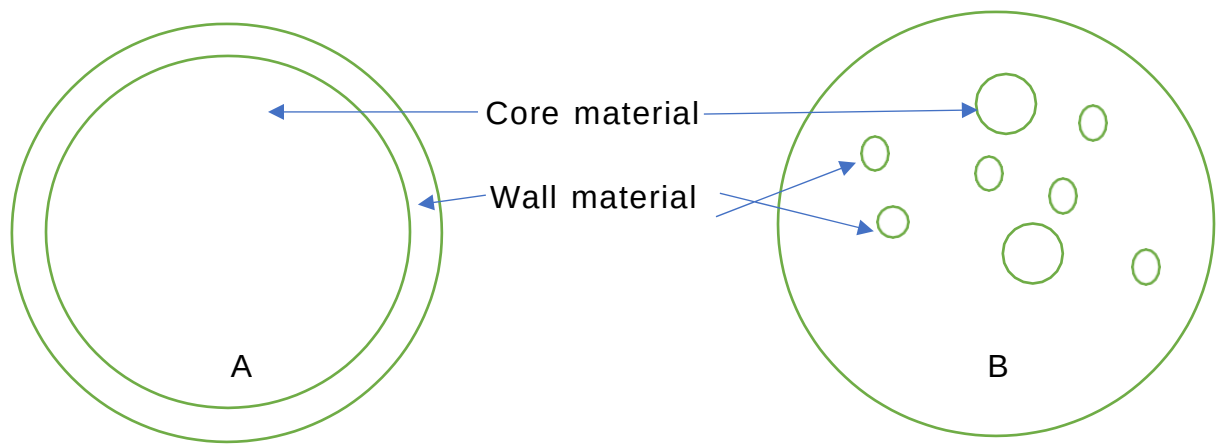


Fig 2.1 Microcapsules based on A) mononuclear and B) polynuclear designs

3 Aim and Scope of the present investigation

3.1 Aim

To extract purify and capsule essential fatty acid from local fishes.

3.2 Scope

- 1) Analyze, purify, and classify the extracted oil.
- 2) To suggest varieties of fish with a high nutritional and therapeutic value
- 3) To study the impact of omega-3 fatty acid in humans.
- 4) To encapsule omega 3 fatty acid from low cost and high nutritional value marine fish.
- 5) Rapid aging population connectivity and production of By-product

4 Materials and Methods

4.1 FISH OIL EXTRACTION

Several studies have been developed around the extraction and quality analysis of oil obtained from different fish species, as well as by-products of their processing, in which different techniques are used, such as supercritical fluids, wet pressing, extraction using solvents, and fish silage employing enzymes present in fish or from other sources

4.1.1 Extraction using conventional methods

The extraction of fish oil by wet pressing is the most commonly used method for production on an industrial scale, and is basically carried out in four stages: fish cooking, pressing, decantation and centrifugation. Drastic temperature and pressure conditions used for protein coagulation and subsequent oil release may partially modify the PUFAs present, due to degradation reactions. The fish sample is selected with the high content of omega-3 fatty acids and low cost and locally available fish from Sholinganallur fish market. Two types of fish were collected from market 1) Sardine (mathi) 2) fresh water pomfret fish (vaval).



Fig 4.1. Fatty acid rich fishes



Fig 4.2. Sardine



Fig 4.3. Fresh Water Pomfret

4.1.2 Steam process /wet pressing method (sardine)

Rinse the fish under running tap water to remove and dirt or sand particles. Remove the fish thorns from fish, clean it properly and remove the head and tail. Cut them in small pieces and add 1 liter of water to cove3r and cook on pressure over medium heat for 5 to 15 minutes. After 15 minutes strain using a fine metal strainer when cool enough pour in the glass jar. After it set, the oil starts to float on top of water transfer the oil to centrifuge tube and keep for centrifuge for 15 min in 5000 rpm then the oil will be raised and can be separated in a clean container then can be given for further fatty acid analysis.



