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Valorization Technologies of Marine By-Products

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Abstract

Generally, in different countries, strategies to improve food security have focused on increasing food production, which contributes to climate pollution and increases stress on scarce natural resources such as water and land. Due to the increase of world population (estimated to be 9 milliards in 2050), to the limited biological resources and to the increase of environmental pollution, there is a need in innovation in food industry. This can be done by improving food quality through new technologies for valorization of food and food by-products. According to Food and Agriculture Organization (FAO), one third of world food production is lost or wasted along the food supply chain. In the sector of fisheries and aquaculture, 35% of the world's harvest is lost or wasted each year. Thus, the valorization of marine by-products should be an obligation to assure the world food security and to satisfy the growing demand for fishery products. The objectives of this study are: First to review the sources of by-products and their characteristics and second to describe and evaluate the different technologies that are or can be used to valorize marine by-products in production of marine oils and concentrated fatty acids.

Keywords: by-products, valorization, technologies, fish, marine oil, n-3 fatty acids

1. Introduction

According to the FAO, world fishery production has reached around 179 million tons in 2018, of which 156.4 million tons were intended for direct human consumption. This is equivalent to an annual supply estimated at 20.5 kg per inhabitant. While world capture fisheries production stagnates at around 96.4 million tons, aquaculture is experiencing continuous growth in the supply of fish for human consumption, contributing with 46% of the total supply [1].

Unfortunately, despite the expanding demand for fishery and aquaculture products and their importance to the food security of many populations, a large part of the catch is wasted [2–4]. From the catch to the finished product, unused secondary products are generated. Today, some of these by-products are used but a great amount is wasted [5]. In 2016, Jackson & Newton estimated that 11.7 million tons of by-products produced in processing plants around the world are not collected for the production of marine ingredients [6].

In the past, marine by-products were often discarded as waste, used directly as feed for aquaculture, livestock, pets or used in silage and fertilizers [2, 7]. However, in past two decades, other uses of marine by-products have appeared based on their important characteristics and their contents of high value molecules. In some

cases, compounds from by-products are identified higher in value than the starting material [8, 9]. Furthermore, with improved processing technologies, marine by-products can be now used differently and more efficiently.

Several reviews have been previously published discussing the possibilities of using marine by-products to produce high added value compounds [8, 10–15]. Different methods have allowed to produce useful molecules like proteins, gelatin, collagen, enzymes [7, 16, 17], biodiesel and biogas [18–24], natural pigments [25], minerals [26, 27], hydroxyapatite [28], chitin and chitosan [29, 30], creatine and taurine [31, 32].

The possible valorizations of marine by-products can be divided into three main categories: production of marine proteins (fishmeal, silage and hydrolysates), oils rich in polyunsaturated fatty acids (PUFAs) and preparation of high value compounds such as vitamins, enzymes, minerals, taurine and creatine, hydroxyapatite, biodiesel and biogas for human and animal nutrition, industrial or pharmaceutical uses. In this review, we mainly present the sources of marine by-products, their characteristics, and the possible technologies that can be used to produce marine oils and concentrated n-3 fatty acids.

2. Marine by-products and their characteristics

There is no one definition of marine by-products. In the past, marine by-products have been often considered as fish offal or waste [5, 7]. Actually, the term by-products designates all unused parts that can be recovered during production operations. They designate viscera, heads, trimmings, bones, cartilage, tails, skin, scales, blood, shells, carcasses, or damaged fish. Depending on the fishing period, reproductive elements such as eggs, milt or soft roe may be among these by-products [33].

In some works, the definition of by-products was reserved for feed. In others, the terms fish waste [34–37], waste streams [38], and rest raw material [5] have been used. In all cases, the biomass of by-products can be used to generate an added value unlike waste which has to be composted, burned or destroyed [5].

Generally, by-products can result from all aquatic food processing industries on-shore or even during transformation on board. Marine by-products often constitute more than 50% of the body weight of processed fish [2, 4, 7, 39, 40]. However, this amount can reach up to 70% of the catch depending on catching species and area, postharvest conditions and industrial preparation processes [2, 7, 8, 11, 34, 41–44]. Processing operations like filleting, salting and smoking generate the most important amounts of by-products (50–75% of processed fish) [10], followed by the fish canning industry (30–65% of processed fish) and finally, the processing of crustaceans and mollusks [45]. It's estimated that the quantities of fish by-products generated by the processing industries will continue to increase due to the increasing demand for fishery products as source of valuable nutrients and a balanced diet for health [11, 45].

Knowledge of the properties of by-products allows their valorization into highly valuable products that could be higher in value than the fish fillets [8]. Analysis of the composition of by-products has revealed their richness in potentially valuable molecules such as proteins, essential fatty acids, oil, vitamins, minerals but also in bioactive compounds [4, 5, 34, 38, 46–48].

The by-products protein fraction is easily digestible and rich in essential amino acids. It can be used for production of peptides and amino acids, hydrolysates, gelatin and collagen, thermostable protein dispersions and protamine. While marine oils contain n-3 fatty acids [36, 37, 49–51], phospholipids, squalene, fat-soluble

vitamins, and cholesterol. Additionally, other valuable components can be extracted from marine by-products including nucleic acids, calcium, phosphorous, and hydroxyapatite [14, 28, 35] and other bioactive compounds such as astaxanthin [8], chitin and chitosan [25, 30], creatine and taurine [10, 15].

