



Omega-3 fatty acids from fish by-products: Innovative extraction and application in food and feed

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ARTICLE INFO

Keywords:

Omega-3 fatty acids
Health claims
Fish by-products
Natural antioxidants

ABSTRACT

Omega-3 fatty acids (O3FA) are essential nutrients that play a crucial role in maintaining human and animal health. They are known for their numerous health claims, including cardiovascular benefits, contributing to both the prevention and treatment of immunological, neurological, reproductive, and cardiovascular complications, and supporting overall well-being. Fish, especially oily fish, comprise rich source of O3FA. In the fish industry, significant amounts of by-products and waste are generated during processing which are often discarded or used for lower-value applications. However, there is recognition of the potential value of extracting O3FA from these by-products. Various extraction techniques can be used, but the goal is to efficiently extract and concentrate the O3FA while minimizing the loss of nutritional value. To prevent oxidation and maintain the stability of O3FA, natural antioxidants can be added. Antioxidants like polyphenolic compounds and plant extracts help to protect the O3FA from degradation caused by exposure to oxygen, light, and heat. By stabilizing the O3FA, the shelf life and nutritional value of the extracted product can be extended. In summary, this work presents a forward-looking strategy for transforming fish by-products into high-quality oils, which hold great potential for application in food and feed.

1. Introduction

In recent decades, there has been a significant increase in public awareness of healthy diets, leading to a surge in the consumption of nutritional supplements. Omega-3 fatty acids are among the most popular supplements in demand. Currently, oily fish such as anchovy, sardine, and mackerel are the primary source of marine omega-3 fatty acids supplements (Liu & Dave, 2022).

Fish and fish product production has increased fourfold over the past five decades. (FAO, 2020). The rise in fish consumption is accompanied

by a surge in fish waste (Ghaly, Ramakrishnan, Brooks, Budge & Dave, 2013). However, the overfishing of these species has become a growing concern in recent years, leading to the exploration of marine by-products as an alternative source of omega-3 fatty acids. Over 70% of harvested fish worldwide are processed for filleting, heading, or gutting, generating by-products such as heads, frames, trimmings, viscera, skin, and scales, which account for over 50% of the fish's body (Liu & Dave, 2022).

Fish typically contains protein, water, and lipids (Afreen & Ucak, 2023). As a rich source of essential omega-3 fatty acids, including

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<https://doi.org/10.1016/j.fbp.2024.02.007>

Received 24 October 2023; Received in revised form 14 February 2024; Accepted 23 February 2024

Available online 26 February 2024

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icosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), fish consumption can play a significant role in preventing atherosclerotic and thrombotic diseases, and reducing the risk of various causes of mortality (Liu & Dave, 2022; Galanakis, 2022). Some fish species, such as salmon, trout, and tuna, have abundant EPA and DHA in their by-products, making them suitable for the production of fish oil and omega-3 fatty acids products (Liu & Dave, 2022). Thus, there is significant potential for the reuse of these by-products, which contain high levels of bioactive compounds that could be converted into valuable products for the food, feed and pharmaceutical industries (Galanakis, 2022).

In fact, fish and fish oil are a rich source of omega-3 fatty acids, which have been extensively studied for their health benefits. Fish oil in particular has been widely applied for the fortification of animal feeds and different food products, an innovative strategy well accepted by consumers. In recent years, there has been an increasing interest in using fish by-products to extract omega-3 fatty acids, as these by-products can be a valuable source of omega-3 fatty acids, as well as other beneficial compounds such as collagen and minerals. So, the increasing demand for fish oil has led to the use of fish processing by-products for oil production by industry (de la Fuente et al., 2022).

To support a sustainable future, this huge amount of fish by-products must be handled with methods that are safe, eco-friendly and efficient with respect to the recovery of valuable resources, ensuring zero waste (Khiari, 2022). These by-products have various nutritional potentials and can be reused either in flours or for oil extraction (Pinela et al., 2022; Boronat et al., 2023). On an industrial scale, pressing is the conventional technique globally applied to obtain fish oil. However, there are other options such as ultrasonic-assisted, microwave-assisted or Soxhlet extraction (de la Fuente et al., 2022).

The composition of fish oil is mostly based in triglycerides, free fatty acids, phospholipids, sphingolipids and oxidized lipids. The oil has several benefits for human health, due to its anti-inflammatory, antioxidant and antimicrobial potential (Pinela et al., 2022; Venegas-Calderón & Napier, 2023). However, omega-3 oils have a high susceptibility to lipid oxidation, leading to a decrease in nutritional value and shelf life of foods. Antioxidants are commonly used to protect these oils against oxidation due to their effectiveness and affordability. However, synthetic antioxidants have been found to be highly toxic and carcinogenic. As a result, there is a growing interest in exploring natural sources of antioxidants as safer alternatives, like plant-based antioxidants (Rashid, Wani, Manzoor, Masoodi & Dar, 2022).

So, the objective of this review is to show that the use of fish by-products for the extraction of omega-3 fatty acids not only provides a sustainable solution to the problem of fish waste disposal but also offers a cost-effective means of producing high-quality oils that can be used in food and feed industry. Furthermore, the application of innovative extraction techniques can enhance the efficiency and sustainability of the extraction process, and the plant-based antioxidant addition can enhance fish oil quality, making their promising avenues for future research and development.

2. Omega-3 fatty acids and associated health claims

Fatty acids can be defined as organic compounds formed by a hydrocarbon chain and a carboxylic group and, depending on the nature of the hydrocarbon chain, they can be distinguished into saturated or unsaturated (which in turn can be mono or polyunsaturated) (Rubio-Rodríguez et al., 2010). Intake of adequate amounts of polyunsaturated fatty acids (PUFA) from the diet is increasingly challenging due to an uneven distribution of dwindling resources, including food, among a growing global population (Karageorgou et al., 2023).