Fig 4.4. Washing



Fig 4.5. Cutting



Fig 4.6. Fish chunks



Fig 4.7. Before autoclave



Fig 4.8, cooking under pressure



Fig 4.9. After cooking



Fig 4.10. Straining



Fig 4.11. Extracted liquid



Fig 4.12. Extracted liquid in to centrifuge tube



Fig 4.13. Tubes arranged in opposite manner inside the centrifuge machine



Fig 4.14. Centrifuge set for 4500rpm for 15 minutes



Fig 4.15. Oil and water are separated



Fig 4.16. Pure oil collected in fresh tube

4.1.3 Sun-dry oil extraction (pomfret)

Cut off the head and tail from the fish with finlet knife. Insert the tip of the knife into the vent of the fish slice down towards the neck of the fish. pullout the gut and entrail from the inside of the fish. cut the fish into small chunks, place the chunks of fish into a plastic container with a lid. Put the container under the sun for 14 days. a liquid will be collected inside the container that should be strained with cheese cloth. Pour the strained liquid into a clean clear jar and cover it. allow the container to sit for 1 day so the oil separate then scoop out the separated oil into a clean container further giver for fatty acid analysis.



Fig 4.17. Freshly purchased pomfret



Fig 4.18. Pomfret chunks



Fig 4.19. Before Sun drying



Fig 4.20. Sundried for 7 days



Fig 4.21. After 14 days sun drying



Fig 4.22. Straining of fish (cheese cloth)



Fig 4.23. Straining the liquid



Fig 4.24. Extracted liquid in clean Tube for oil separation



Fig 4.25. Separated oil.

4.2 Purification of fish oil

4.2.1 Reflux process

Initially oil sample is taken for reflux process using methoxy sodium hydroxide and methoxy H_2SO_4 . It is the process to quantify the lipid in the form of ester. The process consists of heating mandel with round bottom flask connected to distilling pipe where water flows from bottom to top.

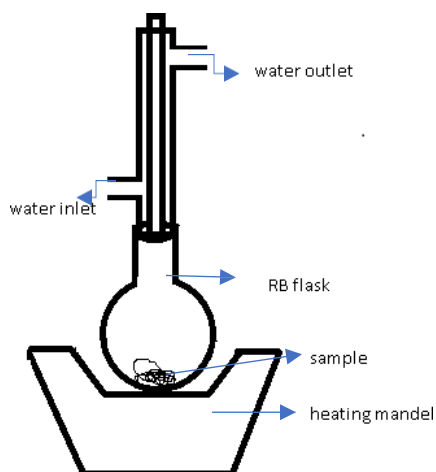


Fig 4.26. Diagrammatic representation of reflux process

Procedure: -

Add 0.5ml to 1ml of the fish oil sample to the round bottom flask and keep it at low temperature and evaporate the sample. Add glass beads in order not to splash the sample. After fully evaporates sample add 15ml of methoxy NaOH to the sample RB flask and set the tube on the top and start the reflux process for 20 minutes.

After the process cool down for few seconds and add 15ml of Methoxy H_2SO_4 to the sample and start the reflex process for 20 minutes. (Heating with the medium amount in the mandel). once done and cool down to room temperature and convert the sample to the tube. And 1ml of heptane and 1ml of Hplc water in ratio of 1:1 in the sample.

Keep the tube without getting disturbed. A layer will be formed on the sample

after adding the ratio. Upper layer is taken out and filled in the GC-vials and it further taken to GC.

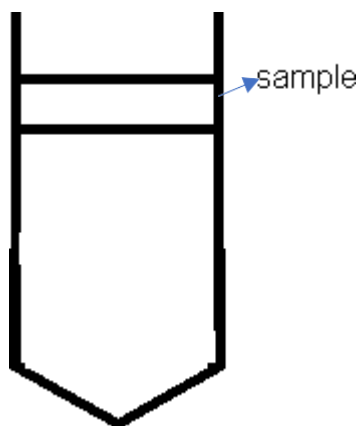


Fig 4.27. Layer formed

4.2.2 GC Analysis

The main components are

Mobile phase

In gas chromatography, usually, three types of gases are employed namely –

1. **Carrier gas** – This is needed for the transfer of the injected sample to the separation column. They are also responsible for the subsequent transfer of separated components to the detector. Common examples: Nitrogen, helium, or hydrogen
2. **Fuel gas** – They support the flame in Flame ionization detector (FID) detector such as Hydrogen.
3. **Zero air** – These are the purified air that plays the role of oxidant to support the combustion of flame in the detector. Before being led to the gas chromatographic system, the above three are intermixed in the desired proportion.

