CURRENT TRENDS IN PRODUCTION AND PROCESSING OF FISH OILS & ITS CHEMICAL ANALYTICAL TECHNIQUES: AN OVERVIEW

Susan Catherin Vaska[1], Pavankumar Muralakar[2]*, Arunkumar H.S[1], Manoj D[1], Shenbagam R[2], Jeevitha D[3], Umesh Chimmalgi[3], Vinay TV[3], Nagaraju M[1]*

Abstract:
Fish oil is an industrial product of high nutritional value because of its Omega-3 Polyunsaturated fatty acids content, currently valued for its beneficial effects on health. Several studies and advances were made since the year 2000 on fish oil extraction from several fish species. Extraction techniques range from traditional to more recently proposed technologies such as SFE, Enzymatic hydrolysis, PEF, UAE, HHP, MAE and pH shift method. Moreover, refining is performed to significantly lower certain undesirable Compounds that impair the oil's stability, quality, and acceptability, without losing the PUFAs. The high susceptibility of fish oils rich in omega-3 fatty acids to oxidation produces Undesirable taste and odour which limits the use of fish oils. So, Organic acids, radical Scavengers and antioxidants are used to maintain the oxidative stability of oils. On the other hand, monitoring the degree of oxidation in marine oils is a typical technique for regulatory and quality control objectives. In this review we discussed characterization of fish oil like GC-MS, ATR-FTIR and Raman main bands Even though various new techniques like MAE, UAE, PEF, SFE, *etc* are developed for improving oil recovery, the capital investment of these techniques is uneconomic in industrial-scale production. Since these modern techniques are cost ineffective most industries choose traditional techniques. However, SCF is primarily employed in the extraction of fish oil since it gives the highest recovery and safe product of all the other methods.

Key Words: Fish oil, global production, PUFAs, extraction methods, ATR-FTIR

[1]*Department of Food Processing Technology, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore-641114 Tamilnadu, India
[2]*Department of Applied Chemistry, Karunya Institute of Technology and Sciences, Coimbatore-641114 Tamilnadu, India
[3]Department of Agriculture, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore-641114 Tamilnadu, India

*__Corresponding author:__
1. Nagaraju M
Assistant Professor, Department of Food Processing Technology, Karunya Institute of Technology and Sciences, Karunya Nagar, Coimbatore, 641 114, Tamil Nadu, India, Email: nagaraju@karunya.edu

2. Pavankumar Muralakar
Assistant Professor, Department of Applied Chemistry, Karunya Institute of Technology and Sciences, Karunya Nagar, Coimbatore, 641 114, Tamil Nadu, India. Email: pavankumar@karunya.edu

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1. Introduction

One of the three main categories of foods, together with carbohydrates and proteins, is edible fats and oils (Peyronel, 2019) which contain large amounts of saturated, monounsaturated, and polyunsaturated fatty acids (Pereira et al., 2016). Edible fats and oils can be extracted from terrestrial or marine animals, from plant seeds or leaves, from pulp, or from the nut or stones of many fruits (Peyronel, 2019).

Worldwide, edible oils are frequently used for dietary enrichment, industrial food production, and other purposes. They are the major source of unsaturated fats in the human diet. Edible oils derived from marine sources, such as fish and krill oils and plant-based microalgae oil, are of utmost significance in the nutritional context due to their high concentrations of omega-3 polyunsaturated fatty acids (PUFAs) such as EPA (eicosapentaenoic acid) (C20:5 n-3) and DHA (docosahexaenoic acid) (C22:6 n-3) (Amorim et al., 2021; Jaiswal et al., 2022).

High levels of Long Chain (LC) omega-3 PUFAs are a distinguishing feature of fish oils (Adriana et al., 2021) produced by the processing of fish (IFFO, 2020).

Fish oil is primarily used in aquaculture, particularly for carnivorous fish like salmonids, and marine species (Pike & Jackson, 2010) and to produce refined fish oil for human use (fish oil capsules) (Pike & Jackson, 2010; Fish oils n.d.2015). However, over the past ten years, the use of nutraceuticals has grown at a rate of about 10% annually, which is even faster than that of aquaculture (EUMOFA n.d.2021; García-Moreno et al., 2014) due to their positive impact on public health (Kh. Albashr et al., 2022; Lamas, 2022; Sahena et al., 2010). These fish oils are marketed asdietary supplements under the following brand names such as Coromega, Solgar Omega 3 700, Nature Made, Spring Valley, Bounty, Barleans, LifeFitness DHA, and Nature Made DHA, and can be purchased as capsules or oils (Fleisher, 2018). In view of the increasing demand for fish oils, the main objective of this review is to give a clear understanding of techniques employed to produce fish oils and to identify a suitable method to meet the increasing demand.

1.1. Worldwide fish oil market

Fish oil and its derivatives are marketed and promoted in three main segments such as nutraceuticals and licensed pharmaceuticals, fortified traditional foodstuffs with fish oil as a major element and purified oils which are used as nutritional supplements (Lister, 2008). On average, between 0.8 and 1.3 million tonnes of fish oil are produced annually. The highest level ever observed was in 2018 when fish oil production came close to 1,3 million tonnes. To a greater or lesser extent, fish oil is produced in worldwide markets. The top nine producers including Peru, Vietnam, Chile, USA, Japan, China, Norway, Denmark and Iceland produced about 70 per cent of global fish oil (Figure.1) in 2019. Production of fish oil in the US and Europe reached 90.000tonnes, and 120.000 tonnes respectively, in 2019 (EUMOFA, 2021)

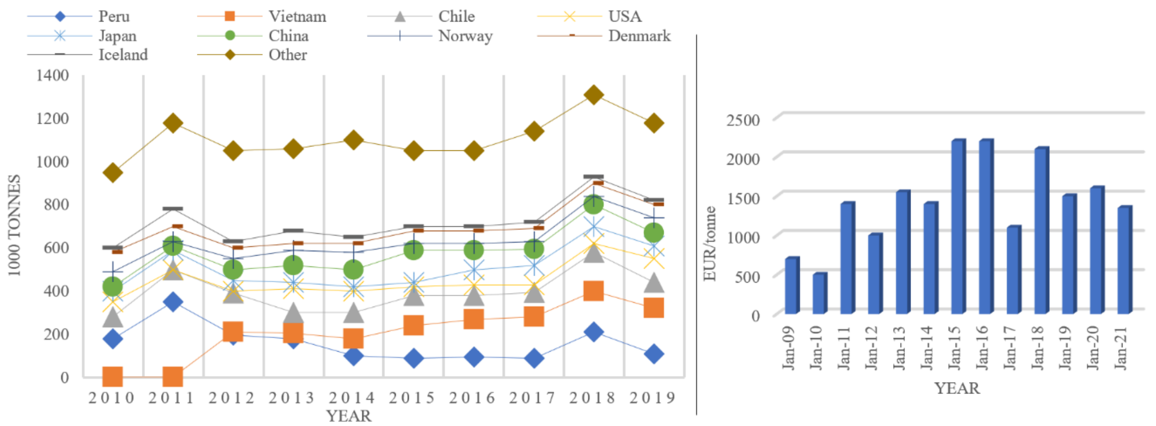

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Fig.1 World's major producers and Peruvian prices of fish oil (EUMOFA, 2021)
Where, a. Major global producers of fish oil; b. Peruvian prices of fish oils

1.2 Global Prices of fish oil

Since the beginning of the 21st century, the prices of fish oil have tripled due to the rapidly growing demand (Aitta et al., 2021). Fish oil prices are estimated to rise again and continue to move upward over the projection period, increasing by

16 per cent nominally by the year 2030 as a result of robust global demand. Since, Peru remained the world's top producer and exporter of fish oil (FAO, 2018) Peruvian prices have had a significant influence on how fish oil prices change globally. However, prices seem to change in accordance with the balance between supply and demand. The average export price for actual fish oil climbed 115 per cent to 1.406 EUR/tonne between January 2009 and January 2021 which is depicted in Figure.1 (EUMOFA, 2021).