There are significant compositional differences between parts composing by-products [38]. In cases where the separation between the different parts of marine by-products is possible, the valorization will be optimal. For example, prioritizing the extraction of protein derivatives from the skin of the fish or oil from viscera and/or heads. Fatty fish by-products present an important raw material for the fish oil extraction industries especially during the high fat season. Aidos and co-authors studied the possibility of oil extraction and quality of oil from salted by-products of the maatjes herring (heads, frames, skin, viscera, etc.) by demonstrating that salt does not prevent the production of an oil of good quality [47]. A recent study showed that sardine cooking condensate and cooked by-products have a high potential for the recovery of oil with yields that can reach 32.9% during the fatty season [52].

The greatest valorization of these by-products depends on their handling according to the hygiene rules applied for food production [33]. Special care must be taken to maintain the temperature low during storage and transport to avoid alteration and to preserve their nutritional qualities as marine by-products are highly sensitive to degradation (oxidation, microbial spoilage and enzymatic reactions) [53].

3. Main valorization technologies of marine by-products

3.1 Production of marine oils

Marine oils are rich in PUFAs, especially, eicosapentaenoic acid (EPA, 20: 5 n-3) and docosahexaenoic acid (DHA, 22: 6 n-3) [52, 54–59]. These n-3 fatty acids have valuable benefits and medicinal properties. Numerous articles have described the benefits of n-3 fatty acids in regard to blood pressure, prevention and treatment of coronary artery disease [60, 61], atherosclerosis and thrombosis [62–64], hypertriglyceremia [64, 65], schizophrenia and memory [66], stress and depression [67] and foetal development [57, 68–71]. However, the most widely discussed benefits relate to cardiovascular health [61, 65, 72–79] and the prevention and treatment of inflammatory diseases [57, 80–82].

These fatty acids are of marine origin, found mainly in fatty fish and seafood. They are obtained by consumption of algae, fungi and phytoplankton [83]. However, certain human groups, such as premature babies and ill people, are unable to synthesize them. Even in people not belonging to these groups, the amount of EPA and DHA synthesized by the body may not be enough because the biosynthesis of these two acids becomes slow with age as well as with bad habits such as smoking, alcohol intake and poor fitness habits [11, 84]. In this case, a diet based on marine lipids (fish and its derivatives) provides the needed intake of EPA and DHA [85, 86].

Marine oils are mainly composed of mixtures of fatty acids esterified with glycerol in triacylglycerides [11]. They are the main natural source of n-3 PUFAs particularly, EPA and DHA [37, 50, 51]. **Table 1** summarizes some variation intervals of EPA and DHA in certain oils extracted from fatty marine by-products. The variation depends on type of by-products used, the species, the catching season and the processing technology used for extraction and purification.

Species	By-products	EPA	DHA	Process	Ref
<i>Sardinella maderensis</i>	Liver skin	4.7 20.5	4.8 4.2	Solvent extraction	[87]
<i>Sardinella aurita</i>	Liver skin	1.8 10.4	1.4 2.5		
<i>Cephalopholis taeniops</i>	Liver skin	1.6 3.1	1.1 6.9		
Cod (<i>Gadus morhua</i>)	Liver Viscera Trimming	8.6–11.4 10.6–12.6 14.2–16.5	11.8–16.2 20.0–25.6 30.4–33.8	Solvent extraction [88]	[89]
Saithe (<i>Pollachius virens</i>)	Liver Viscera Trimming	10.3–11.5 7.3–13.6 10.5–17.1	15.5–15.9 9.5–23.2 11.3–35.5		
Haddock (<i>Melanogrammus aeglefinus</i>)	Liver Viscera Trimming	13.1–14.8 11.7–12.0 14.6–16.6	15.2 23.2–23.7 33.3–35.7		
Tusk (<i>Brosme brosme</i>)	Liver Viscera Trimming	5.0 7.0 6.8	14.2 25.5 34.8		
<i>Sardina pilchardus</i>	cooked by-products	20.5–25.0	4.6–10.2	Batch hydraulic pressing	[52]
Tuna (<i>Thunnus obesus</i>)	Skins	4.2	23.6	CO ₂ supercritical extraction	[90]
	Scales	4.8	23.5		
	Bones	5.1	21.6		
	Skins	3.6	21.8	Hexane Soxhlet extraction	
	Scales	4.5	21.5		
	Bones	4.7	20.0		
<i>Sardina pilchardus</i>	Heads, gut content, fins	14.20	18.59	Wet reduction method	[91]
Skipjack tuna	Precooked heads	0.1	25.5	Wet reduction method	[92]
	Non precooked heads	0.1	18.8		