Sample injector

The injector is a heated block where the sample is injected. Through the carrier gas

stream, the sample is spontaneously vaporized and led to the column. With the help of a gas-tight syringe, the liquid sample mixtures are injected whereas, with the help of automated injection valves, the gaseous mixtures are injected.

Column

This is filled with the stationary phase or its walls are covered with a liquid adsorbent. This is done for selective absorbance and retention of the sample components. Commonly used: Packed columns and Capillary columns (More popular)

Component of the column-oven

The column is enclosed by a column oven which is responsible for maintaining a constant temperature during isothermal operation. This temperature when temperature programming is needed can be increased in a controlled way for acquiring effective separation of mixture components possessing different volatilities.

Detector

This is employed for the identification and quantification of components. Here, the regions of individual peaks created relate to their concentrations and their retention times are representative of their identity. Common examples: Flame ionization detector, Thermal conductivity detector (TCD), and Electron capture detector (ECD)

Data System

It is a set of dedicated software that provides control over many important operational parameters like injection sequence, wash cycles, over-temperature control, the flow rate of gases, detector wavelength, etc. Simultaneously, the data station calculates and displays the parameters.



Fig 4.28. GC-FID machine

4.3 Encapsulation

4.3.1 Encapsulation Using the Spray Drying Technique

Encapsulation is a technique whereby small particles or droplets of active substances that have several attractive characteristics are enclosed within a coating in order to protect them against environmental factors such as oxygen, light, moisture and interactions with other compounds. Encapsulation is typically used to obtain particles with a diameter of 1–1000 μm of food ingredients or other materials. In addition, this technique can enable the controlled release of the encapsulated core when certain conditions are met [Gharsallaoui et al.,2007]. Encapsulation using a spray dryer has frequently been used for food production on an industrial scale since the late 1950s, primarily for fats and oils, and flavourings and colourings [Pellicer et al.,2019]. Moreover, encapsulation is widely used in the food industry to incorporate oil aromas in a spray-dried form, because it is inexpensive, flexible, can be used in continuous operation, and produces particles of good quality [Fang & Bhandari,2010]. The process can be conducted by changing the slurry emulsion from a liquid form into a powder in a continuously operating procedure. The basic principle of this method is to dissolve the core/wall materials in water to prepare an emulsion in liquid form, and then to feed this emulsion into a hot medium (100–300 °C) to evaporate the water. The final dried product can be collected in powder form, or as agglomerated particles, depending on the nature of the materials used in the feed, the design of the dryer's operation, and the

operating conditions. The high temperature of the drying chamber facilitates the water evaporation from the droplets [Fireston,1989]. Despite the energy used in the spray-drying process in terms of heat, spray drying has been shown to be more efficient than freeze drying for the encapsulation of oils, with a 30–50 times lower cost [Jessie et al.,2013]. This technique does, however, require high-temperature conditions and access to air. Although the temperature of the spray dryer is high, the wet-bulb only requires a short duration of exposure (few seconds), and water vaporization will take place in the range of 30 to 50 °C [Schuck et al.,2009]. A schematic diagram of the encapsulation process using the spray drying technique is presented in Figure 4.28. According to Bakry et al. [Moffat et al.,1993], encapsulation using spray-drying consists of four main stages: (i) preparation of a stable emulsion; (ii) homogenization of the dispersion; (iii) atomization of the emulsion; and (iv) dehydration of the atomized particles. Typically, the first stage is carried out by dissolving the wall materials in distilled water and emulsifying, or dispersing using a magnetic stirrer overnight at 25 °C to ensure full saturation of the polymer molecules, and to prevent any variations caused by temperature changes. Before starting the second stage, core materials mixed with an aqueous solution of the wall materials and then the emulsifying agent can be added, depending on the emulsifying characteristics of the wall materials. The formed emulsion containing the wall materials and the core substances must be stable until the drying stage [Gharsallaoui et al.,2007].