2. Sources

Marine oils refer to fish liver oils, fish body oils, marine mammal oils, crustacean oils and cephalopod oils. Fish body oils are the most significant product in this category. It represents almost all of the production with small amounts of marine mammal and squid oils (Jacobsen, 2015). Major sources for the production of fish oils are tuna, anchovies, sea squirt, freshwater crap, sprat, blue whiting, alaska pollock, pilchard, capelin, menhaden and sardine (Hashim et al., 2019). Of these menhaden and sardine are the most prominent oils since ancient times (Alexander, 1954). Krill, crustaceans (Lister, 2008) squid and marine mammals to a lesser extent are the other species used for the extraction of marine oils (Jacobsen, 2015).

Fish species have varying levels of oil depending on species, age, gender, location, and a variety of environmental factors, including temperature and species origin characteristics such as spawning or migration seasons (Boran et al., 2006). Fresh fish flesh is one of the major sources of fish oil production. However, Fish by-products are loaded with nutrients and bioactive components like proteins and omega-3 polyunsaturated fatty acids (Zhang et al., 2021; Y. Zhang et al., 2021; Hathwar et al., 2011). So, fish oil can also be extracted from the fish by-products such as heads, viscera, frames, skins (Šimat et al., 2019), trimmings, blood, scales, air bladders and other underutilised fish parts (Hashim et al., 2019; X. Zhang et al., 2022).The oil content ranges from 1.40% to 40.10% in fish by-products based on the species and the tissue from which oil is extracted (Y. Zhang et al., 2021a). About 51 per cent of global fish oil production comes from fish by-products and the contribution of various by-products for the extraction of fish oil is depicted in Figure.2 (Dr Enrico Bachis, 2022). Of the raw materials used to produce fish oil, by-products of pangasius and salmon account for 15.3 per cent and 14.3 per cent, respectively, which means that these two species' combined contribution makes up more than a quarter of the total production. Secondly, tuna by-products contribute 2.2 per cent of the world's fish oil supply, and finally, tilapia waste contributes 1 per cent as shown in Figure.2 (Dr Enrico Bachis, 2022).

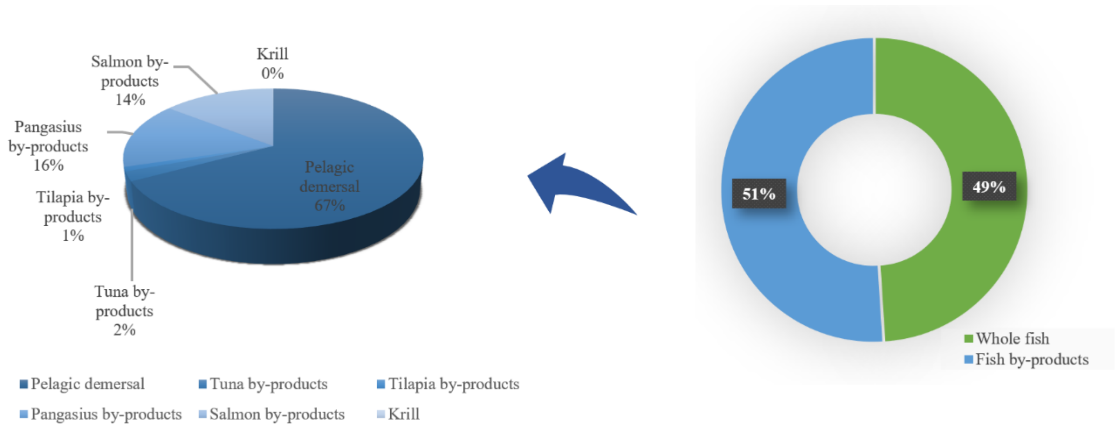

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Fig.2 Major source of fish oil extraction and Average production of fish oil contributed by various fish by-products(Dr Enrico Bachis, 2022)
Where, a. Fish oil production contributed by fish by-products; b. Major raw materials for fish oil production.

About 90 per cent of the fatty acids in fish oil are long-chain fatty acids (Fish oils 2015).These dominant PUFAs of fish oil, particularly, EPA and DHA, are influenced by intrinsic factors like species, size, age, gender and sexual maturity of the fish and extrinsic factors such as feed, habitat temperature, fishing season, area and extraction methods (Özyurt et al., 2013).

The average fatty acid composition of major fish oils along with their applications is given in Table 1.

Table. 1 Average LC- PUFA concentration and Major sources of fish oils and their applications

Sources	Name of the oil	Major long chain fatty acids (%)								Applications	Reference
		DHA C22:6	EPA C20:5	Myristic acid C14:0	Palmitic acid C16:0	Palmitoleic acid C16:1	Oleic acid C18:1	Eicosenoic acid C20:1	Eurucic acid C22:1		
Brevoortintyrannus	Menhaden	9.1	11	7.3	19	9.1	13.2	2	0.6	Medicinal and pharmaceutical	(Nichols, 2007; Shahidi, 2007; Shireman, 2003)
Sardinopsmelanostictus	Pilchard	12	21	7.3	16	9	10	1	2	Aquaculture	(Patterson, 2011; Nichols, 2007; Shirai, 2011)
Mytilus edulis	Capelin	6	8	7	10	10	14	17	14	Health Supplements	(Jacobsen, 2015; Shahidi, 2007; Adriana et al., 2021)
Clupea arengusmembras	Herring	7	7.5	6	11	7	10	13	21	Aquaculture	(Patterson, 2011; Nichols, 2007; Aitta et al., 2021)
Engraulisringens	Anchovy	8.8	17	7.5	17.5	9	11.6	1.6	1.2	Aquaculture	(Nichols, 2007)
Gadus macrocephalus	Cod liver oil	10.5	5	3.5	10.4	12.2	19.6	14.6	13.3	Pharmaceutical (Medicinal and Clinical application), pet foods	(Patterson, 2011; Bimbo, 2011; Selvaraj, 2011; Case et al., 2011; Ohyama&Shinki , 2016).
Rastrelligerkanagurta	Mackerel	8	7	8	14	7	13	12	15	Antioxidant, Antidiabetic, Pharmaceutical , Medical and Poultry feed	(Jacobsen, 2015; Ali et al., 2021)
Salmo salar	Salmon	15.7	7.1	4.2	15.7	5.1	16.5	3.3	2.5	Industrial use (gelatin)	(Nichols, 2007; Ali et al., 2021; Y. Liu & Dave, 2022).
Thunnus *(Katsuwonus pelamis)*	Tuna oil	27.8	12.4	3.9	17.6	5.4	12.4	1.3	0.5	Pharmaceutical , Antioxidants	(Nichols, 2007; Ali et al., 2021).
Average long Chain PUFA Concentrations of fish oil		7–18	6 -13	6 –9	10 –19	5 –10	11–14	4 –17	-	Nutraceuticals, Cosmetic and Therapeutic	(Fish oils n.d.-a2015; S. J. Lee & Ying, 2008; Tse-Hung Huang et al., 2018)