Although most fatty acids can be synthesized by humans, there is a group of PUFA that the human body cannot produce, so they are only achieved by the diet and are classified as essential: omega-3 fatty acids (n-3) and omega-6 (n-6) (WHO/FAO, 1994). Fish and green vegetables

are highlighted as excellent sources of these compounds and recommended to be included in daily diets to help prevent various diseases. For example, studies highlight the Japanese and Mediterranean diets, which are based on the consumption of large amounts of fish, as capable of providing high amounts of n-3 and n-6 to consumers (Ambring, et al., 2006; Kamei, Ki, Kawagoshi & Kawai, 2002). The majority of EPA and DHA is derived from fish of the Clupeidae, Scombridae, and Salmonidae families. However, these sources are increasingly scarce due to overfishing and the loss of ecosystems, and tend to be contaminated with heavy metals (Karageorgou et al., 2023).

In ancient times, regular intake of alpha-linolenic acid (ALA, 18:3n-3) was believed to ensure an adequate supply of long-chain omega-3 fatty acid (LC n-3 FA). However, currently, additional intake of LC n-3 FA is considered indispensable since the degree to which ALA is converted to physiologically essential LC n-3 FA, in particular EPA and DHA, is very limited and therefore insufficient to provide preventive and therapeutic benefits (Schuchardt & Hahn, 2013).

There is a growing body of scientific evidence highlighting the beneficial effects of certain dietary lipids on human health (Harris et al., 2021). PUFAs, particularly omega-3 and omega-6 fatty acids, have been linked to contributing to human health through their structural, developmental and signalling functions. These biologically active lipids have often been classified as nutraceutical fatty acids (Veras et al., 2021).

DHA and EPA contribute to both the prevention and treatment of immunological, neurological, skeletal, reproductive, and cardiovascular complications. Furthermore, PUFAs play a crucial role during fetal development and tumor cell proliferation (Tocher et al., 2019). Table 1 summarizes some of the beneficial effects resulting from fatty acids intake.

3. Fish industry by-products and waste

The production of fish and fish products has quadrupled in the last 50 years. This phenomenon is due not only to the increase in population, but also because people are consuming more fish. According to FAO data, the supply of fish, from capture and aquaculture, is expected to exceed 200 million tons by 2030 (FAO, 2021). The development of aquaculture has played a major role in this increase, as it produces about 100 million tons of fish annually (Ritchie & Roser, 2021).

World fish consumption per capita increased from 9.0 kg in 1961–20.2 kg in 2015, and estimates indicate an increase for the following years up to 20.5 kg (Coppola, et al., 2021). There has also been an increase in annual fish consumption (1.5%), greater than the increase in annual meat consumption (1.1%) (Galanakis, 2022). Currently about 7% of total protein consumption comes from fish and fish products (Ritchie & Roser, 2021).

The existence of scientific evidence about the benefits of fish consumption in the human diet due to its nutritional composition is one of the reasons associated with this growth. In general, fish consists mostly of protein (between 15% and 30%), lipids (0–25%) and water (50–80%), however, these values vary according to some characteristics such as the type and age of the animal (Afreen & Ucak, 2019). It is a rich source of essential fatty acids, such as EPA and DHA, important in preventing atherosclerotic and thrombotic diseases and with benefits in decreasing

Table 1
Health benefits that can result from fatty acids intake.

Healths benefits	References
Anti-inflammatory	Calder, 2017
Blood cholesterol	Zibaenezhad et al., 2017
Cancer	Eliseo & Velotti, 2016
Cardiovascular benefits	Tocher et al., 2019
Hypertension	Colussi et al., 2016
Immunological	Tocher et al., 2019
Neurological	Tocher et al., 2019
Maternal and child health	Akerele & Cheema, 2016

various causes of mortality (Galanakis, 2022; Greggio, et al., 2021).

The desire of consumers to improve their eating habits as well as the recognition of its health benefits has led this food to represent about 17.6% of the world consumption of animal protein and 6.5% of the total protein consumption (Ghaly, et al., 2013; Galanakis, 2022; Pupovac, et al., 2022).

The most recent data available, from 2018, indicates that approximately 85 million tons of fish were caught at sea that year and the total production value, from catch and aquaculture, was 178 million tons (FAO, 2020), of which 156 million tons were for human consumption and the remaining 22 million tons for fishmeal and fish oil production.

The European Union (EU) has an important weight in the world fish and seafood market. According to data from EUMOFA (European Market Observatory for Fisheries and Aquaculture Products), in 2020, fish consumption in Portugal was approximately 57.7 kg per capita, being the country that consumes more fish in the European Union (European Commission, 2022).

The world population is growing and is projected to exceed 9 billion by 2050. Population growth naturally leads to increased food production, particularly fish and fish products (Galanakis, 2022). Simultaneously, there is the concern about the effect of environmental impact on food production.

The food industry has increasingly sought to invest in technologies and alternatives that reduce the waste generated to take advantage of the by-products that are generated during processing, not only for economic reasons, but mainly because of their negative impact on the environment. Like fish, their by-products are an important source of nutrients such as protein, fatty acids and minerals and can amount to approximately 20 million tons, equivalent to 25% of the world's production from marine capture fisheries (Coppola, et al., 2021). The transformation of food by-products into ingredients or products that can be reintroduced into value chains is a sustainable and economic alternative that helps reduce this impact (Galanakis, 2022), usually being re-introduced in the production of fish meal and oil, fertilizers, or raw material for aquaculture.

The increase in fish consumption also translates into an increase in fish waste. More than 70% of the fish caught undergoes some processing, where high amounts of by-products and waste are generated that can range from 20% to 80%, depending on the type of processing and other variables such as the species of fish concerned (Coppola, et al., 2021; Ghaly, et al., 2013).

There is, however, much potential in the reuse of these by-products, allowing their conversion into high-value products due to their richness in bioactive compounds, which can be interesting in various markets such as the food, feed, pharmaceutical or cosmetic industry (Ghaly, et al., 2013; Galanakis, 2022).