4.3.2 Unit Operation of Spray Drying

The process of spray drying is illustrated in Figure 1. The steps of the spray drying process involve: (a) atomization of fluid feed; (b) drying of the medium; (c) drying of the feed and spray contact; and (d) separation of the product from air. The properties of the final product are directly influenced by all of the steps, including their operational parameters. Spray drying is a very rapid and reproducible drying method, due to the very large surface area created by the atomization of the liquid feed [Sosnik A & Seremeta,2015].

4.3.3 Feed Atomization

Atomization involves converting the fluid feed (emulsion) into small droplets of uniform size resulting in a balance of heat and mass transfer in the drying stage, as well as increasing the surface area and allowing a good distribution of the feed within the dryer chamber. The size enlargement of the particles (surface area) leads to fast water evaporation and formation of a crust, drying the feed in seconds. Different types of atomizers, including rotary atomizers, pressure nozzles, pneumatic nozzles and sonic nozzles, exist to accomplish feed liquid collapse [Sosnik A & Seremeta,2015]. To choose the atomizer type based on the feed needing to be converted to dried powder and also on the desired particle size of the final product.

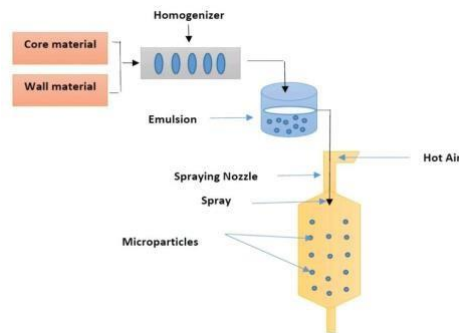


Fig 4.29. Schematic representation of the encapsulation process by spray-drying.

4.3.4 Air Flow Contact

Air flow contact is influenced by the fluid spray and the air contact time, because both factors determine the drying rate and the intensity of the drying. At this stage, the air flow contact starts from the drying step and continues during atomization. The atmosphere inside the heating chamber of the spray dryer is controlled by the operating parameters of the air heating and filtration system. The gas used during the process is usually nitrogen or another gas chosen based on the sensitivity or instability of the feed in the presence of oxygen [Sosnik A & Seremeta,2015]. In general, two types of feed-drying air flow conditions are available:

(a) The co-current drying design: in this configuration, the atomizer is located in the top of the drying chamber along with the drying gas stream inlet. The feed is sprayed in the same direction as the hot air, and thus it is preferred for heat sensitive composites. The inlet temperature is usually 150–220 °C and rapid vaporization takes

place; the outlet temperature is 50–80 °C, which limits the thermal degradation. This design exposes heat-sensitive materials to the lower exit air temperature only.

(b) The counter-current drying design: this system is not popular and has limited applications for dry products. The emulsion and the drying air are introduced at opposite ends of the dryer. Although this configuration is more thermally efficient than the co-current drying design as it exposes the dried powders to high temperatures, it has limited use for products that are sensitive to heat. As a result, of the complicated flow, the drying phenomena are not clear in these systems [Gharsallaoui et al.,2007; Edris et al.,2016].

4.3.5 Drying and Particle Formation

The third step is drying and particle formation by convection. The heat is transferred from the drying medium to the droplets, where it is converted to latent heat during evaporation of moisture content from the droplets. The temperature and mass transfer rate are based on the diameter of the droplets and the relative velocity of the air and the droplets. Quick moisture evaporation immediately happens when the droplets come into contact with the drying air. Initially, the droplet surface exhibits a continuous mass transfer of moisture from within the droplet. At last, a dried shell is formed and moisture evaporation continues at a slower rate until the final product is formed [Gharsallaoui et al., 2007; Munin and Edwards-Levy, 2011].