3. Production/extraction of fish oil

Extraction methods range from traditional techniques like wet pressing, and solvent extraction to more recently proposed alternatives like supercritical fluids and enzymatic extraction (Bonilla-Mendez & Hoyos-Concha, 2018). Good-quality fresh raw materials rich in omega-3 PUFAs as well as a suitable extraction method are the prerequisites for the production of high-quality fish oil (Y. Zhang et al., 2021; Oterhals & Vogt, 2013). The most common methods for the production of fish oils are the wet rendering method (Bonilla-Mendez & Hoyos-Concha, 2018), dry rendering method, hydrolysis, silage production or autolysis, and solvent extraction (Rizliya & Mendis, 2013). However, these traditional extraction methods employ high levels of mechanical stress and temperature to maximise oil recovery, but doing so can degrade the final product's quality and prevent the selective extraction of desirable components. So, several novel techniques such as (Naliyadhara et al.,2022) enzymatic hydrolysis, supercritical fluid extraction, (Ivanovs & Blumberga, 2017) microwave-assisted extraction, high hydrostatic pressure extraction, pulsed electric field extraction and ultrasound-assisted extraction are developed which seemed to permit a maximum recovery of diverse elements from the food matrix than the traditional techniques (Naliyadhara et al., 2022).The extraction rate of traditional methods and more recently proposed conventional methods is given in Table 2.

Table 2 Comparison of most commonly used traditional and new conventional extraction methods.

Method	Extraction conditions	Sources	Recovery of oil (%)	Inference	References
Traditional method					
Wet rendering	Temp: 85°C; Time: 1 h	Acipenseridae	52.51	• Commonly used extraction method. • Energy-intensive process. • The thermal method, using high temperature affects the nutritive value and induces oxidative degradation.	(Hao et al., 2015; Jamalluddin et al., 2022; García-Moreno et al., 2014)
Dry rendering	Temp: 50°C; time:2 h	Catfish	45.17	• The oils contain high amounts of dissolved phospholipids. • It produces dark coloured oil.	(Suptijah et al., 2020; Rizliya&Mendis, 2013)
Solvent extraction	Solvent: ethanol; Temp: 80˚C	*Parexocoetusbrachypterus*	75	• High extraction efficiency. • Uses potential hazardous solvents. • Solvent residual risks. • Non- eco-friendly.	(Yunus et al., 2010;Jamalluddin et al., 2022; Routray et al., 2018; Alfio et al., 2021)
Conventional methods					
Enzyme assisted extraction	Enzyme: Neutral protease; Temp: 40˚C; Time: 2 h	Acipenseridae	83.63	• Environment friendly. • Low energy requirement. • Low capital investment. • Require further treatment to remove undesirable compounds.	(Hao et al., 2015; Routray et al., 2018;Alfio et al., 2021)
Super critical fluid	Pressure: 31,600 kPa	Acipenseridae	97.25	• Recovers high-quality products. • Cost ineffective. • Highest yield and extraction rate.	(Hao et al., 2015; Rubio-Rodríguez et al., 2012),
Microwave assisted extraction	MAE: 110 W; Time:1 min; Enzymatic hydrolysis: Alcalase 2%, 2 h, 120 rpm	Catfish (*Pangasianodon gigas*-*Pangasianodon hypothalamus*)	Improved extraction yield and oil quality	• Extraction efficiency is similar to or greater than traditional methods.	(Chimsook &Wannalangka, 2015; Alfio et al., 2021)
Ultrasound assisted extraction	UAE: 400 W; Temp:50 ˚ C, 57 min	Bighead carp (*Hypophthalmichthys nobilis*)	94.82	• Oil recovery is similar to or greater than traditional methods. • Low extraction time. • Low solvent consumption. • High power consumption.	(Alfio et al., 2021; (Tu et al., 2015)
Pulsed electric field extraction	PEF: 16 kV/cm, 240 pulses	Pacific white shrimp (*Litopenaeusvannamei*)	Improved recovery (30.34 g/100 g)	-	(Gulzar &Benjakul, 2020)

3.1 Traditional methods

3.1.1 Wet rendering

Wet rendering is the most widely used technique for producing fish oil on an industrial scale, and it essentially involves four steps: heating, pressing, decanting, and centrifuging (Bonilla-Mendez & Hoyos-Concha, 2018). The process is briefly depicted in Figure.3a. In this technique, extreme temperature (85–95°C) (Rubio-Rodríguez et al., 2012) and pressure conditions (Bonilla-Mendez & Hoyos-Concha, 2018) are employed for the extraction of oil (Mozuraityte et al., 2016). The extraction of oil is typically influenced by these extreme conditions that cause protein coagulation and result in subsequent oil release (Bonilla-Mendez & Hoyos-Concha, 2018).

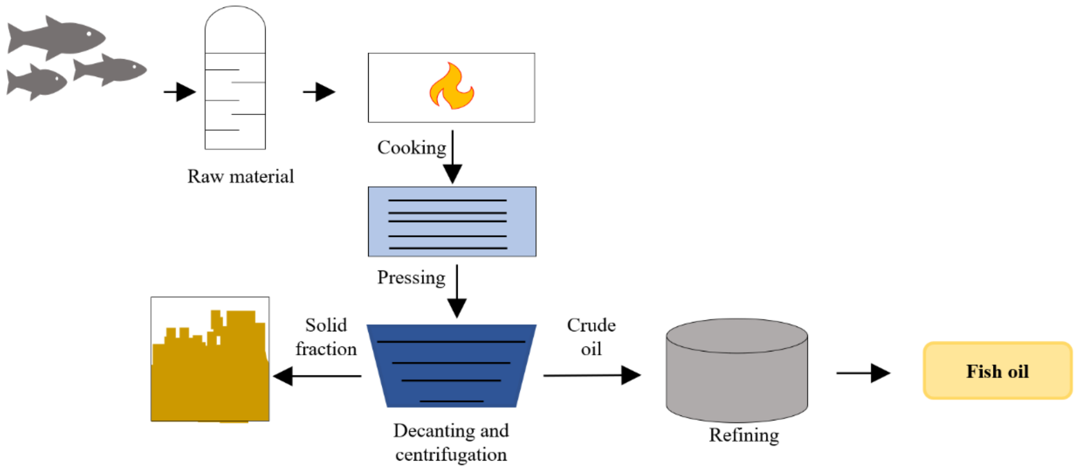

Fig.3a Extraction of fish oil using wet rendering method (FAO, 2018)

3.1.2 Dry rendering

Dry rendering is not frequently utilised in the production process. The process mainly involves cooking and drying raw material to lower the moisture content followed by subjecting it to a hydraulic press to extract the oil (Rizliya & Mendis, 2013).