4. Extraction of omega-3 fatty acids from fish by-products

Given the current concerns of consumers in their healthier food options, the food industry has been developing several products (namely nutritional supplements and functional foods) enriched with omega-3 fatty acids to help to obtain the desired levels of these compounds in the body (Sprague, Betancor & Tocher, 2017). Tuna, salmon, sardines and mackerel are some of the oily fish highlighted as important sources of these fatty acids (Hamed, Özogul, Özogul & Regenstein, 2015; Pateiro et al., 2020). The sources of LC omega-3 fatty acids that have by far the greatest quantitative significance for humans are cold-water fish such as salmon, mackerel, herring and tuna. Natural fish oils contain approximately 18% EPA and 12% DHA, meaning that each molecule of triacylglycerides (TG) contains one LC omega-3 fatty acids (Schuchardt & Hahn, 2013).

The species, size, age, genus, diet and temperature of the habitat are some of the factors that affect the quantity and quality of fatty acids possible to obtain from fish and their residues. Likewise, the selected methodology and extraction conditions will also affect the extraction of

fatty acids, namely, omega-3 fatty acids (Kim & Mendis, 2006; Shavandi, Hou, Carne, McConnell & Bekhit, 2019).

Fish oil is one of the examples of ingredients incorporated in food to ensure this goal, as this oil is recognized as an excellent natural source of omega-3 fatty acids (Jacobsen, Let, Nielsen, & Meyer, 2008). However, only 5% of the world's production of this oil is used to extract omega-3 fatty acids intended for use as food ingredients or supplements (Ciriminna, Meneguzzo, Delisi & Pagliaro, 2017). The remaining fractions generated are used for animal feed (FAO, 2020).

Several methodologies are used to extract fatty acids from fish and their derivatives. The most traditional methods use organic solvents for oil extraction, however, due to restrictions for application in the food industry, the use of petroleum ether, methanol/water and ethanol/water as extraction solvents has been explored. These processes for obtaining fish oil have good yield results with high oil content when using oil-rich fish such as herring, tuna, sardines and salmon, among others (Rubio-Rodríguez et al., 2010).

Wet pressing is the conventional methodology most used by the industry. This extraction includes different steps such as cooking the fish, pressing, decanting and centrifuging. In this way, the fish, after cooking, is pressed, and then the mixture is centrifuged to separate the fatty and aqueous fractions. Finally, the oil phase (which contains omega-3 fatty acids) is refined in several steps: neutralization, followed by bleaching, degumming or winter preparation and deodorization (Simat et al., 2019; Gulzar, Raju, Nagarajao & Benjakul, 2020).

Traditionally, these types of methodologies include two steps: extracting the oil from the raw material and refining. When extracted from a natural material, the oil is a mixture of several compounds, namely free fatty acids, glycerides, phospholipids, sterols, pigments, or tocopherols. Thus, in the production of edible oils it is considered necessary to remove non-triglycerides, dyes, and toxic compounds through the refining process (Rubio-Rodríguez et al., 2010). More innovative processes for obtaining omega-3 concentrates for the food and pharmaceutical industries include enzymatic methods and some use supercritical fluids.

The long extraction times and the high consumption of solvents (some of which have toxicity levels) are the main disadvantages presented by these techniques, which have led to the search and exploration of more viable and sustainable methodologies to guarantee quality, purity and stability of the extracted valuable compounds. To circumvent this problem and explore processes considered more viable and more sustainable, the industry has been developing new alternative processes that guarantee purity and stability while being considered eco-friendly (Al Khawli et al., 2019; Chemat et al., 2020). Additionally, the high temperatures required by these methodologies are associated with the degradation of heat-sensitive natural compounds and negative impacts on the environment since the extraction requires a significant amount of heat, with a risk of leakage of organic solvents into the environment (Adeoti & Hawboldt, 2014).

Ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and pulsed electric fields (PEF) are some of the most used examples of green and safe technologies. Despite having clear advantages over conventional methodologies, it is considered that optimization studies are still needed to avoid the development of oxidative processes that reduce the quality of the products obtained (Pateiro et al., 2020). The use of enzymes in industrial processes is quite recent, but it has become a good alternative to traditional methods, as it can be simpler and cheaper in relation to the investment cost and energy expenditure. Furthermore, this technology does not require organic solvents or high temperatures (Rubio-Rodríguez et al., 2010).

Although UAE is associated with the enrichment of oils with compounds of high added value, some research shows that this technology can increase lipid oxidation (Gulzar & Benjakul, 2018). To circumvent this limitation and guarantee the quality of the oil obtained, some authors have been applying UAE under a nitrogen atmosphere or adding

antioxidant compounds during the extraction process or combined with enzymatic extraction pre-treatment (Pateiro et al., 2020).

In turn, the MAE appears in the literature as an extraction technique with greater efficiency than traditional fish oil extraction techniques. This methodology is based on the use of microwaves to heat solvents in contact with the solid matrix to extract the content of the sample solution (Adeoti & Hawboldt, 2014). This technology is defined as fast and with low consumption of solvent and temperature, in addition to water being identified as the most efficient solvent, which made this method very attractive from an economic and environmental point of view (Alfio, Manzo, & Micillo, 2021). However, heat generation, which can lead to the oxidation of unsaturated fatty acids and, when resorting to volatile solvents, has low efficiency (Adeoti & Hawboldt, 2014).

Currently, SFE is widely applied in the industry, namely for the extraction of fish oil with supercritical carbon dioxide. This methodology uses supercritical fluids to separate the extractor from the matrix using SC-CO₂ as a solvent. This technology has been standing out when compared to conventional methodologies since the yields obtained with SFE are high, without solvent residues and smaller amounts of impurities, especially heavy metals. Additionally, the protein fraction obtained at the end of the extraction can be reused for application as animal feed or even valued as a source of bioactive compounds (Melgosa, Sanz & Beltrán, 2021). However, the equipment is of high cost and complexity, the pressures required are high, and as CO₂ is highly selective, it is not possible to extract polar substances. Furthermore, the CO₂ must be clean, and the methodology requires a high energy consumption (Ivanovs & Blumberga, 2017).

PEF is defined as an innovative oil extraction technology or used as a pre-treatment in combination with other technologies to improve the extraction yield and guarantee oxidative stability due to the inactivation of oxidative enzymes (Gulzar et al., 2020).