4.3.6 Separation of Product from the Drying Air

The last step involves the separation of the product from the drying air. The spray drying begins when the dried particles fall to the bottom of the drying chamber and are collected through gravitational effects; while fines entrained in the drying air are parted from the atmosphere and separation occurs after the dried particles recovered in a cyclone filter that is located outside the dryer with collection bottle [Gharsallaoui et al.,2007]. The liquid emulsion consists of the wall and core materials and is converted through the spray drying process into dried particles in powder form. The dried particles are spherical in shape with a size range from 10 to 100 micrometers[Munin & Edwards-Levy,2011].

5 Results and Discussion

5.1 Result

The fish oil extracted from the tissues of *Sardinella* employing direct steaming method produced an average of 20 ml for every 1000g of *Sardinella* fish tissues and oil extracted from the tissues of Fresh Water Pomfret employing direct from sun-dry method produced an average of 25 ml for every 1000g of Pomfret fish tissues. The loss of oil removed as impurities in each step of purification was calculated. Initially 50ml of extracted water was collect from sardinella fish tissue and 60 ml of extracted water was collected from pomfret fish tissue after centrifuge 20ml and 25ml of oil was separated out respectively. From both the samples 1-2 ml of oil was collected for the fatty acid profiling test. The weight of the oil progressively decreased through various steps of purification, might be due to the removal of FFA and other impurities.

5.1.1 Proximate composition of the fishes

Lipid content extracted from the fish muscles varied between species. The major fatty acids found in *Sardinella* fish oil are Myristic Acid Methyl Ester (C14:0), Palmitic Acid Methyl Ester (C16:0), Palmitoleic Acid Methyl Ester (C16:1), Stearic Acid Methyl Ester (C18:0), Oleic Acid Methyl Ester (C18:1n9c), Linoleic Acid Methyl Ester (C18:2n6c), cis-11,14-Eicosadienoic Acid Methyl Ester (C20:2), cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester (C20:5n3), Nervonic Acid Methyl Ester (C24:1n9), cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester (C22:6n3). The major fatty acids found in Fresh Water Pomfret fish oil are Caprylic Acid Methyl Ester (C8:0), Lauric Acid Methyl Ester (C12:0), Tridecanoic Acid Methyl Ester (C13:0), Myristic Acid Methyl Ester (C14:0), Myristoleic Acid Methyl Ester (C14:1), Pentadecanoic Acid Methyl Ester (C15:0), Palmitic Acid Methyl Ester (C16:0), Palmitoleic Acid Methyl Ester (C16:1), Stearic Acid Methyl Ester (C18:0), Oleic Acid Methyl Ester (C18:1n9c), Linoleic Acid Methyl Ester (C18:2n6c), cis-11,14-Eicosadienoic Acid Methyl Ester (C20:2), cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester (C20:5n3), Nervonic Acid Methyl Ester (C24:1n9), cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester (C22:6n3), Linoleic Acid Methyl Ester (C18:2n6c), Heneicosanoic Acid Methyl Ester (C21:0), cis-11-Eicosenoic Acid Methyl Ester (C20:1n), Tricosanoic Acid Methyl Ester (C23:0)+ Meth, Nervonic Acid Methyl Ester (C24:1n9), cis-4,7,10,13,16,19-Docosahexaenoic

Acid M, Erucic Acid Methyl Ester (C22:1n9).

5.2 Discussion

In recent years, fish oil has been allowed for human use as a nutritional supplement and as a component in meals. This justifies a high demand for fish oil. Several hygienic and scientific approaches were used to enhance the quality of fish oil in traditional meal factories and other industrial operations where fish oil is a byproduct. Direct steaming and sun drying produced significant yields of oil recovered from *Sardinella pomfret* samples. Both processes are regarded as good old traditional and economical ways of oil extraction. The current experiment supports the notion that oil extraction by direct steaming is simpler, less expensive, faster, and more affordable to the general people. Taking a fish oil supplement could enhance the health and quality of life for those suffering from diseases that may be prevented with adequate dietary intake of omega-3 fatty acids. Our findings suggest that there are a few supplements that are more affordable than others. Choosing an affordable supplement is possible but requires that consumers are aware of content of supplements as well as recommendations for health benefits. Fish oil supplementation has the potential to not only save lives, but also to save billions of dollars that are currently spent on preventable diseases.