3.1.3 Hydrolysis

The principal element in the hydrolysis or the hydrolytic production of fish oil is the proteolytic enzyme. Proteolytic enzymes are naturally present in the fish or they are obtained from other sources like animals, plants, microbes *etc*. The proteolytic enzymes result in the breakdown of proteins into smaller units and thereby improve the release of oils. Hydrolysis is also achieved chemically by the action of acids or alkalis (Rizliya & Mendis, 2013). The schematic diagram of hydrolytic extraction is shown in Figure.3b (Adeoti & Hawboldt, 2014).

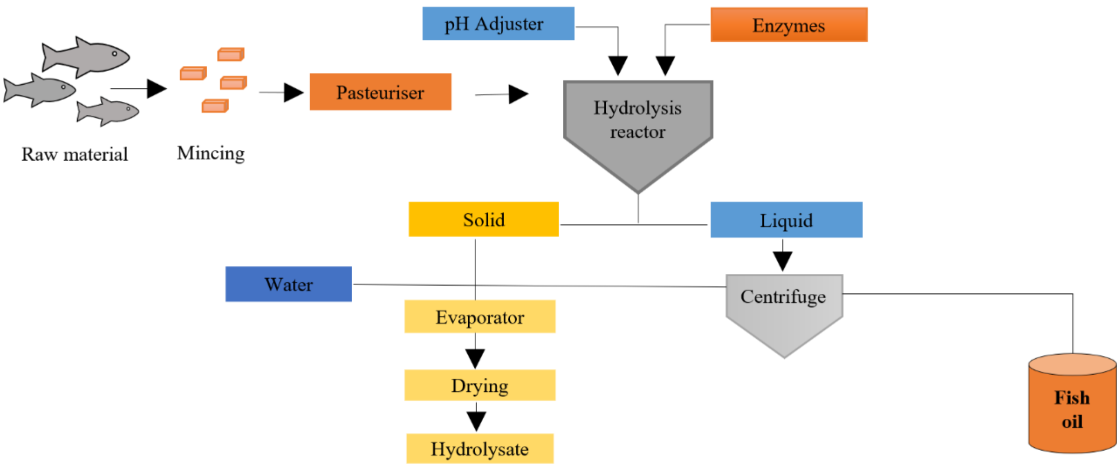

Fig.3b Hydrolytic extraction of fish oil(Adeoti&Hawboldt, 2014)

3.1.4 Autolysis

Autolysis also known as the silage process is a simple cost-effective hydrolytic process. The fish gut is naturally acquainted with enzymes that aid in oil extraction (Rizliya & Mendis, 2013). In this process whole fish or parts of fish, together with acids (chemical silage), enzymes (silage or enzymatic extraction) or lactic acid bacteria (biological silage) are added, causing protein hydrolysis resulting in the formation of semi-liquid product, fish silage (Bonilla-Mendez & Hoyos-Concha, 2018).

3.1.5 Solvent extraction

The use of organic solvents for extraction is another widespread extraction technique. Organic solvents extract the oil from the cells by rupturing their cell walls or by interfering with the interactive forces that bind lipids to the tissue matrix (Adeoti & Hawboldt, 2014). Owing to the lipids' solubility in organic solvents and their insolubility in water, this technique allows for the extraction of lipids from water as well as other soluble elements including proteins, carbohydrates, and minerals (Bonilla-Mendez & Hoyos-Concha, 2018).

Using the proper chemical solvents, the maximum amount of fat is removed during this process. However, the efficiency of extraction of oil from the raw material is strongly influenced by two parameters such as the partition coefficient of the lipids into the organic phase and the lipid composition of the raw material (Kumoro et al., 2022). For efficient oil extraction, the solvent must completely penetrate the lipid cells and the polarity of the targeted molecules must match (Adeoti & Hawboldt, 2014). The factors that have to be taken into consideration when selecting an organic solvent are solubility, a low boiling point, economics, toxicity, availability, and reusability. Hexane is one of the few solvents with these properties thus it is employed extensively for oil extraction (Adeoti & Hawboldt, 2014). Although hexane is an organic solvent, the Food and Drug Administration (FDA) approves its application in the food industry (Castejón & Señoráns, 2019). Furthermore, this technique allows the extraction of high-quality oil (Rizliya & Mendis, 2013). However, the low availability of food-grade solvents, large solvent volume requirements, and high capital investment limits the use of this technique (Rizliya & Mendis, 2013).

3.2 Conventional methods

3.2.1 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) has emerged as a crucial separation technique in the field of food and nutraceutical applications. With the aid of this technique, it is feasible to extract heat-sensitive, readily oxidised substances like PUFAs (Follegatti-Romero et al., 2009). SFEhas been highly efficient in extracting omega-3 fatty acids from marine sources (Pateiro et al., 2021). The rate of separation and extraction of fish oils by SFE is much faster, more reliable and more efficient than other methods because the extraction is carried out at low temperatures and utilises non-toxic solvents (Ivanovs & Blumberga, 2017). SFE is effectuated under lower oxidizing conditions such as mild temperatures, a non-oxidant atmosphere and a dark place (Rubio-Rodríguez et al., 2012). Supercritical carbon dioxide (Sc-CO_2) is a pressurised form of CO_2 with moderate critical pressure and temperature (Jamalluddin et al., 2022). Since Sc-CO_2has relatively mild critical values for the operating parameters (critical temperature of 31˚C (304.15 K) and critical pressure of 7.38 MPa), it has been widely used in supercritical fluid extraction as a non-toxic, cost-effective, and inflammable "green" solvent (Kuvendziev et al., 2018). These characteristics enable the extraction of thermally unstable bioactive components such as polyunsaturated fatty acids successfully (Kuvendziev et al., 2018;Sahena et al., 2010). Moreover, by altering the operating conditions, it is simple to separate CO_2 from the extracts, thus, Sc-CO_2 extraction produces a safe and clean product (Lee et al., 2022).

Supercritical carbon dioxide (Sc-CO_2) extraction is a method that has gained popularity owing to its speed, efficiency, and minimal solvent toxicity (Sánchez-Camargo et al., 2012) and reduced environmental impact (Pateiro et al., 2021). In addition to this, the Food and Drug Administration and European Food Safety Authority also classified Sc-CO_2 as (GRAS) Generally Recognised as Safe (Jamalluddin et al., 2022). The instrumentation of Sc-CO_2 is depicted in Figure.3c (Follegatti-Romero et al., 2009).