5. Refinement of omega-3 fatty acids from fish by-products

In literature, several studies reported that oil with good quality could be obtained using fish by-products as raw material through conventional refinement procedures (Šimat et al., 2019; Soldo et al., 2019; Song et al., 2018a) and using green refining technologies (Lamas, 2022). Despite the raw material used to obtain fish oil, crude fish oil must undergo a refinement process before being consumed (Lamas, 2022). At the end of the extraction process, fish oil contains impurities, such as moisture, phospholipids, free fatty acids, primary oxidation products, minerals, pigments, off-flavours, and even persistent organic pollutants (POP) (Šimat et al., 2019). A higher presence of impurities was reported in oils from fish by-products affecting their stability and making them highly perishable (Šimat et al., 2019). These impurities reduce fish oil quality and should be eliminated while preserving the omega-3 and other PUFAs, guaranteeing oil purity and stability (Lamas, 2022).

The conventional refinement process was applied to crude fish oil obtained from different fish by-products (Šimat et al., 2019; Soldo et al., 2019; Song et al., 2018a). Conventionally refinement of fish oils includes the following steps: (1) degumming using 1% phosphoric acid to eliminate phospholipids and gums, (2) neutralisation with 1 M NaOH followed by centrifugation to eliminate free-fatty acids by decreasing the oil acidity, (3) bleaching with a combination of adsorbents to remove pigments and other contaminants, and (4) deodorisation by steam distillation under vacuum to remove volatile compounds (Marsol-Vall et al., 2022; Vaisali et al., 2015). Washing with 10% hot water (90–95 °C) and drying procedures (90–95 °C) could also be necessary (Crexli et al., 2010; Lamas, 2022).

The effect of the refining process on the quality of crude fish oil extracted from tuna and anchovies' by-products (viscera, skins, and skeletons) was evaluated by Song et al. (2018b). In another study, the sardine canning by-products (heads, gut content, and fins) were also explored as an alternative source of raw materials for fish oil production (Soldo et al., 2019). Both studies validate the feasibility of the refining

process for removing the undesirable volatile components and free fatty acids of fish by-products oil, enhancing oil quality without scarifying oil's nutritive value. The same conclusion was achieved in another study investigating the changes in the characteristics of fish oil obtained from tuna, seabass and gilthead seabream by-products and tuna liver during a four-stage refining process (Šimat et al., 2019). However, according to Šimat et al. (2019), using temperatures higher than 100 °C for a short time (less than 1 hour) during the deodorisation step could be necessary for more effective removal of volatile components from fish oils. Instead, according to Song et al. (2018), the control of temperature and heating time in the deodorisation step were pointed out as essential to prevent omega-3 fatty acids oxidation and consequent formation of volatile compounds and should be optimised. However, in the study of Song et al. (2018b), a higher temperature (220 °C) was applied in comparison to the studies of Šimat et al. (2019) and Song et al. (2018b) that used 95–97 °C both. According to another study of fish by-products oil refining, 160 °C for 1 h and 200 °C for 1 h were recommended for the tuna by-product oil deodorisation (de Oliveira et al., 2016).

More recently, alternatives to conventional fish oil refining have been proposed (Lamas, 2022; Marsol-Vall et al., 2022; Melgosa et al., 2021). These technologies have been described as more efficient and with lower environmental impact than traditional methodologies that use chemical products or high temperatures (Lamas, 2022). Regarding the degumming process, one of the technological alternatives studied is the use of enzymes, namely phospholipase – type enzymes which hydrolyse the ester bonds of phospholipidic salts (Marsol-Vall et al., 2022). Recently, this technology was applied to an oil sample from multispecies fish by-products using the enzymes phospholipase A1 or acyltransferase (Lamas, 2022). The treatment using the enzymes reduced the phosphorus content more efficiently than the traditional process without compromising the EPA and DHA contents and improving the oil colour. The use of acids, such as orthophosphoric acid, lactic acid and acetic acid, was also explored in the degumming of sardine oil. The orthophosphoric acid (5%) showed to be efficient in the reduction of phospholipids, also reducing the metal ions content (copper, iron, and mercury) of oils (Charanyaa et al., 2017).

Concerning the neutralisation of fish oils, the most common alternative to the conventional procedure is enzymatic esterification with ethanol or glycerol using a commercial lipase (Marsol-Vall et al., 2022). This procedure allowed the enrichment of PUFAs and removing the free fatty acids. The use of membrane-assisted solvent-extraction processes was also evaluated by other works (Charanyaa et al., 2017). Until now, the use of deep eutectic solvents to replace traditional solvents has only been studied in vegetable oils (Marsol-Vall et al., 2022). In bleaching, alternative processes such as short-path distillation or supercritical fluids have been investigated to produce bleached fish oil. The short-path distillation involves using a high vacuum at high temperatures (200 °C) during short residence times; on the other hand, the apolar nature of CO₂ turns supercritical fluid into a suitable solvent to remove the commonly non-polar persistent organic pollutants (Marsol-Vall et al., 2022; Melgosa et al., 2021). However, the operational costs of short-paths distillation and the high costs of the supercritical fluid equipment have drawbacks to their industrial implementation (Marsol-Vall et al., 2022; Oliveira & Miller, 2014). Indeed, the use of solid adsorbents, if optimised, is the most convenient method for bleaching fish oils (Marsol-Vall et al., 2022). In literature, some studies reported optimisation studies of the bleaching step of fish by-product oils (Monte et al., 2015). A response surface methodology tested different percentages of adsorbent to the viscera carp oil mass and activated carbon to the total adsorbent mass to optimise its colour, carotenoid content and thiobarbituric acid (TBA) value (Monte et al., 2015). The optimum conditions (2% adsorbent and 10% activated carbon) revealed a lower carotenoid loss, a reduction in the TBA value, and oil darkening.