5.2.1 Performance Analysis

<Chromatogram>

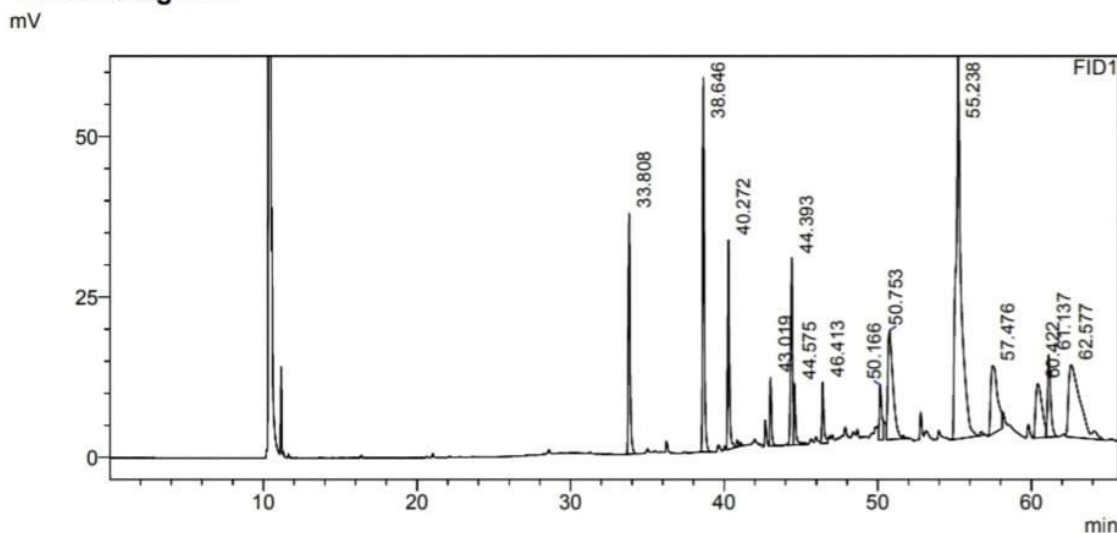


Fig 5.1. GC-FID peak for sardinella sample

Table 5.1. Peak table (Sardinella)

Peak	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	33.808	285509	37539	0.000	%		Myristic Acid Methyl Ester (C14:0)
2	38.646	426520	58206	0.000	%		Palmitic Acid Methyl Ester (C16:0)
3	40.272	251103	32558	0.000	%	SV	Palmitoleic Acid Methyl Ester (C16:1)
4	43.019	75080	10657	0.000	%	V	Stearic Acid Methyl Ester (C18:0)
5	44.393	248850	29107	0.000	%	V	Oleic Acid Methyl Ester (C18:1n9c)
6	44.575	77675	9457	0.000	%	SV	Oleic Acid Methyl Ester (C18:1n9c)
7	46.431	81353	9497	0.000	%	V	Linoleic Acid Methyl Ester (C18:2n6c)
8	50.166	90698	8577	0.000	%	V	cis-11,14-Eicosadienoic Acid Methyl Ester (C20:2)
9	50.753	395897	16968	0.000	%	SV	cis-11,14-Eicosadienoic Acid Methyl Ester (C20:2)
10	55.238	1361117	61487	0.000	%	S	cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester (C20:5n3)
11	57.475	323206	10612	0.000	%		Nervonic Acid Methyl Ester (C24:1n9)
12	60.422	241968	8409	0.000	%		cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester (C22:6n3)
13	61.0137	177341	12802	0.000	%	V	cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester (C22:6n3)
14	62.577	574007	11248	0.000	%	SV	cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester (C22:6n3)
Total		4610328	317124				