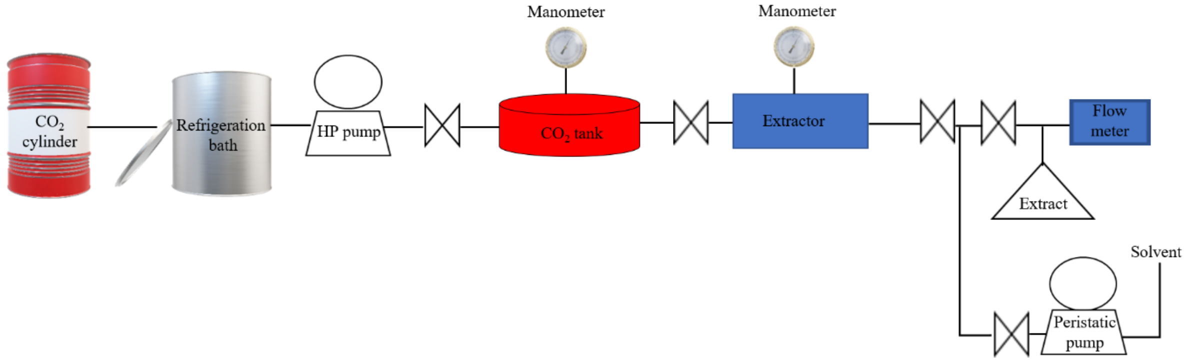

Fig.3c Instrumentation of SFE (Follegatti-Romero et al., 2009)

3.2.2 Enzymatic hydrolysis

Enzymatic hydrolysis is another approach that has been gaining popularity as a non-thermal method for extracting biomolecules from food matrices (Ali et al., 2021). Enzymatic techniques present an alluring substitute for conventional chemical methods (Kamal et al., 2015) as these enzymes are biodegradable, highly specific and easily removable biological catalysts (Lamas, 2022)and the process can be carried out under very mild conditions without producing undesired by-products (Kamal et al., 2015). In comparison with the wet rendering method, it is a highly efficient process as the obtained product has better biological, digestible, and functional properties (Carvajal et al., 2015). Enzymatic hydrolysis employing specific enzymes facilitates the simultaneous extraction of oil from, the whole fish and fish by-products (Aitta et al., 2021). The principle involved in oil extraction is enzyme-mediated protein hydrolysis (Routray et al., 2018). A schematic diagram of the enzymatic extraction is depicted in Figure.3d. During enzymatic hydrolysis, enzymes cleave the peptide bonds in proteins releasing the oil that was previously bound in the protein network. Endogenous or synthetic enzymes can be employed to carry out enzymatic hydrolysis, some majorly used proteases (Liu et al., 2021) together with their optimum conditions are given in Table 3. However, proteolytic enzymes especially alkaline protease is widely used for lipid extraction (Y. Zhang et al., 2021a; Ali et al., 2021; Hathwar et al., 2011).

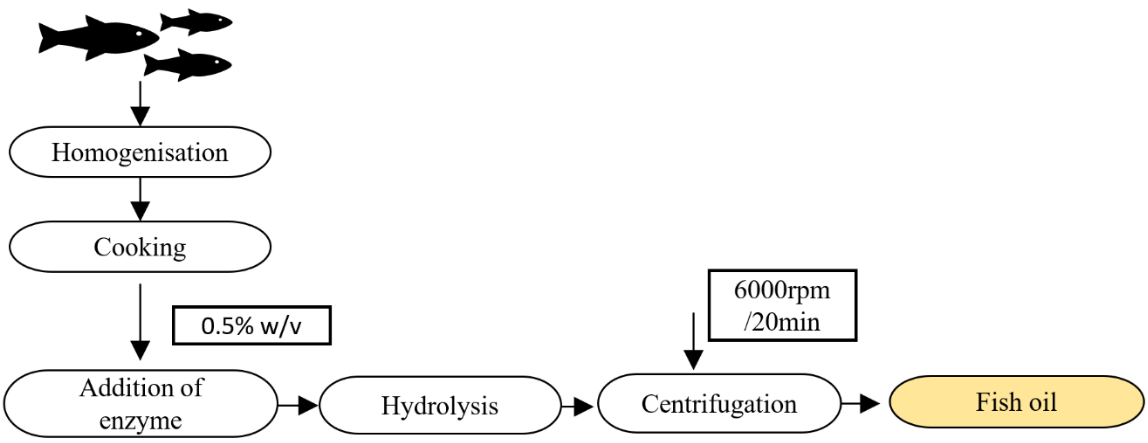

Fig.3d Enzymatic extraction of fish oil (Hathwar et al., 2011)

Table 3 Optimum conditions for the activity of the enzyme

Enzyme	Optimum temperature (°C)	Optimum pH	References
Alcalase	30–65	7.0–9.0	(Valencia et al., 2014)
Flavourzyme	50	7.0	(Bruno et al., 2019)
Neutrase	50	7.0	(B.-Y. Liu & Zhu, 2016)
Protamex	22	7.5	(Pramualkijja et al., 2021)
Papain	70–90	5.0–7.5	(Elavarasan&Shamasundar, 2018)
Bromelain	55	5.5	(Ee et al., 2019)

3.2.3 Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction is a non-thermal extraction technique (Verzera & Condurso, 2012) that employs (UAE), ultrasound waves to generate mechanical energy that is applied to the samples (Weggler et al., 2020). Ultrasound waves used in the food sector are of two main types low-intensity, high-frequency ($f > 100$ kHz) and high-intensity, low-frequency (20 kHz$\leq f \leq$100 kHz) ultrasound waves. Low-intensity ultrasound does not change the material's chemical or physical makeup. However, as a result of the bubble cavitation, high-intensity shock waves produce extreme pressures and temperature gradients that shatter the matrix (Herrero et al., 2012).

Ultrasound is based on the principle of cavitation or oscillation phenomenon. Vibrations caused by ultrasonic waves can result in voids that transfer energy to the solid particles submerged in the extraction. The rupture of the cell wall is caused by cavitation bubbles that are getting closer to the solid surface and collapsing at a larger amplitude speeding up the transfer of desirable substances trapped inside to the solvent media (Thilakarathna et al., 2022). UAE is a promising method of extraction since it is less expensive than other techniques and requires few instruments. An ultrasonic probe system or an ultrasonic bath is used for extraction (Mussatto, 2015) as shown in Figure.3e.

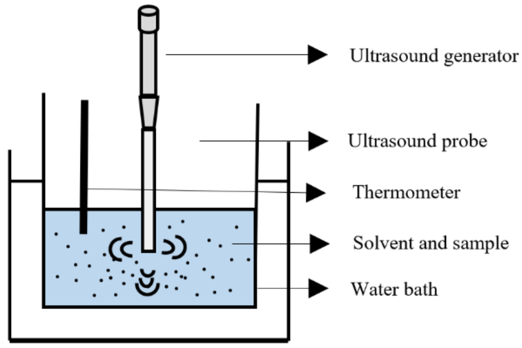

Fig.3e Schematic representation of UAE (Thilakarathna et al., 2022)

The application of UAE in food processing and analysis is of significant interest to the industrial sector's growing demand for sustainable development since, it improves the extraction of components with a higher recovery, allows the replacement of organic solvents with safer solvents, reduces the amount of solvent used, and shorten the extraction time (Pingret et al., 2012).