Some alternative deodorisation methodologies have already been applied to refining oil from fish by-products to replace the traditional

high-temperature methods, like steam distillation and molecular distillation (Monsiváis-Alonso et al., 2020; Song et al., 2018b). Fish oil's alternative deodorisation strategies are short-path distillation (Messina et al., 2021), nanofiltration (Fang et al., 2018), alkaline ethanol extraction and solid-phase adsorption (Song et al., 2018b). Short-path distillation has been studied in farmed gilthead sea bream viscera by Messina et al. (2021). On the other hand, Song et al. (2018b) evaluate the effect of alkaline ethanol extraction and solid-phase adsorption on volatile components and fatty acids in the oil from tuna and anchovies by-products to replace the industrially implemented molecular and steam distillations. Song et al. (2018b) also investigated a green tea polyphenol treatment. Among the alternative techniques investigated, alkaline ethanol achieved oils with superior quality, with the advantages of being a simplified procedure easily implemented industrially, that operates at low temperatures, and that solvent can be recovered.

At last, after the refining process, fish oils could pass by an enrichment of omega-3 fatty acids (EPA and DHA) to obtain PUFA-concentrated products. Alternative techniques, such as liquid and supercritical fluid chromatography, have also been tested as potential replacers of the most common methodologies, such as urea complexation, low-temperature crystallisation, and enzymatic purification (Marsol-Vall et al., 2022).

In conclusion, the refinement process reduced the potentially higher level of contaminants in the oil produced using fish by-products, pointing out that oil fraction from fish by-products could be valorised, promoting the sustainable and efficient utilisation of fish. Nevertheless, alternative methodologies at different levels of the refining process should also be adopted in order to achieve fish by-product valorisation schemes environmentally and economically more sustainable.

6. Stabilisation of omega-3 fatty acids with natural antioxidants

Omega-3 oils have a high susceptibility to oxidation due to their high content of long-chain PUFAs, a high number of double bonds, and their position in the fatty acid chain, causing deterioration of their biological function, nutritional value, and production of toxic compounds (Hrebien-Filisińska, 2021; Wang et al., 2021).

Lipid oxidation occurs through different mechanisms, namely enzymatic oxidation, photo-oxidation, and autoxidation. Photo-oxidation occurs when light and/or photosensitizers are present and significantly impacts the oxidation process and the distribution of primary oxidation compounds. The most common photosensitizers are pheophytins, chlorophylls, riboflavin, and myoglobin. They may participate in lipid oxidation through the generation of excited state singlet oxygen, which will react with unsaturated lipids and form hydroperoxides. Enzymatic oxidation occurs in the presence of lipoxygenase enzymes, and PUFA is transformed into their corresponding hydroperoxides. This mechanism mainly affects vegetal oils. Autoxidation is the most common mechanism in lipid oxidation and occurs when oxygen molecules react with unsaturated lipids (Wang et al., 2021). Autoxidation is a chain reaction divided into three steps: initiation, propagation, and termination. It starts with the formation of very reactive lipid free radicals or alkyl radicals through the loss of a hydrogen atom by an unsaturated lipid in the presence of initiators such as light, heat, and transition metal ions. Then, lipid peroxy radicals are formed by reacting the very reactive lipid free radicals with oxygen. Once these products are very unstable and reactive, they react with another unsaturated lipid, producing lipid hydroperoxides (i.e., peroxides). Peroxides are termed as primary oxidation products, and they are tasteless. However, in the presence of heat or metal ions, peroxides can be decomposed into secondary volatile oxidation products, which are responsible for the loss of the organoleptic properties of lipids through the production of unpleasant flavours and odours. The lipid oxidation is over when the newly formed radicals react with each other (Jacobsen et al., 2013; Rahmani-Manglano et al., 2020). Several factors can affect the rate of lipid oxidation, such as fatty acid composition and its positional distribution, the presence of catalysts and

compounds with pro-oxidant and antioxidant properties, and processing and storage conditions (Wang et al., 2021).

Omega-3 fatty acid oxidation can be prevented or retarded using antioxidants. Antioxidants are compounds that can improve oxidative stability by inactivating peroxides, scavenging free radicals and reactive oxygen species, inhibiting pro-oxidative enzymes, chelating metal ions, and quenching secondary oxidation products (Mishra et al., 2021). Antioxidants can be classified into primary antioxidants and secondary oxidants based on their mode of action. Primary antioxidants can neutralize free radicals by donating a hydrogen atom to lipid alkyl radicals and scavenging the lipid peroxy radicals, breaking the chain reaction of oxidation, while secondary antioxidants can remove oxidation promoters such as singlet oxygen, metal ions, pro-oxidative enzymes, and other oxidants (Mishra et al., 2021; Rahmani-Manglano et al., 2020). The effectiveness of the antioxidant is dependent on structural features, such as, its concentration, bulk oil system temperature, and the presence of pro-oxidants and other antioxidants. Furthermore, once the lipid oxidation can occur by different mechanisms and can be influenced by different intrinsic (i.e., degree of saturation, free fatty acids) and extrinsic (i.e., temperature and light) conditions, there is no single antioxidant that can prevent all lipid oxidation (Jacobsen et al., 2013; Mishra et al., 2021). There are already several antioxidants used in food products to prevent or retard lipid oxidation, such as synthetic antioxidants (i.e., tertiary-butylhydroquinone (TBHQ), propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT)), tocopherols, phenolic compounds, ascorbates, spices, and spice extracts (Wang et al., 2021). Synthetic antioxidants are fat-soluble compounds that have long been used to stabilize fish oils, vegetable oils, and fried and baked goods. BHA and BHT are considered primary antioxidants once they become potent peroxy radical scavengers and oxidation chain disruptors (Wang et al., 2021). However, there is some concern about the potential toxicological effects of these antioxidants on human health, so natural antioxidants have been attracting wide attention (Hrebien-Filisińska, 2021). Furthermore, natural antioxidants may improve its health-promoting properties while protecting the product from oxidation.

Tocopherols and tocotrienols are among the most well-known natural antioxidants used to stabilize edible oils (e.g., omega-3 oils). They act as scavengers of free radicals once they compete with unsaturated fatty acids for lipid peroxy radicals (Mishra et al., 2021). However, their effectiveness depends on the concentration and type of isomer used, as well as on the presence of other components. If the concentration of tocopherols is too high or in the presence of metal ions, they can act as pro-oxidants. Therefore, the combination with other compounds (e.g., ascorbic acid, citric acid, and lecithin) can improve their antioxidant capacity (Hrebien-Filisińska, 2021). Ascorbic acid and its derivatives are hydrophilic antioxidants that can act by quenching several forms of oxygen, neutralizing free radicals, and regenerating primary antioxidants (Mishra et al., 2021). Moen et al. (2017) developed a mixture of different natural antioxidants (i.e., tocopherol, ascorbyl palmitate, rosemary extract, and green tea catechins) to improve the oxidative stability of industrial marine oil concentrates. The authors observed a clear synergy between ascorbyl palmitate and tocopherol and between ascorbyl palmitate and rosemary extract. The mixture developed was added to omega-3 concentrates and tested against tocopherol in omega-3 concentrates. The mixture added to the concentrates has only about half the amount of antioxidants when compared to the sample with tocopherol. The mixture of antioxidants presented an extended induction period in the weight increase curves, peroxide value, and formation of secondary oxidation products over time compared to the concentrates with tocopherols. These findings could be attributed to ascorbyl palmitate's ability to regenerate -tocopherol from its oxidized form, thereby reducing the amount of tocopheryl radicals available for oxidation, as well as the antioxidant mixture's ability to prevent the formation of primary and secondary oxidation products.

Polyphenols are another type of natural antioxidants widely used to

stabilize omega-3 fatty acids in fish oil. Quercetin (Liu et al., 2021), catechin (Feng et al., 2018), flavonoids, and curcumin are some of the polyphenolic compounds most studied as antioxidants to stabilize omega-3 fatty acids in fish oil. They can also be used as single antioxidants or in combination with other antioxidants, namely caffeic acid (Hrebić-Filišinska, 2021). Huang et al., (2017) studied the oxidative stability of fish oil in the presence of curcumin, its two synthetic analogues, and α -tocopherol for 70 days at 4 °C and 25 °C. The authors observed that curcumin and α -tocopherol had higher antioxidant efficacy than the two synthetic curcumin analogues at 4 °C, but both analogues had higher antioxidant efficacy at 25 °C, indicating that temperature is an important factor in lipid oxidation during fish oil storage. Therefore, the selection of antioxidants should consider the storage conditions, namely temperature.

Spices and plant extracts can also be added to fish oil as natural antioxidants once they present reducing power, metal chelating capacity, and radical scavenging activity. Rosemary, sage, and tea extracts present several phenolic compounds with antioxidant properties; however, they have a detectable flavor (Rahmani-Manglano et al., 2020). Yeşilsu & Özyurt, (2019) evaluated the effect of rosemary, thyme, and laurel extracts on the stability of fish oil during the microencapsulation process and heat-induced degradation. The authors observed that the microencapsulated fish oil presented lower peroxide values in the presence of rosemary extract when compared to the microcapsules with BHT. Furthermore, microcapsules with rosemary and laurel extracts showed a lower value of thiobarbituric acid than the microcapsules with BHT. Therefore, rosemary and laurel extracts showed potential as antioxidants to improve the oxidative stability of fish oil during microencapsulation by spray drying.

7. Omega-3 fatty acids in food and feed

The regular consumption of adequate quantities of omega-3 fatty acids is claimed to provide a broad spectrum of health benefits (Section 2). The most beneficial omega-3 fatty acids for a healthy lifestyle are EPA and DHA, which can be obtained from marine foods. Shorter chain omega-3 fatty acids can be found in terrestrial plants as alpha-linolenic acid (18:3n-3), particularly in nuts, linseed, flaxseed, rapeseed, and perilla seeds, being the precursor for the synthesis of long-chain omega-3 fatty acids as EPA, DPA and DHA (Brenna et al., 2009, Abad & Shahidi, 2020). As humans and animals cannot efficiently synthesize long-chain omega-3 PUFA from alpha-linolenic, they must source these essential fatty acids through food (Gerster, 1998). For adult humans, the minimum daily consumption of EPA and DHA varies from 0.25 to 0.5 g (WHO, 2003; SACN, 2004; EFSA, 2010), but it may increase by two to three-fold when following a low-fat diet (Kris-Etherton et al., 2009). Additionally, diets should have an optimal 1:1–4:1 omega-6:omega-3 fatty acids ratio (Rimm et al., 2018, Rizzo et al., 2023). However, the increased consumption of foods rich in omega-6 and poor in omega-3 fatty acids, such as animal fats and vegetable oils, has promoted the shift of this ratio up to 45:1 (Singh et al., 2017). Animal feeds follows a similar trend, the omega-6: omega-3 fatty acids ratio in diets typically being much higher than the optimum of 4:1 (Rimm et al., 2018). As omega-6 and omega-3 long-chain fatty acids are formed from C18 precursors (linoleic and alpha-linolenic acids, respectively) by the same biosynthetic pathway and enzymes, greater omega-6 fatty acids intake leads even to less EPA, docosapentaenoic acid (22:5n-3, DPA) and DHA endogenous formation and to higher production of pro-inflammatory eicosanoids (Patel et al., 2022, Rizzo et al., 2023).

Fish and fish oils constitute the main food sources of EPA and DHA, as these essential fatty acids are mainly produced at the base of aquatic ecosystems, namely by phytoplankton and heterotrophic unicellular organisms, being concentrated at higher trophic levels (Ganesan et al., 2014). Conversely, terrestrial foods are poor sources of omega-3 fatty acids. Indeed, the amount of alpha-linolenic acid, the main omega-3 PUFA, constitutes only 0.7–1.4% of the total fatty acids in beef, lamb,

poultry and pork meat (Patel et al., 2022). However, being linoleic acid, the main omega-6 fatty acid, higher in pork and poultry than beef and lamb meat, red meat presents a lower omega-6:omega-3 ratio (Patel et al., 2022). Similar to meat, the main omega-3 fatty acid in milk fat is alpha-linolenic acid (Jensen, 2002). Although higher plants have the ability to synthesize omega-6 and omega-3 precursors (i.e., linoleic and alpha-linolenic acids), they lack the enzymatic machinery to produce long-chain fatty acids as arachidonic (C20:4n-6), EPA, DPA and DHA (Venegas-Calderón et al., 2010). However, a small number of higher plants can synthesize the first step of Δ 6-pathway biosynthesis of PUFA, producing gamma-linolenic (C18:3n-6) and stearidonic (C18:4n-3) acids (Sayanova et al., 1999). High stearidonic acid content may be found in Echim and hemp seeds and oils (Amaro et al., 2015, Rizzo et al., 2023).

Fish oil is considered the golden standard and primary source of omega-3 PUFA for human and animal feeding, but alternative sources are crucial due to the risk of the presence of contaminants, as heavy metals and polychlorinated biphenyls (Jacobs et al., 2004) and to sustainability reasons. Indeed, studies indicate that wild fish capture does not support the growing demand for fish oil (Steinrücken et al., 2017), existing a gap between ecological supply and requirements for omega-3 fatty acids for food and feed. As the majority of wild caught stocks are at maximum levels (FAO, 2012), and taking into consideration the Sustainable Development Goal 14 “Life below water” of the United Nations Agenda 2030 to prevent overfishing, alternative sustainable sources of omega-3 PUFA are required. These include additional marine sources such as microalgae, macroalgae, krill, copepods, and single cell organisms (Cottrell et al., 2020, Orozco Colonia et al., 2020), and genetically modified plant oilseeds such as rapeseed (*Brassica napus*), Thale cress (*Arabidopsis thaliana*) and Camelina (*Camelina sativa*) (Gill & Valivety, 1997, Tocher, 2015, Patel et al., 2021, Patel et al., 2022).

Algae are a diverse group of autotrophic organisms, including micro- and macroalgae, that form the basis of marine food chain. Microalgae are valuable sources of omega-3 fatty acids, growing faster and producing up to 10 times more oil than native higher plants (Sajjadi et al., 2018), which can be further improved through cultivation conditions such as light, temperature, pH, and nutrients (Perdana et al., 2021). Of particular importance, microalgae and heterotrophic microalgae-like organisms are the primary producers of EPA and DHA. Among microalgae *Phaeodactylum* sp., *Nannochloropsis* sp., and *Nitzschia* sp. are the main EPA producers, accounting up to 39% of total fatty acids (Adarme-Vega et al., 2012). On the other hand, DHA is mostly produced by heterotrophic algae-like organisms such as thraustochytrids (e.g., *Schizochytrium* sp.) and dinoflagellates (e.g., *Cryptothecodinium* sp.) that can accumulate up to 65% DHA in their lipids along with EPA and minor amounts of other PUFA (Ji et al., 2015, Barta et al., 2021). Unlike microalgae, macroalgae species have modest lipid content (up to 4% dry matter basis). Nonetheless, its highly unsaturated lipid profile with EPA and DHA comprising from 10% to 50% of total fatty acids (Bocanegra et al., 2021, Rizzo et al., 2023) has prompted attention to macroalgae species. High levels of omega-3 PUFA were reported in red macroalgae, and of EPA in red and brown macroalgae species such as *Analipus japonicus*, *Sargassum thunbergia*, and *Champia parvula* (Bocanegra et al., 2021, Rizzo et al., 2023).

Krill, a shrimp-like crustacean that feeds on algae, is rich in both EPA and DHA. Krill oil is characterized by high phospholipid content (40–60%), with a considerable proportion of these essential fatty acids (30–65%) (Köhler et al., 2015), which contrast to fish oil mostly composed by triacylglycerols with EPA and DHA, preferentially in sn-2 position (Jin et al., 2020). The amphipathic properties of phospholipids promotes emulsification and may contribute to an enhance absorption and bioavailability of EPA and DHA from krill oil (Ulven et al., 2011).

Copepods, one of the most abundant animals within zooplankton, are particularly rich in EPA and DHA (Kabeya et al., 2021). Like krill, long-chain omega-3 fatty acids are the dominant fatty acids in phospholipids, phosphatidylcholine being composed of similar levels of EPA

and DHA (c.a., 35%), while in phosphatidylethanolamine predominates DHA (c.a., 50%) (Kattner & Hagen, 2009). Lower percentages of EPA and particularly of DHA are found in neutral lipids, wax esters and triacylglycerols (Kattner & Hagen, 2009).

Over the last decade, bioengineering has focused on the production of vegetable oils rich in EPA and DHA from genetically modified crops. Algae and yeast desaturases and elongases, needed to produce long-chain omega-3 fatty acids from the C18 precursor, were genetically inserted on native land plants with high alpha-linolenic production thus enabling the production of EPA or DHA or EPA and DHA rich oils (West et al., 2021). First studies were developed in Thale cress (Petrie et al., 2012, Ruiz-Lopez et al., 2013) and then transferred to Camelina and rapeseed transgenic strains, with variable contents of EPA and DHA. Camelina strains with 24% EPA and no DHA and up to 11% EPA and 9% DHA in oil seeds have been reported by Ruiz-Lopez et al. (2014) whereas Petrie et al. (2014), using different constructs, produced oils with up to 12.4% DHA and 3.3% EPA. EPA-rich rapeseed transgenic strains with up to 8.1% EPA and 0.2% DHA productions (Napier et al., 2019), and DHA-rich strains with contents up to 10.5% DHA and less than 1% EPA (Petrie et al., 2020, MacIntosh et al., 2021) were also developed and made commercially available (Sprague et al., 2017, West et al., 2021).

Bacteria can also produce essential omega-3 fatty acids but are less efficient producers than other single cell organisms as microalgae and algae-like fungi (Thevenieau and Nicaud, 2013). Among most significant bacterial omega-3 producers are members of genus *Shewanella* (Dailey et al., 2016). Oleaginous yeast genetically modified are another alternative of omega-3 fatty acids (Abeln & Chuck, 2021). The two most promising yeasts are *Yarrowia lipolytica*, a EPA-rich strain (c.a. 50% total lipids) (Xie et al., 2015), and *Lipomyces starkeyi*, a high DHA (17.4%) and low EPA (1.2%) strain (Salunke et al., 2015).

Despite the potential of the alternative EPA and or DHA sources, they are currently limited, among others, by cost, conservation issues, competition for land resources and public acceptance especially regarding transgenic agricultural production. Thus, alternative strategies such as fortification of foods has been pursued. The fortification of foods with omega-3 PUFA have been well accepted by consumers due to their low cost and accessibility while avoiding the need for changing feeding habits (Patel et al., 2022). In animal feeding, omega-3 fortification is also a promising strategy, particularly in monogastric animals, as the dietary fatty acids profile is the primary factor impacting the profile of the fatty acids absorbed and deposited in tissues and eggs (Kouba & Mouro, 2011). On the contrary, in ruminant animals, dietary lipids are extensively lipolyzed and the unsaturated fatty acids biohydrogenated to monounsaturated and saturated fatty acids by the rumen microbial population, thus reducing the amount of PUFA available for absorption (Jenkins et al., 2008, Amaro et al., 2012, Maia et al., 2012). However, dietary supplementation with linseed oil, fish oil, and marine algae are effective strategies to increase the absorption of omega-3 PUFA in ruminant animals, and thus to enrich meat and milk (Shingfield et al., 2013, Doreau & Ferlay, 2015, Gómez-Cortés et al., 2021). Similarly, pasture and grass silage-based diets, rich in alpha-linolenic acid, promote the omega-3 PUFA of ruminant foods when compared to grain and concentrate based diets, favorably altering the omega-6:omega-3 ratio (Dewhurst & Moloney, 2013). Adoption of management systems toward an enrichment of animal tissues in omega-3 PUFA is performed in both monogastric and ruminant animals not only to increase the nutritive and functional value of animal products for food, but also to benefit the animal health (Ao et al., 2015, Neijat et al., 2020, Ibrahim et al., 2022).

Another approach for obtaining fortified animal products is through nutrient enrichment of processed foods. This strategy has led to the development of several foods, namely a strawberry yogurt fortified with marine fish oil (Estrada et al., 2011), chicken nuggets fortified with encapsulated fish oil and garlic essential oil (Raeisi et al., 2021), mortadella fortified with pre-emulsified fish oil (Cáceres et al., 2008), and cookies and pasta fortified with microalgae (*Nannochloropsis*

oculata) (Babuskin et al., 2014).

Fortification of foods and feeds with long-chain omega-3 PUFA presents a challenge as these fatty acids are extremely susceptible to oxidation (Porter et al., 1995) generating harmful oxidation products and undesirable aromas that reduce product quality and consumer acceptance (Domínguez et al., 2019, Grootveld et al., 2020). Lipid oxidation may occur at several steps, from storage to cooking processes. Rate of oxidation during storage was shown to greatly impact shelf life of meat, as shown by the decrease in PUFA content of goat (Adeyemi et al., 2016) and lamb (Díaz et al., 2011) meat after a 12 days and 6 days chilled storage period, respectively. A wide range of approaches (e.g., controlling storage conditions, packaging, antioxidants) have been developed to increase the oxidative stability of omega-3 PUFA in food and feed (Wang et al., 2021).

More recently, nanotechnology has emerged as a powerful strategy to encapsulate, protect and deliver bioactive compounds in foods, using different nanoscale delivery systems as nanoemulsions, solid lipid nanoparticles, nanostructured lipid carriers, nanoliposomes, nanogels, and nano-particle stabilized Pickering emulsions (McClements & Öztürk, 2021). This technology has been already used in fortification of foods with omega-3 fatty acids to improve the matrix compatibility, physiochemical stability and bioavailability of these essential fatty acids. Indeed, studies have already reported the use of nanotechnology for the enrichment of pasteurized milk with alpha-linolenic acid (Slewa & Mowsow, 2018), low fat probiotic fermented milk (Moghadam et al., 2019) and yoghurt (Ghorbanzade et al., 2017) with omega-3 fatty acids through fish oil nanoencapsulation. In animal feeding, the use of nanotechnology is in his infancy, but a recent study with Tilapia fed diets with nano-encapsulated omega-3 PUFA reported a fortification of flesh with these essential fatty acids and improvement of animal performance, immunity, and disease resistance (Ibrahim et al., 2022).

8. Concluding remarks and future perspectives

The regular consumption of adequate quantities of omega-3 fatty acids, particularly EPA and DHA, is claimed to provide a broad spectrum of health benefits. Although fish and fish oils constitute the main food sources of EPA and DHA, alternatives sources are crucial for security and sustainability reasons. Microalgae, macroalgae, krill, copepods, single cell and genetically modified plant oilseeds have shown potential as EPA and or DHA sources, but they are currently limited for a widely use in human and animal feeding. Fortification of foods with omega-3 PUFA have been well accepted by consumers but strategies are needed to overcome their oxidation.

The effectiveness of antioxidants depends on the antioxidant amount, the presence of other antioxidants, the impurities in the oil, the fatty acid profile of the oil, and storage conditions. There is a growing interest in to develop some combinations of several antioxidants comprising synergists, radical and oxygen scavengers to have higher stabilization, reduction of concentrations, costs and changes in the color and flavor profile. Furthermore, other technologies have been developed to prevent or retard lipid oxidation, such as structural modifications, removal of catalysts and oxygen, encapsulation, and emulsification.

More recently, nanotechnology has emerged as a powerful strategy to be used in fortification of foods with omega-3 fatty acids to improve the matrix compatibility, physiochemical stability and bioavailability of these essential fatty acids.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by national funds through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020) and REQUIMTE (UIDB/50006/2020, DOI: 10.54499/UIDB/50006/2020; and UIDP/50006/2020, DOI: 10.54499/UIDP/50006/2020); CBQF, UIDB/50016/2020 (DOI: 10.54499/UIDB/50016/2020). This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, with DOI 10.54499/UIDB/04469/2020, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020. This work was funded by the European Regional Development Fund (ERDF) through the Competitiveness and Internationalization Operational Program (POCI), within the framework of the Corporate R&D project in Co-promotion HealthyPETFOOD: PetFood Formulations to promote health and quality of life (POCI-01-0247 -FEDER-047073) M.R.G. Maia acknowledges FCT through program DL 57/2016 – Norma transitória (SFRH/BPD/70176/2010). Raquel F. S. Gonçalves acknowledge the Foundation for Science and Technology (FCT) for her fellowship (SFRH/BD/140182/2018).

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