Species	By-products	EPA	DHA	Process	Ref
Hake,	Offcuts	—	—	CO ₂ supercritical extraction/Cold extraction/ Wet reduction/enzymatic extraction	[50]
Orange roughy,	Offcuts				
Salmon	Offcuts				
Jumbo squid	Livers				
<i>Sardinella lemuru</i>	Head	1.84	15.95	Solvent extraction	[37]
	Intestine	1.73	11.87		
	liver	2.76	12.97		
<i>Sardina pilchardus</i>	Heads	9.3	10.3	Enzymatic hydrolysis	[93]
Salmon	Frames without heads	9.3	11.3	Enzymatic hydrolysis	[94]
Black scabbardfish (<i>Aphanopus carbo</i>)	Heads, viscera, frames, skin, trimmings	2.7	6.2	Enzymatic hydrolysis	[95]
Sardine (Sardina pilchardus)	Heads, viscera, frames, trimmings)	—	—	Enzymatic hydrolysis	[96]
Salmon	Belly part	3.17	3.85	Pressing	[97]
	Belly part	4.45	3.62	CO ₂ supercritical extraction	
	Trimmed muscle	3.53	3.46		
	Frame bone	4.27	3.60		
	Skin	3.87	3.26	n-Hexane extraction	
	Belly part	3.12	3.23		
	Trimmed muscle	3.22	3.98		
	Frame bone	3.85	4.32		
	Skin	2.79	3.09		
Indian mackerel (<i>Rastrelliger kanagurta</i>)	Skin	11.91–12.31	13.15–14.47	CO ₂ supercritical extraction	[98]
		12.22	13.86	Soxhlet extraction	

Species	By-products	EPA	DHA	Process	Ref
Salmon	head, skin, viscera, backbone, frames, cuts off	2.46	2.97	Cold pressing	[99]
Salmon (<i>Salmo salar</i>)	Heads	7.7	11.9	Enzymatic hydrolysis	[100]
		8.4	12.1	Solvent extraction	
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Roe	11.3	19.0	Enzymatic hydrolysis	[101]
		11.5	24.0	Solvent extraction [88]	
Rainbow Trout (<i>Oncorhynchus mykiss</i>)	Bones with leftover fish meat, skin, scales, fins	6.49–6.89	14.76–5.72	Isoelectric solubilization/precipitation	[102]
Nile perch (<i>Lates niloticus</i>)	Viscera	3.0	9.0	Enzymatic hydrolysis	[103]
Nile perch (<i>Lates niloticus</i>)	Heads	3.4	7.7	Enzymatic hydrolysis	[104]
Salmon (<i>Salmo salar</i>)	Heads	6.1	8.4		
Sardine	Heads	10.95	13.01	CO ₂ supercritical extraction	[105]

Table 1.
EPA and DHA content (% of total fatty acid) in oil produced from marine by-products by different methods of extraction and purification.

Marine oils are produced from whole fish mainly small pelagic species, but also from by-products generated by the transformation industry. In 2018, it was estimated that between 25 and 35% of the total volume of fishmeal and fish oil produced came from by-products [2]. In production of marine oils, different techniques can be employed such as wet reduction process [52, 91, 92], solvent extraction [37, 87, 89, 106], supercritical fluid extraction [50, 90, 97, 98, 107, 108], urea complexation [108], cold pressing [99] and enzymatic treatment [9, 93–96, 101, 104, 109–112].

3.1.1 Wet reduction process

The traditional process for the production of marine oils is coupled with the production process of fishmeal. It is based on a heat treatment of the raw material which allows breaking the cell membrane to liberate the oil, pressing and separation [15, 106]. The principle of this process is based on the separation of the lipid phase from various fish compounds. The oil is separated by a decanter that separates insoluble compounds from the liquid phase, and a separator, separating oil and water. Another possibility is to use a tricanter, which separates solid, water phase and oil in one operation [8]. This process is mainly used in the treatment of fatty species containing high levels of fats such as anchovies, menhaden, sardines, Atlantic herring or their by-products [13].

On an industrial scale, the wet extraction technique is the most used method to obtain the oil and a substrate rich in proteins [106]. This process consists of 3 main stages:

Cooking, where the biomass is heated in a continuous screw cooker. Oil and water are separated from the solid protein. In order to recover an oil of high quality, the temperature and pressure must be adapted to the type and size of used biomass. Cooking can be done by direct injection of steam or by indirect steam heating in order to denature cell proteins and facilitate oil extraction [113, 114].

Pressing, a screw press squeezes the oil and water from the cooked biomass to separate the liquid phase containing the oil and the solid material. Press juice contains a considerable amount of suspended solids in the form of coagulated proteins, scales, edge fragments which have escaped the pressing. These particles are added to the fishmeal while the liquid phase undergoes centrifugation [115].

Centrifugation, which is now preferable compared to decantation, separates the oil from the aqueous phase. Draining and press water can be treated with live steam, allowing better separation of the oily phase on a horizontal centrifugal decanter [116]. In this step, water can be added also to wash the oil from any remaining impurities. At the end of this operation, the oil and stick water are obtained. The stickwater is evaporated to a concentrate before to add it to the pressing cake [36, 47, 117].

Quality and stability of the oil produced depend on process conditions. On one hand, the quality of oil depends on temperature, pumping speed and centrifugation. The marine oil is more stable if high pumping speed is used [118]. On the other hand, Chantachum and co-authors studied the influence of precooking on the separation and quality of tuna heads oil [92]. The results obtained have showed that cooking at 85°C for 30 min, followed by pressing at 140 tons/m² using a hydraulic press allows better release of the oily fractions which would be slowed down when cooked at a higher temperature due to the coagulation of proteins. The process conditions applied normally depend also on the type and quality of raw material used [117]. Comparison of quality of herring oils produced from three different types of by-products: heads, mixed by-products (heads, frames, skin, viscera, etc.), and headless byproducts showed that heads by-products and its oil presented the highest oxidation levels and the lowest R-tocopherol content. Heads contained the lowest PUFAs level and the highest amount of saturated fatty acids (SFAs) [55].

Oil recovery yield varies during the year depending on fat content of by-products. Extraction of oil from cooked by-products of *Sardina pilchardus* (skin, meat, bones and cooking condensate), using wet hydraulic pressing at 85°C for 30 min and centrifugation, gave an important oil yield varying between 6.0% and 32.9% depending on the fishing period [52]. Another study reported that the percentage of oil extracted from cooked by-products of *Sardinella gibbosa* was 8.96% [119].

Wet reduction process is more suitable to extract oil from fatty fish producing oil with improved quality and high level of n-3 PUFAs [50]. However, the main disadvantage of this technique is the use of high temperature during cooking that degrades oxidative quality of the oil and causes a loss of EPA and DHA contents due to hydrolysis and oxidation reactions [12, 111, 120].

In addition to the conventional extraction process called also wet reduction or hydraulic pressing, the extraction of marine oils could be obtained with several methods, such as enzymatic hydrolysis, physical fractionation, low-temperature solvent fractionation, supercritical fluid extraction and pH adjustment method to the isoelectric point (Table 1).

3.1.2 Enzymatic hydrolysis

The principle of enzymatic hydrolysis is based on the action of specific proteases at low temperature on protein tissue without use of solvents and high temperature [13, 50]. Firstly, the by-products are hydrolyzed by the use of commercial proteolytic enzymes and endogenous enzymes. These enzymes destroy the structure of cells walls. They broke down protein molecules to small peptides and amino acids which allows releasing the oil contained. After inactivation of the enzymes, the oil, hydrolysate and the insoluble residue are separated. The released oil can be centrifuged as previously described in the conventional process. For example, Batista and co-authors extracted oil from black scabbardfish (*Aphanopus carbo*) by-products using enzymatic hydrolysis with 1% Protamex [95]. The percentage of free oil released from the by-products has reached 36% of the total amount.

The enzymatic extraction is influenced by several operating factors, namely: nature of enzymes, temperature, pH, concentration of enzymes during hydrolysis, method and quality of grinding, and water content of the raw material [93, 110, 111, 120]. Optimal conditions of hydrolysis of sardine heads by Protamex were studied (temperature, hydrolysis time and enzyme-substrate ratio) [93]. Results have showed that optimum conditions were found to be similar to recover lipids and phospholipids (29 min, 31°C with 2.6 g.kg⁻¹ enzyme). The same study has showed that hydrolysis could increase the extraction of lipids and phospholipids by 27% and 50%, respectively compared to classical extraction.

Šližyte and co-authors studied the enzymatic hydrolysis of mixtures of cod (*Gadus morhua*) by-products using Flavoenzyme and Neutrase [110]. The results demonstrated that the most important factor influencing the extraction yield is the added water regardless the type of enzyme. On the other hand, using an enzymatic process based on a proteolytic extraction of oil from crude tuna heads followed by a urea complexation (−5°C, 20 h) has allowed to obtain a mixture of DHA and EPA with a purity of 85.02% and a liquid recovery yield of 25.10% [121].

The enzymatic hydrolysis of salmon frames with Protamex, was able to separate the salmon frames into an aqueous fraction rich in soluble nitrogen (fish protein hydrolysate), an insoluble nitrogen fraction, an emulsion fraction, salmon oil and a bone fraction rich in protein and minerals. This process allows to separate oil from protein fraction that can be valorized to recover peptides, essential amino acids or other molecules [94].

Batista and co-authors studied the use of 0.5% of three commercial enzymes (Alcalase, Neutrase, and Protamex TM) and a water/fish ratio of 1:1 for production of protein hydrolysates and oil from raw and cooked sardine by-products from the canning industry [96]. Results have showed that the highest nitrogen solubilization and degree of hydrolysis were obtained with Alcalase and Protamex. The raw by-products were more easily hydrolyzed by these enzymes than the cooked sardine. The highest percentage of oil released was obtained from raw sardine, and Alcalase and Protamex were the most efficient. In another study, enzymatic extraction of oil from ground salmon heads at 55°C using different commercial enzymes (Alcalase, Neutrase, and Flavourzyme) showed that the highest oil recovery (17.4%) was obtained after 2 h by using Alcalase [111]. Same results were obtained later by other researchers [100, 101].