<Chromatogram>

mV

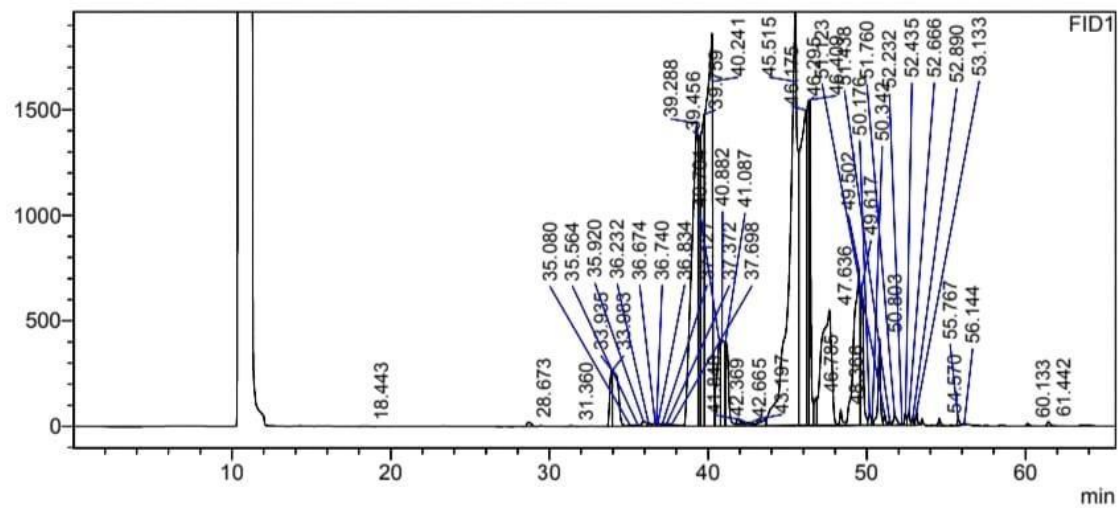


Fig 5.2. GC-FID peak for pomfret sample

Table 5.2. Peak table (pomfret)

Peak	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	18.443	74398	1976	0.000	%	V	Caprylic Acid Methyl Ester (C8:0)
2	28.673	370174	18597	0.000	%		Lauric Acid Methyl Ester (C12:0)
3	31.360	76006	2868	0.000	%		Tridecanoic Acid Methyl Ester (C13:0)
4	33.935	2798662	267874	0.000	%		Myristic Acid Methyl Ester (C14:0)
5	33.983	7102133	267558	0.000	%	SV	Myristic Acid Methyl Ester (C14:0)
6	35.080	105736	4917	0.000	%	T	Myristoleic Acid Methyl Ester (C14:1)
7	35.564	122648	6821	0.000	%	TV	Myristoleic Acid Methyl Ester (C14:1)
8	35.920	433374	21168	0.000	%	TV	Myristoleic Acid Methyl Ester (C14:1)
9	36.232	214991	16867	0.000	%	TV	Pentadecanoic Acid Methyl Ester (C15:0)
10	36.674	170858	11954	0.000	%	TV	Pentadecanoic Acid Methyl Ester (C15:0)
11	36.740	83725	12051	0.000	%	TV	Pentadecanoic Acid Methyl Ester (C15:0)
12	36.834	150277	11807	0.000	%	TV	Pentadecanoic Acid Methyl Ester (C15:0)
13	37.127	111738	9563	0.000	%	TV	Pentadecanoic Acid Methyl Ester (C15:0)
14	37.372	170109	8263	0.000	%	TV	Pentadecanoic Acid Methyl Ester (C15:0)
15	37.698	94660	6513	0.000	%	TV	cis-10-Pentadecenoic Acid Methyl Ester (C15)
16	39.288	38105038	1434002	0.000	%	V	Palmitic Acid Methyl Ester (C16:0)
17	39.456	11819624	1374929	0.000	%	V	Palmitic Acid Methyl Ester (C16:0)
18	39.759	23040394	1476976	0.000	%	V	Palmitoleic Acid Methyl Ester (C16:1)
19	40.241	51953686	1857125	0.000	%	V	Palmitoleic Acid Methyl Ester (C16:1)
20	40.704	6235182	397496	0.000	%	V	Palmitoleic Acid Methyl Ester (C16:1)
21	40.882	7039274	406250	0.000	%	V	Heptadecanoic Acid Methyl Ester (C17:0)
22	41.087	7011454	397230	0.000	%	SV	Heptadecanoic Acid Methyl Ester (C17:0)
23	41.848	411161	23413	0.000	%	T	cis-10-Heptadecenoic Acid Methyl Ester (C17)
24	42.369	83599	7881	0.000	%	TV	cis-10-Heptadecenoic Acid Methyl Ester (C17)
25	42.665	72831	6346	0.000	%	TV	cis-10-Heptadecenoic Acid Methyl Ester (C17)
26	43.197	211364	18438	0.000	%	T	Stearic Acid Methyl Ester (C18:0)
27	45.515	70603804	2086596	0.000	%	V	Linolelaidic Acid Methyl Ester (C18:2n6t)
28	46.175	42761670	1493324	0.000	%	V	Linoleic Acid Methyl Ester (C18:2n6c)
29	46.295	13125163	1534894	0.000	%	V	Linoleic Acid Methyl Ester (C18:2n6c)
30	46.409	10421706	1541504	0.000	%	V	Linoleic Acid Methyl Ester (C18:2n6c)
31	46.785	1445918	130468	0.000	%	V	Linoleic Acid Methyl Ester (C18:2n6c)