3.2.4 Microwave-assisted extraction (MAE)

The microwave-assisted extraction (MAE) technique is a novel innovative promising extraction technique due to the minimal initial capital investment in comparison to other new technologies and the significant effect of the microwave equipment to achieve high yields of bioactive compounds while minimizing the use of solvents and the extraction time(Gomez et al., 2020). MAE technique employs the use of microwave energy which is nonionizing radiation with a frequency range of between 300 and 300,000 MHz and conventional solvent extraction (Llompart et al., 2019). MAE principally utilizes microwave radiation to heat the extraction solvent, which improves the solvent's dispersion into the sample and speeds up the partitioning of the compounds from the sample to the solvent (Pacheco-Fernández & Pino, 2020). MAE is a three-step process involving an initial rise in temperature and pressure that causes the solutes to separate from the active sites of the sample matrix. Next, the solute is released into the solvent from the sample matrix, and finally, the solvent diffuses across the Sample matrix (Rehman et al., 2020). The MAE method offers various benefits such as protection of thermo labile components, better recovery in a shorter extraction time (Afolabi et al., 2018) when compared to traditional techniques (Singh et al., 2020), minimized temperature gradients, smaller equipment (Rehman et al., 2020) and a significant reduction in consumption of organic solvent (Cecilia et al., 2000). MAE uses a noncontact heat source, thereby increasing the efficiency and selectivity of the heating process (Gomez et al., 2020). This method is also regarded as a green technology because it requires less organic solvent (Rehman et al., 2020).

3.2.5 High hydrostatic pressure (HHP)

High hydrostatic pressure is a novel non-thermal and environmentally friendly extraction technique. This method involves exposure of the sample to 400–600 MPa (58–87,000 psi) pressures for a specified period of time at room temperature (Zhao et al., 2019). Application of such high pressure to the cells, results in cell permeability, thereby ensuring a high extraction of valuable compounds and is found to be effective in improving the extraction rate of valuable compounds. HHP is also used in the marine industry to extract omega-3 PUFAs from fish by-products. (Ali et al., 2021).

In comparison to conventional methods, it offers a number of benefits, including faster extraction times, minimal solvent consumption, higher purity extracts, and increased extraction yields. Additionally, the procedure could be carried out at room temperature, protecting thermo-sensitive molecules from thermal damage (Grassino et al., 2020).

3.2.6 Pulsed electric field (PEF)

Pulsed electric field (PEF) extraction is one of the electrical-based extraction techniques. It is an emerging novel technology that has the ability to produce foods with desirable sensory quality, great nutritional value and shelf-life (Gómez et al., 2019). PEF technology is a green, non-thermal, selective extraction technique (Lakka et al., 2021) that is widely employed in the marine industry. The schematic arrangement of PFE is given in Figure.3f. PEF technology is more effective than traditional thermal technologies because of its great adaptability, quick processing time, low energy and temperature expenses, quick responsiveness, and high extraction efficiency (Ali et al., 2021), reduced energy utilisation, capital investment, environmental impact, minimized heat-sensitive compound degradation (Lakka et al., 2021) together with the structural preservation and improved quality of end product (Naliyadhara et al., 2022). PEF technology is based on the intermittent application of direct-current high-voltage pulses (kV), over very brief time intervals (microseconds to milliseconds) (Martínez et al., 2020) via a sample of marine animal by-products sandwiched between two electrodes (Ali et al., 2021)**.**The intensity of the electric field produced by this voltage depends on the distance between the electrodes and the applied voltage. When the electric field is strong enough, a process known as electroporation takes place (Martínez et al., 2020)which disrupts cell membranes by increasing the number of pores(Ali et al., 2021) thereby increasing the cytoplasmic membrane's permeability (Martínez et al., 2020;Lakka et al., 2021)for the release of intracellular contents.(Ali et al., 2021)**.** During the processing, muscle fascicles are contracted and the liquids present in cells are extracted which allowed for the extraction of oils from fish or marine sources (Sitzmann et al., 2017).

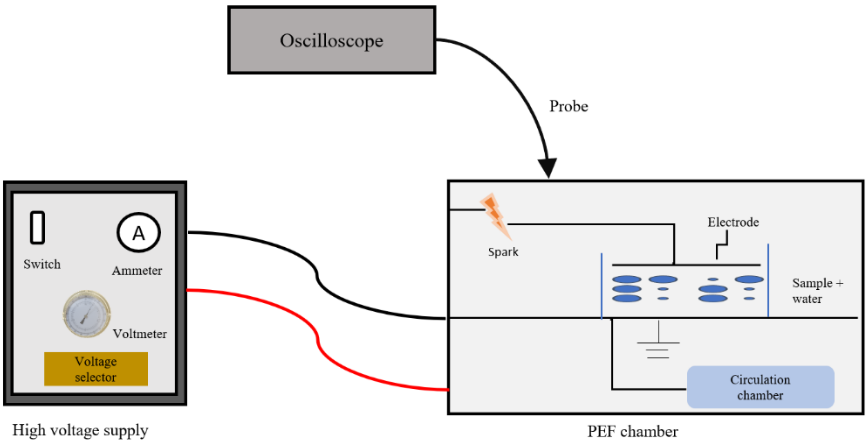

Fig.3f Pulsed Electric Field arrangement(Gulzar &Benjakul, 2020)

3.2.7 pH shift method

The technique, known as acid and alkali solubilization/pH shift process/isoelectric solubilization and precipitation, solubilizes the proteins using acid and alkali, then precipitates them at their isoelectric pH (Surasani, 2018). The so-called pH-shift method has seemed to show great potential for direct recovery of functional protein from complex raw materials like by-products of fish processing. (Abdollahi & Undeland, 2020; Jiang et al., 2018).

It is a promising yet underutilised approach for the production of fish oil under cold conditions (Abdollahi & Undeland, 2020). This technique employs a high (> 10.5) or a low (3.5) pH to solubilize the muscle proteins in water, followed by centrifugation thereby separating the solubilized proteins from undissolved low- and high-density material (Abdollahi & Undeland, 2020; Abdollahi et al., 2016;Meshre et al., 2021). The oil-containing emulsion layer floats to the top during the procedure allowing for the oil extraction (Abdollahi & Undeland, 2020)**.** Even though various new techniques like MAE, UAE, PEF etc are developed for improving oil recovery, the capital investment of these techniques is very high in industrial-scale production. Since these modern techniques are cost ineffective most industries choose traditional techniques particularly wet rendering and solvent extraction. However, SCF is being used extensively for oil extraction when compared to other novel techniques. By comparison of the various techniques, it is perfectly clear that SCF gives the highest recovery and safest product.