The enzymatic process can be chosen to extract the oil for many reasons. First, it is conducted under mild conditions protecting PUFAs from oxidation. Second, this technique does not use chemical solvents in addition to short time of hydrolysis [120, 122]. However, this process focuses more on the yield and production of protein hydrolysates than on the production of high quality oil. This disadvantage is due to the presence of lipase in the by-products which still active during the hydrolysis therefore it affects the oil quality [96]. Furthermore, this effect is more important if the hydrolysis is done in the presence of oxygen which increases significantly the free fatty acids in the oil, compared with the conventional method [8]. The main product from this process is the protein hydrolysates that are produced with higher yields compared with the traditional process [8].

3.1.3 Supercritical carbon dioxide

Extraction with supercritical carbon dioxide (SC-CO₂) is a promising technique, as CO₂ is nontoxic gas, nonflammable and clean solvent [123]. This technique can be carried out under mild operating conditions in an oxygen-free environment and at a moderate temperature, preventing degradation of PUFAs [12, 50, 90, 97, 98, 105]. CO₂ used is a green solvent. At or above critical temperature and pressure (31.1°C, 7.39 MPa), CO₂ is in a liquid state while at ambient temperature and pressure, CO₂ becomes a gas and evaporates [120].

This technique allows the extraction of lipids of low polarity, avoids the extraction of impurities and reduces the heavy metal content [12, 50, 90]. Rubio-Rodriguez and co-authors proposed to couple this technique with extraction-fractionation process to remove free fatty acids and improve fish oil quality, alternatively to physical and chemical refining [50]. Same authors compared different oil extraction methods from fish by-products, cold extraction or centrifuging, wet reduction, enzymatic extraction and supercritical fluid extraction. This study has showed that SC-CO₂ is an interesting method, operating conditions are suitable to prevent lipid oxidation and to reduce the amount of certain pollutants such as some arsenic products [50].

Another research has also compared 3 different oil extraction methods (supercritical carbon dioxide, n-hexane and traditional pressing) from Atlantic salmon by-products (belly part, trimmed muscle, frame bone and skin). The maximum oil yield was obtained by n-hexane extraction (total oil), followed by supercritical CO₂ extraction (highly selective technique extracting non-polar compounds) and the traditional pressing that has showed the lowest yield. Likewise, differences were noted in the oil quality parameters between the 3 studied techniques, the longer oxidative stability was obtained in the oil extracted by supercritical fluid CO₂ [97].

This technique was also compared to soxhlet extraction using hexane to produce oil from skins, bones and scales of bigeye tuna (*Thunnus obesus*). This study

has confirmed improved quality parameters of oil obtained by supercritical CO₂ technique extraction (low heavy metal content in the oil) [90].

Using the ground skin of Indian mackerel (*Rastrelliger kanagurta*), various techniques of supercritical CO₂ were studied by varying pressure (20-35 MPa) and temperature (45–75°C). This study has showed that oil yield increased with pressure and temperature and the highest yields were 24.7, 53.2, 52.8, and 52.3/100 g sample (dry basis) for the continuous, cosolvent, soaking, and pressure swing techniques, respectively, at 35 MPa and 75°C [98].

Supercritical fluid technology coupled with membrane, enzymatic or adsorption process have been shown to produce high-quality oil with best reduction of levels of contaminants compared to traditional refining of oils [124, 125].

Faced with all these advantages, the main drawback of this method is the high cost of the application on an industrial scale [12, 50, 124]. In this contest, the effect of supercritical CO₂ techniques on CO₂ consumption was studied. The results have showed that the total amount of CO₂ consumption decreases significantly with temperature and increases with pressure in all extraction modes using supercritical CO₂ method. A higher amount of CO₂ was needed for the continuous technique, compared to the techniques of cosolvent, soaking and pressure swing regardless of levels of pressure and temperature. Consequently, the best extraction technique of the oil with least amount of CO₂ consumption was achieved with pressure swing mode at 35 MPa and 75 C [98].

3.1.4 Solvent extraction

Solvent extraction methods are numerous. They have been studied by several researchers applied on marine by-products [37, 87, 89, 106]. Unfortunately, these techniques have many disadvantages. Large amount of hazardous solvent and important energy are required. Besides, marine oils are oxidized exhibiting a strong red-brown or brown color when extraction of oil is done at high temperature and longtime [90]. Generally, extraction by solvent is only carried out on a laboratory scale for analytical purposes [124]. Among the widely used techniques, Bligh and Dyer method is the most recommended for the total extraction of lipids from biological tissues [126]. Most of the published data on lipid content are related to this method [105, 127–129]. The effectiveness of the Bligh and Dyer method was evaluated compared to Soxhlet method. The results have showed that the Bligh and Dyer extraction method is more effective in extracting polar and non-polar lipids from fish compared to the Soxhlet technique [130].

3.1.5 Cold pressing extraction

Other innovative processes are coming to the market such as cold pressing extraction, a patented process originating from the olive oil production industry [13]. This technique allows protection of PUFAs content, producing high quality marine oil from different types of by-products [50, 99]. This process is well known to produce a lower-yield, but higher quality of oil [131].

Due to its high degree in PUFAs, marine oils are very sensitive to oxidation. The degree of oxidation increases in the presence of air (oxygen), light and heat during extraction and storage [8, 47]. This phenomenon mainly reduces the shelf life of marine oils [53]. All techniques using high-temperature or toxic solvents can induce degradation and loss of nutritional qualities of marine oil.