32	47.636	23612519	546548	0.000	%	V	Arachidic Acid Methyl Ester (C20:0)
33	48.366	779855	74059	0.000	%	V	cis-11-Eicosenoic Acid Methyl Ester (C20:1n
34	49.502	19221783	738827	0.000	%	V	Heneicosanoic Acid Methyl Ester (C21:0)
35	49.617	8186659	623012	0.000	%	V	Heneicosanoic Acid Methyl Ester (C21:0)
36	50.176	629917	57894	0.000	%	V	cis-11,14-Eicosadienoic Acid Methyl Ester (C
37	50.342	238542	32208	0.000	%	V	cis-11,14-Eicosadienoic Acid Methyl Ester (C
38	50.803	4177169	411866	0.000	%	V	Behenic Acid Methyl Ester (C22:0)
39	51.123	454144	52317	0.000	%	V	Behenic Acid Methyl Ester (C22:0)
40	51.438	140115	19770	0.000	%	V	Behenic Acid Methyl Ester (C22:0)
41	51.760	724784	49115	0.000	%	V	cis-8,11,14-Eicosatrienoic Acid Methyl Ester
42	52.232	31528	4243	0.000	%	V	Erucic Acid Methyl Ester (C22:1n9)
43	52.435	419890	56259	0.000	%	V	Erucic Acid Methyl Ester (C22:1n9)
44	52.666	604759	58594	0.000	%	V	cis-11,14,17-Eicosatrienoic Acid Methyl Este
45	52.890	236547	30344	0.000	%	V	Tricosanoic Acid Methyl Ester (C23:0)+ Meth
46	53.133	498831	63051	0.000	%	V	Tricosanoic Acid Methyl Ester (C23:0)+ Meth
47	54.570	340139	34856	0.000	%	V	Lignoceric Acid Methyl Ester (C24:0)
48	55.767	290508	29703	0.000	%		Nervonic Acid Methyl Ester (C24:1n9)
49	56.144	85048	7629	0.000	%	V	Nervonic Acid Methyl Ester (C24:1n9)
50	60.133	156354	13226	0.000	%		cis-4,7,10,13,16,19-Docosahexaenoic Acid M
51	61.442	285936	20890	0.000	%	S	cis-4,7,10,13,16,19-Docosahexaenoic Acid M
Total		357536385	17780078				

6 SUMMARY AND CONCLUSION

The omega-3 polyunsaturated fatty acids (PUFA) are highly valued at present for their beneficial effects on health. Fish oil is recognized as the primary source of these fatty acids, mainly eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids.

Some studies employ the extraction of fish oil by traditional methods, such as wet pressing and conventional solvents, but recent research is opting for the application of technologies such as supercritical fluids and fish silage. Refining methods are still based on traditional stages, although there is an important potential for the application of new technologies such as enzymatic and physical refining.

Likewise, for the concentration of PUFA different methods have been proposed, such as enzymatic and chromatographic methods, winterization, supercritical fluids, complex formation with urea and filtration by membranes. However, currently the combination of different techniques seems to offer a good alternative to increase the purity and performance of these components.

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