4. Refining of fish oils

Crude fish oil falls short of the standards for consumption as it contains contaminants and undesirable substances such FFAs, heavy metals, and cholesterol. In addition to this, the biomagnification process introduces significant amounts of contaminants, such as heavy metals and persistent organic pollutants (POPs) into marine fish which due to their lipophilic nature tend to accumulate in their lipids, which are then transferred to the oil produced from these species. These POPs have potentially harmful effects on both people and animals, such as developmental disorders, neurobehavioral abnormalities, and reproductive and endocrine system disruptions. As refining can significantly lower the levels of environmental pollutants in finished fish oil products, it is vital to refine fish oil for human consumption (X. Zhang et al., 2022). Crude fish oil typically goes through processing stages before being deemed acceptable for eating or usage as a food supplement (Song, Zhang, et al., 2018; Abdollahi & Undeland, 2020; Lamas, 2022). Additionally, fish oils must also be refined to eliminate some undesirable substances that impair the oil's stability, general quality, and customer acceptability, without reducing the PUFAs (Šimat et al., 2019). Therefore, the primary purpose of refining is to remove various kinds of chemicals, hydrocarbons, pigments, phospholipids, oxidation products of fatty acids, vitamins, sterols, mono- and di-acylglycerols, free fatty acids(Lamas, 2022)and other degraded or denatured materials such as protein, suspended mucilaginous and colloid-like matters (Adeoti & Hawboldt, 2014; Šimat et al., 2019).

The conventional refining procedure entails a number of steps, such as degumming, neutralisation also known as alkali refining, bleaching and deodorization together with antioxidant addition (Adeoti & Hawboldt, 2014) and, in certain cases, winterization, a PUFA concentration method is also employed (Bonilla-Mendez & Hoyos-Concha, 2018). Every step is particularly crucial and plays a specific role as depicted in Figure.4.

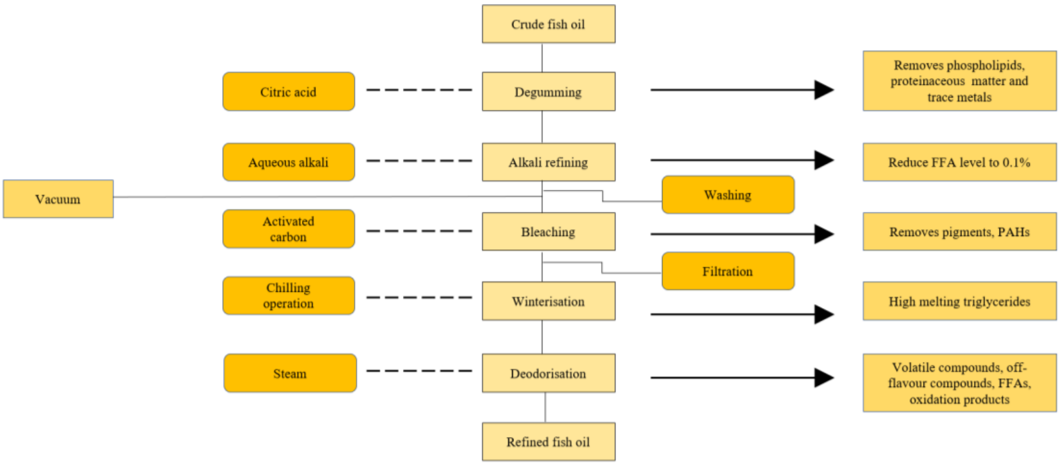

Fig.4 Schematic representation of fish oil refining (Oterhals & Vogt, 2013a)

5. Quality parameters of fish oils

Fish oils containing Omega-3 fatty acids are known to exert many positive effects and are chiefly used in the fields of aquaculture, animal nutrition, feed, supplements, functional food, pharmaceuticals and others (Ali et al., 2021; Sahena et al., 2010). The highly unsaturated nature of these fatty acids, however, leads to lipid oxidation, a deteriorating process which results in the production of undesirable oxidation products and free fatty acids that can significantly impair the oil's nutritive and organoleptic qualities (García-Moreno et al., 2014). Monitoring the degree of oxidation in marine oils is a typical technique for regulatory and quality control objectives. Usually, various oxidation markers, notably peroxides and carbonyls, are measured to achieve this (Ye et al., 2020; Asieh Habibi & Javad Keramat, 2016).

The biomarkers of lipid oxidation are peroxide value and anisidine value while the degree of lipid hydrolysis is indicated by the acid value (Editha Giese & Jan Fritsche, 2021). Physically, refined fish oil is liquid at room temperature (20 °C) yet it could be partially solid (Fish oil, 2015). The recommended quality parameters as per CODEX and GOED and physical parameters are provided in Table 4 (Fish oil, 2015). Furthermore, recent advancement in technology has led to the development of spectroscopic techniques such as 1 H NMR, MIR, and NIR spectroscopy that have become more and more crucial in the examination of culinary oils and animal fats. These are quick, non-destructive procedures that can deliver all relevant data, in a single analysis and require little to no sample preparation, minimal volumes of sample, and may even be automated (Cheng et al., 2013; Editha Giese & Jan Fritsche, 2021).

Table 4 Recommended quality standards of fish oil

Chemical parameters	**CODEX**	**GOED**
Acid value	≤ 3 mg KOH/g	< 3
Peroxide value	≤ 5 meq of active oxygen/kg oil	< 5 meq O2 /kg oil
p-Anisidine value	≤ 20	< 20
Total oxidation value (TOTOX)	≤ 26	≤ 26
Physical parameter	**Specified standard**	
Appearance	Amber coloured oil	
Odour	Characteristic fish odour	
Molecular weight	EPA: 302.45, DHA: 328.49; other oils vary	
Melting point	10–15 °C	
Flashpoint (fatty acids)	Approximately 220 °C	
Boiling point	>250 °C	
Specific gravity at (30 °C)	0.91 (s.g. of water at 30 °C is 0.996)	
Refractive index (n D 30)	1.46–1.48	
Viscosity (cp) at 20 °C	60-90	
Specific heat (cal/g)	0.50–0.55	
Heat of fusion (cal/g)	ca. 54	
Calorific value (cal/g)	ca. 9,500	

Source: Chemical parameters (CODEX, 2017;GOED ,2017;Bannenberg, 2017) Physical parameters (Fish oil, 2015; Rizliya&Mendis, 2013).

6. Stability of fish oils

Fish oils' chemical composition, quality, and stability are influenced by certain factors such as extraction technique and the raw material's quality. In addition to this storage conditions (temperature) and endogenous enzymes which synthesise free fatty acids are shown to have a great influence on the stability and quality of fish oils (Mozuraityte et al., 2016; Boran et al., 2006). Further to this fish lipids are extremely vulnerable to oxidation due to the high concentration of double bonds in PUFAs and their location in the fatty acid chain (Hrebień-Filisińska, 2021). The process of oxidation produces reactive oxygen and free radicals, which can cause negative health consequences (D. G. Zhang et al., 2021; Chang et al., 2018) such as inflammation, cancer, mutagenesis, DNA changes, ageing, and cardiovascular illnesses (Albert et al., 2013).