For this, looking for gentle extraction of marine lipids or using non-heat processes might generate more stable lipid fractions [8]. It is also necessary to protect the oil by stopping or slowing down the oxidation process during the production

and during storage. Adding antioxidants to the oil is one of the most used methods [132]. Using a modified atmosphere packaging [133], or encapsulation, which keeps marine oil away from oxygen and light [133, 134] can be also used. In addition to protecting the oil, the use of microencapsulation technology provides consumers with supplements n-3 fatty acids ready to consume.

3.2 Production of n-3 fatty acids concentrates

Another valorization of marine oils (produced from whole fish or from fish by-products) is their use in production of concentrated n-3 fatty acids in the form of free fatty acids, methyl and ethyl esters or acylglycerols [135]. Several processes can be used, the most important are urea complexation [108, 121, 136], molecular distillation [137], supercritical fluid extraction [98, 108], winterization [138, 139], fractionation by chromatography [120] and by enzymatic processes [111, 112, 140]. These techniques have been reviewed by many authors [13, 124, 135] and recently by [12]. The main challenge in the choice of concentration technique at industrial level is to reach higher yield and purity at lower cost [13, 124, 138]. **Table 2** outlines some methods to produce n-3 fatty acids concentrates with levels achieved of enrichment in EPA and DHA.

Concentration by winterization allows elimination of SFAs present in the oil, which crystallize at low temperatures [139]. Winterization is primarily designed for oils with a high content of SFAs. It allows elimination of stearic phase, by cooling the oil to 0–4°C. The degree of concentration of PUFAs by this process is evaluated as low as these interesting fatty acids could be lost in the stearic fraction [13]. However, this method produces n-3 PUFAs concentrate in natural form [138].

An alternative solvent winterization and enzymatic interesterification was studied to concentrate n-3 fatty acids in cod liver oil [138]. The optimization parameters considered were separation method, solvent, oil concentration, time and temperature of winterization. Likewise, enzymes used were examined for interesterification efficiency under different system air condition, time and temperature. Authors proposed the optimal conditions of the technique via winterization (0.1 g/mL oil/acetone, 24 h, –80°C, precooled Büchner filtration) and interesterification (Lipozyme TL IM, N₂ flow, 2.5 h, 40°C) improving n-3 fatty acid content to 43.20 mol%.

In another study, winterization was carried out on a bleached oil by a progressive cooling (30–5°C) in three phases. The effect of solvent type, solvent proportion, and agitation in the second cooling stage was studied. The results have demonstrated that using hexane has improved content of PUFA of 64.3% with 13% as a decrease percentage in level of SFAs compared to the fatty profile of bleached oil [139].

In addition to what is explained previously for CO₂ supercritical fluid extraction, this technique could be used to concentrate fatty acids. It's based on the use of CO₂ which, in the supercritical state, behaves like an extraction fluid and entrains fatty acids. In several passages, their concentrations therefore increase [13]. This method can achieve high concentration levels of n-3 PUFAs.

Supercritical carbon dioxide was used for simultaneous extraction and fractionation of fish oil from Tuna by-products [143]. The obtained oil was divided into six fractions based on molecular weight and the chain length of triglycerides in terms of fatty acid constituents. The results showed that the three first separated fractions were rich in SFAs followed by monounsaturated fatty acids (MUFAs), then PUFAs. While the three last fractions contained high levels of MUFAs and PUFAs.

Melgosa and co-authors studied the use of supercritical CO₂ as solvent in the lipase-catalyzed ethanolysis of fish oil. The effect of initial substrate ethanol/oil molar ratio (2–38), pressure (7.5–30 MPa), and temperature (323.15–353.15 K) on equilibrium conversion, reaction rate and oxidative status of the products were

Species	Type of oil	EPA	DHA	Process	Ref
Tuna (<i>Thunnus albacares</i>)	Tuna oil	EPA + DHA (85.02%) recovery 25.10%		Concentration by urea complexation	[121]
Rainbow sardine (<i>Dussumieria acuta</i>)	Oil from white muscle	15.39	17.45	Extraction [88]	[108]
	Concentrate oil	—	—	CO ₂ Supercritical extraction	
		19.47	29.61	Urea complexation	
		17.74	25.51	Low-temperature crystallization with ethanol	
Pacific sardines (<i>Sardinops sagax</i>)	Crude oil from Skin-on fillets)	28.2	16.7	—	[140]
	Refined unhydrolyzed oil	26.86	13.62	Extraction and refining	
	Concentrate oil	33.74	29.94	Lipase-catalyzed hydrolysis	
Atlantic salmon (<i>Salmo salar</i>)	Crude Salmon by-products oil	3.71	9.02	—	[112]
	Salmon oil 40% hydrolysis	4.72	8.94	Lipolysis, filtration	
	Permeate	5.16	9.91		
	Esterified permeate	5.64	10.30	Enzymatic re-esterification	
Salmon	Oil from salmon heads	3.6	9.9	Enzymatic hydrolysis	[111]
	Permeate	5.6	11.6	Lipolysis	
	Permeate re-esterified	5.06	11.90	Re-esterification	
Arctic cod	Arctic cod liver oil	10.53	7.63	—	[138]
	Winterized oil	21.08	17.83	Alternate solvent winterization and enzymatic interesterification	
Tuna (<i>Thunnus thynnus</i>)	Tunafish oil	4.6	18.3	—	[141]
	Tunafish oil ethyl ester	5.3	23.7	Supercritical fluid chromatography	
Menhaden	Menhaden oil	13.5	12.6	—	[136]
	Menhaden oil after urea inclusion	29.4	41.8	Urea inclusion method	
Sardine (<i>Sardinops sagax caeruleus</i>)	Refined oil	14.51	12.55	—	[142]
	n-3 PUFAs concentrate	34.17	39.47	Chemical hydrolysis + urea complexation	
	n-3 PUFAs concentrate	46.26	40.32	Enzymatic hydrolysis + urea complexation	

Table 2.
Some methods of production of concentrated n-3 fatty acids in oil.

tested. The results have revealed the importance role of employing CO₂ in improving reaction kinetics by reduction of mass transfer limitations and prevention of n-3 PUFAs oxidation due to displacement of oxygen [144].

The results of supercritical fluid extraction applied on Rainbow sardine oil have showed that the highest decrease in SFAs and MUFAs were obtained at 50–60°C and 350 bars [108]. This technique was evaluated selective even in the fractionation of fish oil with lower content of EPA (4.6%) and DHA (6.7%) under conditions of lower pressure (100 and 200 bar) [145]. In another study, conditions (pressure, temperature and supercritical CO₂ flow rate) influencing concentration of fatty acids in fish oil by supercritical carbon dioxide were studied [146]. The results have demonstrated that fractionation by a supercritical fluid under optimal conditions: a pressure increase (at 5 kg/h flow rate) and flow rate increase (at 150 bar pressure), both determined a higher EPA + DHA concentration and decreased the EPA/DHA ratio. The same authors proposed to carry out a urea adduction during preparation to decrease the amount of SFAs in the starting oil before supercritical fractionation.

With regard to optimization conditions, numerous researches have worked on modeling, simulation, and optimization of the CO₂ supercritical fractionation of EPA and DHA esters in fish oil [147, 148].

Optimal conditions of application by using adjuvant material and modifying CO₂ volumetric density and temperature were also investigated in other studies to get the highest fractionation yield of EPA and DHA [149]. Likewise, Antunes-Corrêa and co-authors and Davarnejad and co-authors studied the optimal operating conditions (pressure and temperature) to fractionate fish oil. In the first study [150], the best results based on oil solubility were obtained using 7.8 MPa and 301.15 K. While in the second research [151], the maximal solubility of the fish oil (0.921 g of oil/100 g of CO₂) was obtained at optimum conditions of 40°C and 27.2 MPa. In both studies, EPA fractionation was recorded not possible and low, respectively.

The experience of CO₂ supercritical fluid chromatography was transferred to be used in laboratory in a pilot plant to produce EPA enriched mixtures. Fractionation was done on a silica adsorption column using CO₂ as supercritical solvent [152]. This allowed to achieve best purity of 93% in EPA ethyl ester fraction with a 24.6% yield. The study of the technical and economic feasibility to produce n-3 PUFAs ethyl ester concentrates from trans esterified fish oil using CO₂ supercritical fluid extraction has revealed that process cost is around 550 U.S \$/kg DHA and EPA ethyl ester concentrate [141].

Other investigations have studied the use of enzymatic hydrolysis in production of n-3 PUFAs concentrates. This technique involves the use of specific enzymes (lipases), able to catalyze reactions such hydrolysis, ethanolysis or transesterification of triglycerides [124].

Concentration of Pacific sardines (*Sardinops sagax*) oil was carried out using lipase-catalyzed hydrolysis [140]. The results of this study have showed that hydrolysis with 250 U from *Candida rugosa* lipase has increased EPA concentration to a relatively constant level of 33.74% after 1.5 h. DHA levels were also significantly increased from 13.62% to 29.94% with 500 U after 9 h. This technique uses mild conditions (neutral pH and low temperatures), very important to preserve EPA and DHA from oxidation [140, 153].

Salmon oil produced from by-products of this species by controlled enzymatic procedure with Neutrase has followed a selective enzymatic hydrolysis under mild conditions, using Novozyme SP398 to enrich the n-3 PUFAs. The process used consist of a lipolysis, filtration in flat membrane device and enzymatic re-esterification with glycerol and Immobilized 1,3-specific lipase IM60 (Lipozym IM). This method induced a significant increase in the amount of PUFAs from 39.20 mol% of total fatty acids in the crude oil to 43.29 mol% in the re-esterified permeate [112].

The proteolytic extraction of oil from salmon heads using three different types of enzymes (Alcalase, Neutrase and flavourzyme) and the lipolysis of this oil to concentrate PUFAs were carried out. Lipolysis was done with Novozym SP398 to obtain a mixture of free fatty acids and glycerol (24 hours 45% hydrolysis). The mixture was then filtered. This process has allowed an increase of the PUFAs content from 41.6% in the crude oil to 46.5% in the permeate. Likewise, DHA and EPA percentages have increased from 9.9% to 11.6%, and from 3.6 to 5.6%, respectively [111]. The same authors used a re-esterification in the permeate with Lipozyme IM which permitted obtention of 5.06% and 11.90% in EPA and DHA contents, accordingly [111]. Moreover, other authors proposed combination of enzymatic or chemical hydrolysis with urea complexation to produce high concentrates of n-3 PUFAs. The enzymatic hydrolysis followed with urea complexation of refined sardine oil has increased the level of EPA and DHA from 14.51% to 46.26%, and from 12.55% to 40.32%, respectively [142].

Another technique, short path distillation was tested to purify Alaskan Walleye Pollock (*Gadus chalcogrammus*) and New Zealand Hoki (*Macruronus novaezealandiae*) liver oils [154]. Certainly, this process has reduced free fatty acids and lipid oxidation parameters, which is appreciated to produce purified oils. Consequently, the conduct of this operation at high temperatures may cause degradation of PUFAs or development of new undesirable compounds. The short path distillation was coupled to a previous enzymatic glycerolysis of sardine oil with glycerol [155]. This work showed that short path distillation is able to concentrate n-3 PUFAs in monoacylglycerols at suitable evaporator temperature (125°C) Same technique aided by a working fluid was evaluated efficient in removal of persistent organic pollutants in marine oils (PCDD/PCDF, dl-PCB and ndl-PCB) [156].

When comparing the effect of using urea complexation on the concentration yield compared with dry fractionation and low temperature solvent crystallization, results revealed that n-3 fatty acids were enriched in liquid fractions of all methods except by dry fractionation. The highest enrichment was achieved with the urea complexation method (83.00%) [157]. In the same context of valorization of marine by-products, application of urea crystallization on tuna oil recovered from liquid waste by-product from a tuna canning process allowed an increase in the concentration of n-3 PUFAs [158]. In another study conducted on concentration of fatty acids in sardine oil, the highest PUFA concentrations in low-temperature crystallization with ethanol were attained at -5°C, with EPA and DHA purities equivalent to 17.74 and 25.51%, respectively [108].

These authors also compared three different concentration techniques, supercritical fluid extraction (T = 40, 50, 60°C and 150, 250, 350 bar), Urea complexation (T = 1, -5, -10°C) and low-temperature crystallization with ethanol solvent (T = 10, 0, -5°C). The optimal conditions for each technique were determined. Nevertheless, the highest reduction of SFA and MUFA, the best increase in PUFA and the highest n-3 yield (47.53%), were obtained at -10°C in urea complexation method [108].

There are still several techniques used for the concentration of n-3 PUFAs, among which there is the use of polymeric membrane separation [159]. Optimal conditions of this method were found to be at the temperature of 36.19°C, pressure of 4.82 bar and stirring rate of 43.01 rpm with a desirability value of 0.99. With these conditions, a concentration of n-3 PUFAs of 34.98% was achieved.

Synthesized poly-vinylidene fluoride (PVDF) asymmetric membranes are also tested in concentration of n-3 PUFAs [160]. Conditions of preparation of PVDF membranes influences significantly results. In this work, PVDF membrane prepared at a coagulation bath temperature of 0°C resulted in the best n-3 PUFAs enrichment (40.4%) at 5 bar and 30°C.

4. Conclusion

Marine by-products (viscera, heads, trimmings, bones, cartilage, tails, skin, scales, blood, shells, carcasses, damaged fish, eggs, milt or soft roe), generated by marine transformation industries, constitute a good opportunity of valorization into highly valuable products. Their characterization determines the choice of the most suitable and efficient valorization method among all possibilities available, production of marine proteins (fishmeal, silage and hydrolysates), oils rich in polyunsaturated fatty acids (PUFAs) and preparation of high value compounds such as vitamins, enzymes, minerals, gelatin, collagen, chitin and chitosan, taurine and creatine, hydroxyapatite, natural pigments, biodiesel and biogas.

In this context, several studies have been carried out to explore possible technologies that can be used in the valorization of the marine by-products into marine oils and concentrated fatty acids. In addition to the conventional extraction process called also wet reduction process or hydraulic pressing, solvent extraction, supercritical fluid extraction, urea complexation, cold pressing or enzymatic hydrolysis processes could be used to transform these by-products into marine oils highly rich in PUFAs very demanded by food, nutraceutical and pharmaceutical industries.

For more advanced enhancement, the concentration of fatty acids in marine oils is also widely practiced. Several techniques can be used such as winterization, urea complexation, short path distillation, supercritical fluid extraction, low temperature solvent crystallization, fractionation by chromatography or by enzymatic processes. Combined methods were also tested like solvent winterization and enzymatic interesterification, urea adduction before a supercritical fractionation. Many studies have focused on comparison between these techniques to provide differences, advantages, disadvantages, or even optimal conditions of operating.

The main challenge in the choice of extraction and concentration techniques at industrial level is to reach higher yield, purity, quality, stability at lower cost and low unwanted environmental effects.

Conflict of interest

The authors declare that there is no conflict of interest.

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