Furthermore, the secondary lipid oxidation products that develop from the decomposition of hydroperoxides, primarily aldehydes, ketones, and alcohols, produce some undesirable sensory changes. Four-hydroxy-2-hexenal (4-HHE), four-hydroxy-2-nonenal (4-HNE), and a wide range of isoprostanes are some particular oxidation products emerging from the lipid peroxidation of highly unsaturated fatty acids (Ismail et al., 2016). Due to their extremely low odour thresholds, some of these compounds can have an impact on sensory quality even at very low concentrations (Serfert et al., 2010). Because of the extreme instability and ease of oxidation, it produces hazardous chemicals, undesirable flavour and odour (Lall & Dumas, 2022; Pickova, 2009) that limits the use of those oils in foods (Annamalai et al., 2015; Hashim et al., 2019). In addition to this oxidation also results in the degradation of bioactive compounds like vitamins and other nutrients (Lall & Dumas, 2022; Pickova, 2009).

Given that fish oil is susceptible to oxidative degradation, it has been advised to maintain an anoxic environment during the extraction process (Pateiro et al., 2021)and to use delivery systems such as oil-in-water emulsions to create a physical barrier between the fish oil and oxygen and/or prooxidants. Additionally, to overcome this limitation certain antioxidants are also utilised to prevent lipid oxidation (Ghelichi et al., 2017; Editha Giese & Jan Fritsche, 2021). Furthermore, Organic acids and radical scavengers seem to increase the stabilization of fish oils by the deactivation or removal of free metals and phospholipids as well as by the prevention of the spread of radicals. (Mozuraityte et al., 2016). Additionally, a coating substance to encapsulate or microencapsulate the oil may prevent lipid oxidation (Serfert et al., 2010; Pateiro et al., 2021; Fish oil n.d.2015). Fish oil in the form of microencapsulates can maintain its stability for up to 2 years (Fish oil n.d.2015).However, reducing exposure to ambient air during processing and refining oils under a vacuum and nitrogen gas flushing, are some preventive measures employed to reduce lipid oxidation(Ismail et al., 2016).

7. Characterization of Fish oil

Fish oils have been employed in the past for a variety of purposes, including tanning, weather repellents, fluids, polymers, anti-corrosion agents, and fuel (Windsor, 1982). Today, more than 95% of the developed fish oil developed is used in human nourishment (Young, 1986). Following refinement, the oils are hydrogenation to create solidified cholesterol for use in grease. According to Colin, 1993 Fishing liquid's triacylglycerols were subjected to silver ion analysis by HPLC reported oil is not hydrogenated, but its composition and the one of almost all fish oils can be identified through using the following eight fatty acids: DHA ,EPA (14:0, myristic acid), (16:1 (n-7), palmitoleic acid), (16:0, palmitic acid), (22:1(n-II), cetoleic acid) (18:1(n-9), oleic acid) and (20:1 (n-11), gadoleic acid) . Fish oils' composition of fatty acids is further complicated by the presence of lesser quantities of pentadecanoic acid (15:0), hexadecadienoic acid (16:2), hexadecadienoic acid (16:2), hexadecatetraenoic acid (16:4), octadecanoic acid (18:0, stearic acid), trans-11-octadecenoic acid (18:l (n-7). The presence of pentadecanoic acid (15:0), hexadecatrienoic acid (16:3), hexadecatrienoic acid (16:3), hexadecatetraenoic acid (16:4), octadecanoic acid (18:0) , (18:l (n-7), vaccenic acid), (20:4 (n-6), arachidonic acid), (20:4 (n-3), and cis-7,10,13,16,19-docosapentaenoic acid (225 (n-3), clupanodonic acid). Additionally, methyl-branched fatty acids and the odd number of carbons in the fatty acids heptadecanoic (17: O) and heptadecenoic (17: 1) acid exist in trace amounts.

7.1 Assignment of Functional Groups Found in the Spectrum of Fish Oil Supplement to ATR-FTIR and Raman Main Bands

wavenumber approximate values (cm^{-1})	band assignment (ATR-FTIR)	wavenumber approximate values (cm^{-1})	band assignment (Raman)
3015	ν (C—H) of *cis*-HC=CH—	3015	ν_{as} (=C–H) of *cis*-HC=CH—
2960	ν_{as} (C—H) from methyl (—CH_3) groups	2970	ν_{as} (C—H) from methyl (—CH_3) groups
2920	ν_{as} (C—H) from methylene (—CH_2) groups	2940	ν_{as} (C—H) from methylene (—CH_2) groups
2850–2900	ν_s (C—H) from methyl (—CH_3) and methylene (—CH_2) groups	2860–2900	ν_s (C—H) from methyl (—CH_3) and methylene (—CH_2) groups
1745	ν (C=O) of ester functional groups from triacylglycerols	1750	ν (C=O) of ester functional groups from triacylglycerols
1735	ν (C=O) of ester functional groups from ethyl esters	1740	ν (C=O) of ester functional groups from ethyl esters
1650	ν (C=C) of *cis*-HC=CH—	1660	ν (C=C) of *cis*-HC=CH—
1460	δ_{as} $(CH_2)_{scissor}$ from methylene (—CH_2) groups	1445	δ (CH) from methylene (—CH_2) groups
1380	δ_s (CH_3) and δ_s (CH_2) of lipids	1310	δ (CH) from methylene (—CH_2) groups
1235	γ (CH_2) of lipids	1270	δ (=C—H) of *cis*-HC=CH—
1090	ν_s (C—O—C) of triglycerides and cholesterol esters	1100–1000	ν (C—C) from —$(CH_2)_n$—

Band assignments based on Baeten et al. in 1998 and Vongsvivut et al. in 2012 Macoure et al., 2019 reported that gas chromatography (GC) is used to profile the various fatty acids found in fish oils. It is a technique for analyzing oils to ascertain their fatty acid makeup.

8. Future perspectives:

The market for fish oil is expanding as a result of rising consumer interest in nutritional supplements and functional foods that contain fish oil as well as due to the increasing demands for aquaculture feed (Editha Giese & Jan Fritsche, 2021). Medical and nutritional studies have demonstrated the significant therapeutic and health benefits of marine fish oils (Kh. Albashr et al., 2022) that resulted in consumer demand. Due to growing concern for health, there is growing interested in the fortification of food products with functional substances (Pateiro et al., 2021). In this regard, there is an escalating need for fish oil either as a food ingredient or nutritional supplement(Kh. Albashr et al., 2022).

Conclusion:

A million tonnes of fish oil are produced each year, primarily most of it is obtained from marine by-products. This enables the beneficial long-chain omega-3 PUFAs from the initial inedible fish to be utilized by humans for various purposes such as ingredients, nutraceuticals, supplements *etc*. Fish oils specifically omega-3 PUFAs are currently widely appreciated for their therapeutic impact on health. As a result, fish oil production is enlarging to meet the rising demand. Fish oil extraction is usually carried out by employing traditional techniques However, recent advancements in technology led to the development of non-thermal techniques to enhance oil recovery and prevent the degradation of heat-sensitive compounds. So, recently industries have opted to use non-thermal technologies like supercritical fluids, MAE, PEF, UAE *etc*. However, supercritical carbon dioxide extraction has become Increasingly popular due to its high efficiency and maximum oil recovery.

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Graphical Abstract: