Sustainable Food Technology

ROYAL SOCIETY OF CHEMISTRY

REVIEW

View Article Online
View Journal

Cite this: DOI: 10.1039/d5fb00888c

Advances in fish oil extraction and enrichment: a comprehensive review of conventional and green technologies for high-quality omega-3-rich oil production

Aseel Swaidan,[a] Federica Grasso,[a] Filippo Falco,[a] Federica Turrini *[ab] and Raffaella Boggia[ac]

Omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), are well-known for their therapeutic potential, especially in their cardiovascular, anti-inflammatory, and neuroprotective roles. The primary dietary source of these bioactive compounds is fish oil. However, extraction methods require careful attention to ensure the production of high-quality omega-3 rich oil, while also aligning with green chemistry principles, environmental sustainability, and growing market demands, all of which place industries under pressure to balance between efficiency, safety, and cost. Conventional extraction methods, including mechanical (*e.g.*, wet pressing and cold pressing) and solvent-based approaches (*e.g.*, Soxhlet, Folch, Hara–Radin), are often associated with the oxidation and degradation of thermolabile compounds like omega-3s, in addition to posing environmental and occupational hazards. As a result, a shift toward green and advanced extraction techniques (*e.g.*, ultrasound-assisted, microwave-assisted, enzymatic-assisted, and supercritical fluid extractions) offers a more sustainable alternative, although their industrial application remains limited due to high operational costs and scalability challenges. However, even with milder green technologies, losses of EPA, DPA, and DHA still occur, necessitating an enrichment step to meet regulatory standards and consumer demand for omega-3-rich supplements. Current enrichment strategies such as molecular distillation, membrane-based filtration, and enzymatic purification, have undergone various improvements but still struggle with some challenges that limit their application, such as low DHA selectivity, process optimization, and enzyme instability. This review provides a comprehensive overview of conventional and green extraction methods, as well as enrichment strategies, highlighting their principles, applications in fish oil, advantages, limitations, and industrial feasibility. Additional research is required to further improve these technologies and address current limitations, with the goal of integrating them into scalable systems that support the production of high-quality omega-3-rich oil. This is also particularly crucial when combined with the upcycling of fish by-products, offering a more sustainable and effective approach that aligns with green chemistry and circular economy principles.

Received 7th November 2025
Accepted 21st March 2026

DOI: 10.1039/d5fb00888c

rsc.li/susfoodtech

Sustainability spotlight

The present review stems from the EcoeFISHent project ("Demonstrable and replicable cluster implementing systemic solutions through multilevel circular value chains for eco-efficient valorisation of fishing and fish industry side-streams"), funded under the Horizon 2020 Green Deal – Innovation Action (Grant Agreement ID: 101036428). This review advances sustainable innovation in marine bioresource processing by critically evaluating extraction and enrichment technologies for omega-3 fatty acids (EPA and DHA) from fish oil. It promotes an integrated approach that combines green extraction methods with downstream purification strategies to enhance product quality and reduce environmental impact. By emphasizing the valorization of fish by-products, the manuscript supports circular economy models and aligns with green chemistry principles, contributing to more responsible and scalable production of high-value omega-3 ingredients. The approach supports circular economy principles and aligns with UN SDGs: SDG 12 (Responsible Consumption and Production), SDG 9 (Industry, Innovation and Infrastructure), SDG 14 (Life Below Water), and SDG 3 (Good Health and Well-Being).

[a]*Department of Pharmacy, University of Genova, Viale Cembrano 4, 16148 Genova, Italy*

[b]*National Center for the Development of New Technologies in Agriculture (Agritech), 80121 Napoli, Italy. E-mail: Federica.turrini@unige.it*

[c]*National Biodiversity Future Center (NBFC), 90133 Palermo, Italy*

1. Introduction

In recent decades, consumer interest in functional foods and naturally derived bioactive compounds has increased, driven by

growing awareness of the therapeutic potential of nutraceuticals.[1] This rising demand has inspired various researchers, particularly those interested in the prevention and management of chronic diseases through nutraceuticals. Among these compounds, fish oil—rich in long-chain omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—has gained considerable attention due to its proven properties, including cardiovascular, antihypertensive, and anti-inflammatory.[2–4] These omega-3 PUFAs are considered essential, as they can't be synthesized endogenously in sufficient amounts and therefore must be obtained from dietary sources to achieve the recommended intake levels.[5]

Fish and fish oil are among the richest natural sources of omega-3 fatty acids, especially EPA, DPA, and DHA.[5] Conventional lipid extraction methods such as wet pressing, cold pressing, Soxhlet, maceration, Folch, Bligh and Dyer, Smedes, and Hara–Radin, have been employed for a long time to recover oil from fish and its by-products (Fig. 1). However, these techniques often involve prolonged exposure to high temperatures and large amounts of toxic organic solvents. Such conditions can degrade thermolabile bioactive compounds and alter the nutritional quality as well as the oxidative stability of the recovered oil. Additionally, solvents like hexane, chloroform, isopropanol, and petroleum ether employed in these methods pose significant occupational and environmental hazards, including mutagenicity, carcinogenicity, and ecosystem contamination, which contribute to climate change and exacerbate global warming.[6]

In response to these limitations, green extraction technologies, such as Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE), Enzymatic-Assisted Extraction (EAE), Supercritical Fluid Extraction (SFE), Pulsed-Electric Field (PEF), Deep Eutectic Solvents (DES) and Pressurized Liquid Extraction (PLE), have emerged as sustainable alternatives (Fig. 1). These methods offer several advantages over conventional ones, as they require less solvents and operate under milder conditions and shorter times, thereby preserving the bioactive integrity of the oil.[7] Nowadays, these techniques are increasingly applied in the pharmaceutical, food, and cosmetic sectors, aligning with the principles of green chemistry and environmental sustainability. However, their implementation in small-scale or low-budget industries remains limited due to high equipment costs and the need for process optimization.[8,9]

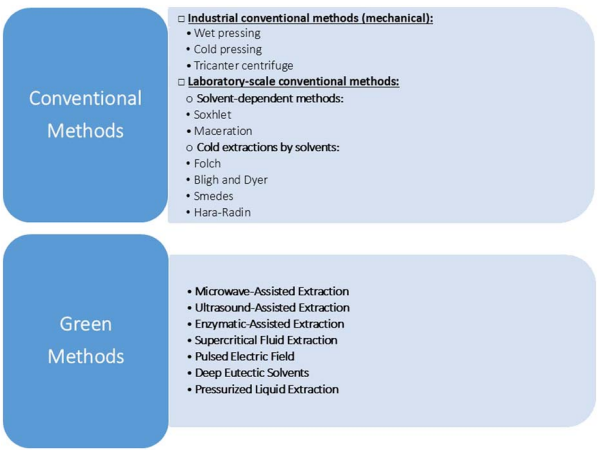

Fig. 1 Summary of conventional and green lipid extraction methods for fish oil recovery.

Among the different bioactive components of fish oil, EPA, DPA, and DHA are commonly recognized for their therapeutic potentials. Although α-linolenic acid (ALA), a precursor to EPA, DPA, and DHA, is commonly found in plant sources like flaxseeds, chia seeds, and walnuts, its conversion efficiency is often limited in the human body (approximately 4–8%). Therefore, direct dietary intake of these PUFAs is deemed essential.[10] The American Heart Association (AHA) recommends the consumption of fatty fish at least twice per week, providing a daily intake of 250–500 mg of EPA and DHA for healthy adults. For patients with coronary heart disease (CHD), an intake of 1 g per day of EPA and DHA is advised, while in patients with hypertriglyceridemia higher doses of 2 g up to 4 g per day are recommended.[11] However, omega-3 content in fish varies significantly depending on species, diet, and habitat (wild *vs.* farmed), and the extraction and quantification methods employed. Consequently, fish oil supplements are commonly marketed to support dietary intakes and ensure meeting the recommended levels to mitigate any risks associated with omega-3 deficiency.[12]

The rising global demand for omega-3 products has placed increasing pressure on marine ecosystems, raising concerns regarding overfishing and resource depletion. Aligning with zero-waste and circular economy concepts, the upcycling of fish by-products, including viscera, heads, skins, and bones, has emerged as an innovative, cost-effective, and sustainable strategy to reduce waste and maintain consistent omega-3 supply.[8]

In this context, the integration of innovative green technologies along with the upcycling of omega-3-rich fish by-products offers a dual opportunity: it supports circular economy concept and environmental sustainability while providing a cost-effective and continuous supply of high-quality fish oil.[13] However, to further enhance the nutritional and therapeutic value of the final product, enrichment with omega-3 fatty acids, especially EPA and DHA, might be necessary, particularly in formulations intended for therapeutic applications.[14]

While several studies have discussed fish oil extraction techniques and omega-3 enrichment, few have provided a comprehensive comparison between conventional and green extraction methods, with particular focus on the oil yield, oxidative stability, and nutritional quality, mainly in terms of EPA and DHA omega-3s. Moreover, the literature often considers extraction and enrichment as separate strategies, lacking an integrated perspective on how extraction techniques, even the optimized green alternatives to conventional methods, require a next step for subsequent purification and enrichment strategies. Therefore, this review aims to provide a comprehensive and detailed evaluation of conventional and emerging green techniques for fish oil extraction, highlighting their impact on fish oil quality, and shedding light on the current

enrichment approaches to provide a holistic overview of the available literature discussing the approaches and challenges of producing high-value, omega-3-rich fish oil.

2. Extraction of fish oil

Fish oil is usually extracted from fatty marine species such as sardines, mackerels, salmon, tuna, and anchovy, which provide natural sources of long-chain omega-3 PUFAs, especially EPA, DPA, and DHA. The quality of the extracted oil is highly dependent on the lipid content and composition of the raw material, as well as on the extraction method and operational conditions applied.[8,12] According to Codex Alimentarius Standards, high-quality fish oil is generally characterized by: low acidity (0.5 to 3.0 mg KOH per g), indicating low levels of free fatty acids due to minimal hydrolysis; low peroxide value (PV) (0.5 to 5.0 meq O_2 per kg), reflecting limited primary oxidation; and a low *p*-anisidine value (AV) (<20), showing reduced secondary oxidation.[15] In addition, elevated concentrations of omega-3 fatty acids further enhance the oil's nutritional and therapeutic value.[10]

However, achieving such quality requires more than lipid-rich raw materials, as the extraction method must also preserve sensitive compounds and minimize oxidation reactions. Conventional techniques often rely on high temperatures, prolonged processing times, and excessive volumes of organic solvents.[16–20] Such harsh conditions can accelerate lipid oxidation and degrade sensitive constituents such as omega-3 fatty acids, thereby compromising the oil's nutritional profile.[6]

To address these limitations, green extraction methods have emerged as sustainable alternatives. Through working under milder conditions, these methods improve oil yield and quality while reducing solvent usage and environmental impact.[21,22] The following sections provide a comprehensive overview of conventional and green extraction methods for extraction of fish oil, with particular emphasis on their influence on oil yield, oxidative stability, and nutritional composition.

2.1. Conventional extraction methods

Conventional extraction methods have long been used for fish oil recovery. These techniques can be classified based on their scale of application, as either industrial or laboratory. This distinction is crucial for understanding the purpose, design, and practical relevance of the methods. Industrial-scale techniques are applied to produce large volumes of oil. Therefore, industries often prioritize cost-effectiveness, continuous and rapid operation, while also ensuring compliance with regulatory standards to deliver a safe and nutritious product for human consumption. Industrial-scale methods are generally mechanical, including wet pressing, cold pressing, and tricanter centrifugation, which rely on mechanical force and applied pressure for oil release and phase separation. These methods are easy to scale and integrate into large-automated industries because they avoid the use of organic solvents, which is a priority when considering industrial scalability, safety, and regulatory compliance, where solvent handling at the laboratory scale poses environmental, occupational, and food safety concerns. However, these methods are costly and require large investment as well as large processing space industries.[23,24] On the other hand, laboratory-scale methods are primarily used for analytical purposes or small-scale extractions, where the objective is to recover lipids for further analysis or characterization rather than high-volume production. These methods often involve high use of organic solvents and time-controlled protocols, and are considered essential for research, quality control, and formulation studies, but are impractical for application at industrial and large-scale production. Laboratory-scale methods can be further divided into two subcategories: solvent-dependent and cold extractions by solvents. Solvent-dependent approaches, including Soxhlet and maceration, utilize organic solvents to dissolve and extract lipids.[7,25] While cold solvent extraction methods, such as Folch, Bligh and Dyer, and Hara–Radin also use organic solvents, they are designed to work under ambient temperatures, thereby avoiding the high thermal treatment which minimizes the degradation of thermolabile compounds like omega-3 and preserves oil oxidative stability.[17–19,26,27] These methods remain the foundation for fish oil extraction, specifically in the industrial and pharmaceutical fields.

2.1.1. Industrial mechanical extractions: wet pressing and cold pressing. Mechanical extraction methods, particularly wet pressing, cold pressing, and tricanter separation, are among the widely applied methods for the extraction of fish oil. The methods differ in terms of their heat utilization, thereby causing some variations in oil yield, oxidative stability, and composition. Although these techniques are widely employed at an industrial scale, several studies have explored simplified or pilot-scale versions of wet and cold pressing to assess the influence of processing parameters on oil quality and composition, and to compare these methods with alternative extraction approaches.

Wet pressing, also known as wet rendering, mainly involves three steps: cooking, mechanical pressing, and oil recovery following the separation of oil and water. This method is commonly used by most industries that produce fish oil across the world due to its efficiency and scalability.[28,29] The process starts by heating fish or fish-byproducts at temperatures ranging from 85 °C to 121 °C to coagulate proteins and ease the release of lipids. This is followed by mechanical pressing of the heated sample, and then the resulting liquid is subjected to separation techniques such as centrifugation, decanter systems to remove fine solids, and separators that isolate fish oil from water (Fig. 2). While this method appears to be relatively simple and solvent-free, high temperatures can compromise oil quality through oxidation and degradation of heat-sensitive compounds such as EPA, DPA, and DHA.[30]

Different studies have applied this method to recover oil from fish samples. For example, Lakmini *et al.* (2022) extracted oil from yellowfin tuna heads by autoclaving at 121 °C for varying durations (15, 30, and 45 minutes), followed by manual pressing and centrifugation at 13 000×*g* at 48 °C for 10 minutes. The highest yield (5.37%) was achieved after 30 minutes. The quality and oxidation parameters of the oils were analyzed.

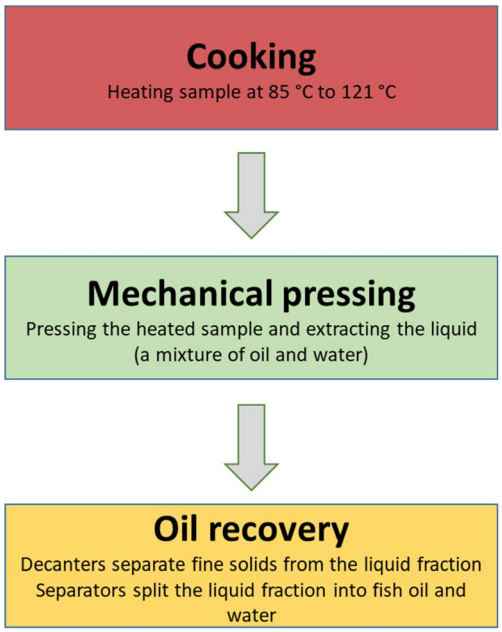

Fig. 2 Wet rendering process for oil recovery.

While PV remained stable across the different times, acidity, AV, and total oxidation value (TOTOX) increased significantly with prolonged heating, reflecting progressive oxidation with increased temperatures. The fatty acid analysis of the extracted oils revealed high PUFA content exceeding those of monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA), where DHA appeared to be the predominant fatty acid.[31] Similarly, another study applied wet pressing to Tilapia (*Oreochromis niloticus*) fish by-products, achieving a maximum yield of 6.44% at 70 °C after 35 minutes. PV, AV, and TOTOX values remained within acceptable limits, implying minimal occurrence of oxidation and acceptable oil quality. EPA and DHA quantification revealed values of 1.15% and 1.03%, respectively.[32] In addition, Chakraborty and Joseph (2015) reported an 8.3% yield from *Sardinella longiceps* using a pressure cooker at 75 °C for 30 minutes. The resulting oil showed high oxidation markers indicated by TOTOX value of 39.96.[23] Furthermore, Rahman *et al.* (2023) extracted Pangus (*Pangasius pangasius*) fish oil by heating the sample at 105 °C for 30 minutes, yielding 19.247% of oil. Although the acidity value (1.523 mg KOH per g) and PV (3.308 meq per kg) were acceptable, EPA (0.214 mg/100 g), DPA (0.618 mg/100 g), and DHA (0.103 mg/100 g) contents were reported as quite low. However, the authors contributed these results with the possible species-specific lipid profile along with the possibility of thermal degradation of these compounds at 105 °C.[33]

On the other hand, cold pressing applies mechanical pressure on fish samples without the use of heat, relying solely on the physical force for oil extraction. The ambient temperature adopted provides an advantage over the wet pressing method, as it helps to preserve the heat-labile compounds and minimizes oxidation, often resulting in better oil oxidation status as well as favorable nutritional value with better retention of omega-3 fatty acids.[34]

For instance, Fouda (2022) applied cold pressing for 200 minutes to various salmon by-products (head, skin, viscera, backbones, trimmings, and cutoffs) achieving oil yields ranging from 9% to 21%, with the highest yields obtained from skin and cutoffs. The extracted oil contained significant amounts of EPA and DHA, indicating good nutritional quality.[24] In addition, Głowacz-Różyńska *et al.* (2016) compared "cold" *versus* "hot" extraction of Atlantic salmon (*Salmo salar*) by-products (head, backbone, and skin). The cold method involved adding water (50 °C) to the frozen samples and blending for 5 minutes, maintaining a controlled temperature below 15 °C. For the hot method, the same steps were applied, followed by an additional heating step at 95 °C for 30 minutes under reduced pressure (0.02–0.04 MPa). Skin yielded the highest oil recovery (95%). Peroxide values in the cold-extracted oil were four times lower than those obtained at a high temperature of 95 °C, indicating favorable oxidative stability of the oil at lower temperature. On the other hand, EPA + DHA content was lower in the cold-extracted oil than the hot (15.4% *vs.* 23.2%).[35]

As the name implies, tricanter centrifugation enables three-phase separation, simultaneously separating two liquid phases (oil and water) from a solid phase based on density differences. This technique offers a significant advantage over the conventional decanters, which only achieve two-phase separation (solid–liquid). The tricanter consists of a horizontal cylindrical bowl with an internal conveyor scroll that rotates at a controlled speed. Separation is achieved under high centrifugal force where the feed enters through the central inlet, and the rotation allows phases separation: the light liquid phase (*e.g.*, oil) is discharged by gravity, the denser aqueous phase is discharged under pressure *via* an impeller, and solids are conveyed to the conical end of the bowl for discharge (Fig. 3). This continuous method enables multiple separation, improving efficiency and reducing processing time. This method has been widely implemented in industrial fish oil processing to achieve continuous three-phase separation (oil, water, solids), improving efficiency and reducing waste. Although tricanter systems offer high oil recovery and scalability, they are costly due to their complex design and high energy requirements.[36]

Overall, it has been demonstrated that while elevated temperatures in wet pressing facilitate the release of lipids from fat cells, resulting in a higher yield, they also increase the risk of lipolysis and oxidative degradation. In contrast, cold pressing, under optimized pressure and duration, results in oil with superior quality. On top of that, the elimination of the need for a heating setup tends to reduce energy consumption and operational costs, enhancing the economic feasibility and

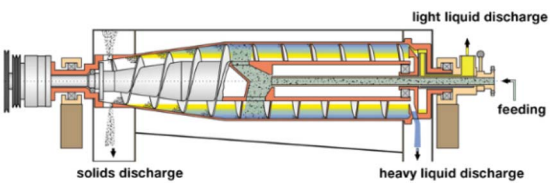

Fig. 3 Tricanter centrifuge for fish oil processing.[37]

scalability of the cold pressing method for fish oil recovery. Tricanter centrifugation offers an advantage by enabling continuous three-phase separation (oil, water, solids) without thermal exposure, improving processing efficiency. However, its high cost limits its application low-to-moderate-budget industries.

2.1.2. Laboratory-scale methods

2.1.2.1. Solvent-dependent methods. In addition to industrial techniques, conventional extraction methods also include solvent-dependent approaches, usually applied at a laboratory-scale, which primarily rely on organic solvents to recover lipids from fish tissues and by-products. The efficiency of these methods significantly depends on the extent of solubility of lipophilic compounds in the chosen solvent system. Based on the "like dissolves like" principle, the solvent of extraction used is typically non-polar to enable the dissolution of the lipid constituents of fish samples. However, the combination of non-polar with mid-polar or polar solvents can also be used to broaden the solubility range, enabling the extraction of more lipid classes (*e.g.*, phospholipids, glycolipids) which possess both polar and non-polar properties, thereby enhancing overall lipid recovery.[38] The extraction is then followed by an oil separation step, usually *via* high-speed centrifugation or rotary evaporation. Despite their reliance on toxic solvents that are both environmentally and occupationally hazardous, methods such as Soxhlet, maceration, and Folch are still widely used in pharmaceutical and industrial fields owing to their efficiency and reproducibility.[7]

2.1.2.1.1. Soxhlet. The Soxhlet extraction method is considered the official reference method for the recovery and quantification of total lipids.[25] The method involves placing the solid sample into a thimble holder and continuously passing a refluxing solvent (*e.g.*, *n*-hexane, petroleum ether, ethyl acetate) over prolonged durations (2–12 hours or more) at temperatures ranging from 60 °C to 130 °C. During this duration, the solvent vapor condenses and passes again through the sample continuously. The cycle is repeated as the siphon brings the solution back to the boiling flask (Fig. 4). As the solvent interacts with the sample matrix, the lipophilic compounds are extracted and collected.[39]

This method has been widely applied to extract oil from fish samples. For example, Rahman *et al.* (2023) utilized the Soxhlet apparatus for recovering Pangus (*Pangasius pangasius*) fish oil using diethyl ether at temperatures ranging from 40 °C and 60 °C for 3 hours. The results revealed an oil yield of 13.50%, with acceptable values of free fatty acids (0.74%), acidity (1.47 mg KOH per g), and peroxide (4.15 meq O_2 per kg). However, EPA and DHA levels were generally low (0.04 and 0.06 mg/100 g, respectively), while DPA was significantly higher (0.54 g/100 g), likely due to a species-specific fatty acid profile or thermal degradation of these long-chain fatty acids during extraction.[33]

Another study evaluated Soxhlet extraction from Japanese Spanish mackerel (*Scomberomorus niphonius*) by-products using *n*-hexane for 12 hours. The highest yield was obtained from skin (51.08%), followed by bones, heads, viscera, and muscles (44.51%, 44.27%, 30.27%, and 29.94%). While peroxide and free fatty acid values of the extracted oils were within acceptable limits, acidity exceeded the recommended Codex limit, with values ranging from 9.66 to 10.84 mg KOH per g. Fatty acid composition analysis showed that muscle-derived oil had the highest EPA and DHA content (5.14% and 14.51%, respectively), followed by viscera, skin, heads, and bones.[40]

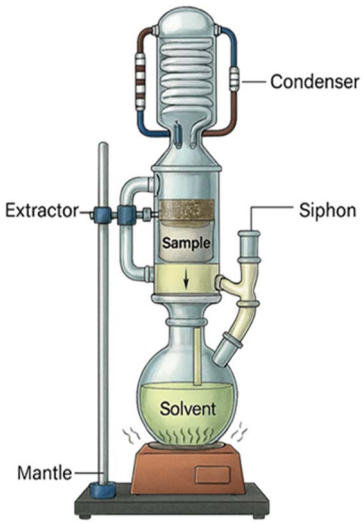

Fig. 4 The Soxhlet apparatus used for lipid extraction.

Furthermore, Soxhlet extraction was applied to salmon (*Salmo salar*) side streams (backbones, heads, and viscera) using *n*-hexane at 80 °C for 6 hours. The values obtained for oil yields were 57%, 56%, and 77% for backbones, heads, and viscera, respectively. Although the oils' oxidation parameters were not evaluated, fatty acid profiling revealed considerable amounts of EPA (3.79% to 6.1%) and DHA (7.3% to 9.3%) across all samples.[20]

Overall, these studies highlight the effectiveness of the Soxhlet extraction method in recovering oil from various fish tissues and by-products. However, the prolonged extraction time combined with elevated temperatures can potentially alter the quality of oil, contributing to elevated acidity and oxidation levels. Additionally, the use of toxic solvents, such as hexane and diethyl ether, raises concerns about the potential solvent residues in the final product. Moreover, the nutritional value, particularly EPA, DPA, and DHA, is difficult to preserve under the harsh conditions applied during this extraction method, making them highly prone to oxidation and degradation, especially in species with naturally low omega-3 levels.

2.1.2.1.2. Maceration. Maceration has been commonly used as a simple solvent-based extraction method. It involves soaking the solid sample in an organic solvent for a certain period, often with continuous or frequent stirring or shaking to enhance mass transfer. The process is usually conducted at ambient temperature (20–25 °C), without any heat treatment, making it suitable for preserving heat-sensitive compounds that might otherwise degrade when exposed to high temperatures. The method's simplicity makes it ideal for small-scale applications. However, its long extraction times and relatively low oil yields

limit its industrial scalability. While no heat treatment is applied, the prolonged exposure to solvents can promote the oxidation and degradation of sensitive fatty acids. In addition, the method requires large amounts of solvents, raising concerns about their toxicity, environmental impacts, and residual traces in the final product.[16]

In this context, several studies applied maceration for fish oil recovery. For instance, Jamaluddin *et al.* (2019) compared maceration and Soxhlet extractions for oil recovery from eel (*Anguilla marmorata*) from Lake Poso. The maceration process lasted 24 hours, where 20 g of sample was soaked in 100 mL hexane, followed by filtration and rotary evaporation. The oil yield was significantly lower in maceration (3.70%) than Soxhlet (28.87%). However, no significant difference in fatty acid content was observed between methods.[41] Additionally, Sasongko *et al.* (2017) found that maceration of 1 kg of fresh eel (*Anguilla bicolor*) in chloroform yielded 5.44% of oil, with an acid value of 17.39 mg KOH per g, peroxide value of 7.02 meq O_2 per kg, saponification value of 111.16 mg KOH per g, and a iodine value of 65.14 WIJS, reflecting a significantly high level of free fatty acids and moderate oxidation.[42] A further study applied maceration to *Chrysichthys nigrodigitatus* fish flesh, where 100 g of oven-dried powder was soaked in 500 mL of hexane for 24 hours with stirring performed three times during this period. After that, the oil was recovered after filtration and rotary evaporation at 40 °C, followed by oven drying at 35 °C for 2 days to remove residual traces of solvent. This resulted in a yield of 5.80%. The oil exhibited hydrolysis and oxidation indicated by acid value of 7.33 mg KOH per g, peroxide value of 4.49 meq O_2 per kg, and AV of 35.43. With regards to the fatty acid composition, SFAs predominated, especially palmitic acid (34.07%). On the contrary, PUFAs values were relatively low, with EPA (1.29%), DHA (2.96%), and total n-3 (5.62%).[43]

2.1.2.2. Cold extractions by solvents

2.1.2.2.1. Folch. Another conventional technique is the Folch method, widely considered a gold standard for extracting lipids from animal tissues, including fish. A solvent system composed of chloroform and methanol in a 2 : 1 (v/v) ratio is usually used.[17,18] The biphasic solvent mixture offers an advantage over single-solvent systems by enabling the extraction of fatty acids with a broader range of polarity, extracting fatty acids with both polar and non-polar characteristics. When applied to fish tissues and by-products, the tissue is usually homogenized in the solvent mixture to facilitate lipid dissolution. This is followed by the addition of an aqueous sodium sulphate solution to induce the separation of the two phases, where the lower chloroform phase contains the dissolved lipids, while the upper aqueous phase is rich in non-lipid components of the sample with polar properties that prevent them from dissolving in chloroform (Fig. 5). The mild and non-thermal conditions of this method help prevent the degradation of heat-sensitive compounds. However, the use of considerable amounts of chloroform poses significant occupational and environmental hazards.[7,16]

In this context, Głowacz-Różyńska *et al.* (2016) applied the standard Folch method to evaluate the lipid yield and quality of oils extracted from processing by-products of both wild and

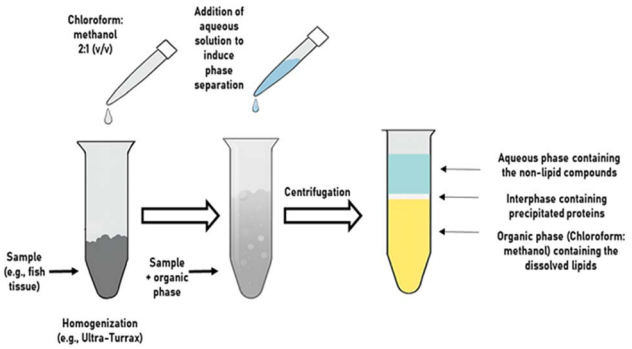

Fig. 5 Folch extraction method for extraction of fish oil.

farmed Atlantic salmon (*Salmo salar*), particularly heads, skin, and backbones. The study found that skin yielded the highest lipid content, with 20.2% in farmed salmon and 8.6% in wild salmon. This was followed by heads (14.8% and 8.0%) and backbones (15.6% and 7.3%), respectively. Regarding oxidative status, peroxide values were lowest in oils from skin (0.88 meq O_2 per kg) and backbones (0.68 meq O_2 per kg), while oils from heads showed higher values (2.56 meq O_2 per kg), which were still below the Codex Alimentarius threshold. The authors attributed the higher value in heads to the presence of blood and haemoglobin in gill tissues, which may have promoted oxidation. Additionally, the free fatty acid content was minimal (acidity value less than 1 mg KOH per g) across all samples. Furthermore, the oils were rich in omega-3 fatty acids, with EPA + DHA content ranging from 16.4% to 24.6% of the total fatty acids, and DPA ranging from 2.7% to 3.9%, highlighting the favorable nutritional value of oil derived from these by-products.[35]

Another study applied the Folch extraction procedure to compare the fatty acid composition of various raw and oven-dried (105 °C for 3 hours) fish by-products, particularly from salmon (*Salmo salar*), red cusk-eel (*Genipterus chilensis*), and yellowtail kingfish (*Seriola lalandi*). For the extraction, 5 g of raw and 1 g of dried samples were mixed with 100 mL and 20 mL of a chloroform : methanol (2 : 1 v/v) solvent system and placed on a magnetic stirrer for 20 minutes. The mixture was then filtered to separate the extract from the solid residues, followed by centrifugation and rotary evaporation of the lower organic phase at 40 °C. When compared to the Soxhlet and Hara–Radin methods, the Folch method yielded the highest lipid recovery: 7.2%, 44.3%, 5.6%, and 5.6% for raw salmon liver, salmon viscera, red cusk-eel viscera, and yellowtail kingfish viscera, respectively. Dried samples yielded even higher values: 22.4%, 65.0%, 27.7%, and 19.1%, respectively. In fact, this study showed that the reduced water content in dried samples enhanced lipid solubility and solvent interaction, highlighting drying as a key preprocessing step for enhancing extraction efficiency. Although no significant difference in oil yield was observed between Folch and the other methods, fatty acid analysis revealed high variability across the studied by-products. The fatty acid profiles were nutritionally rich and showed slight variations between raw and dried samples, where

EPA ranged from 3.06% to 11.30% and 2.83% to 11.20%, DPA from 1.22% to 4.43% and 1.22% to 4.34%, and DHA from 4.00% to 21.90% and 3.95% to 19.52%, respectively. The study concluded that although the Folch method was able to effectively preserve fatty acid profiles, its reliance on large amounts of toxic organic solvents, particularly chloroform, poses health and environmental risks, making this method a less favorable option for routine lipid extraction.[44]

2.1.2.2.2. Bligh and Dyer. The Bligh and Dyer method was introduced shortly after the publication of the Folch method and is also considered a gold standard for lipid extraction.[45] Both methods employ the same solvent system (chloroform/methanol/water) but differ in their ratios: Bligh and Dyer use a lower ratio (1 : 1 : 0.9%) compared to Folch (2 : 1 : 0.8%).[6] Other differences include the solvent volume (approximately 20 times the sample volume in Folch compared to four times in Bligh and Dyer), assumptions about sample moisture content (100% in Folch and 80% Bligh and Dyer), as well as the incorporation of salts (*e.g.*, sodium sulphate) into the aqueous phase in the Folch method to induce phase separation, while they are omitted in the Bligh and Dyer protocol.[6,21]

This reduced chloroform usage in Bligh and Dyer lowers solvent toxicity, although still used and regarded as toxic.

Although Bligh and Dyer method was originally validated using fish muscle, its lower volume of chloroform makes it ideal for the analysis of biological fluids.[6,46] On the contrary, the higher use of chloroform in the Folch method is generally preferred for oil recovery from solid tissues. However, both methods are widely applied for lipid extraction from different sample types. The selection of the appropriate method depends on several key factors, including solvent volume, solid-to-solvent ratio, sample lipid content, sample moisture level, and type of analysis being conducted.[47] In this context, a comparative study evaluated the efficiency of the Bligh and Dyer and Folch methods for lipid recovery from different marine tissues, including pollock, herring, rock sole, rock fish, sculpin, octopus, and squid. The study applied the original protocols of both methods and found no significant differences in lipid recovery from samples containing <2% lipid. However, when applied to samples with >2% lipid content, the Bligh and Dyer method yielded lower lipid amounts, where this lower amount became more evident as lipid content of the sample. This study indicates that the choice between the different methods significantly depends on the lipid content increased.[48] The higher lipid yields observed in Folch method with samples >2% lipid content was attributed to its greater chloroform proportion and larger solvent volume (20 times of sample), compared to Bligh and Dyer (4 times of sample).

2.1.2.2.3. Modified Folch and Bligh and Dyer. In response to the toxic nature of solvents used in the classic Folch and Bligh and Dyer methods, modified versions were developed to substitute these toxic solvents with safer alternatives. For this aim, several solvents such as ethanol, isopropanol, and methyl-*tert*-butyl ether have been introduced and evaluated, particularly as replacements to chloroform.[6] During method development, it became evident that the inclusion of a polar solvent is necessary to facilitate biphasic separation between the aqueous and organic phases. However, excessive water content in the polar phase can result in the co-extraction of not only polar lipids but also non-lipids polar compounds which are regarded as impurities in the oil and can negatively affect its quality and stability.[6,49] Despite ongoing efforts for improvement, many of these solvents remain toxic to both humans and environment. Current research continues to face challenges in identifying a safer, non-chlorinated, and environmentally friendly solvents that can achieve the extraction effectiveness of chloroform-based Folch method.[50–53]

However, Lin *et al.* (2004) developed a method alternative to the classical Folch, which replaced the Folch reagent with non-toxic solvents such as ethyl acetate and ethanol to extract lipids from animal tissues, including pork loin, belly pork, and pork fat. These extractions employed ethyl acetate : ethanol mixtures at 2 : 1 and 1 : 1 ratios, where the resulting oil yields were compared with those obtained using the traditional Folch method (chloroform : methanol, 2 : 1). The results showed no significant difference in lipid yield between the ethyl acetate : ethanol (2 : 1) and chloroform : methanol (2 : 1) mixtures across the tested tissues, highlighting the potential of ethyl acetate : ethanol (2 : 1) as a safer and effective alternative to the traditional Folch.[54]

Furthermore, another study assessed the efficiency of ethyl acetate as a sustainable alternative to the Folch method for the recovery of lipids from different marine species, including *Salmo salar* (Atlantic salmon), *Calanus finmarchicus* (zooplankton), *Porosira glacialis* (microalgae), and *Saccharina latissima* (macroalgae). The results revealed comparable yield for Atlantic salmon fillets and freeze-dried *C. finmarchicus* (p-value >0.05). On the contrary, significantly lower yields were achieved using ethyl acetate for *P. glacialis* (49.5%) and *S. latissima* (27.3%) when compared to the Folch method. Interestingly, lipid profiles showed that ethyl acetate was particularly effective in extracting n-3 PUFAs, especially EPA, from *P. glacialis* and *S. latissimi*. On the other hand, the Folch method demonstrated better performance in extracting DHA and other polar lipid–bound fatty acids. The study concluded that although ethyl acetate method offers a safer choice for lipid extraction from marine species, it lacks the high efficiency across all classes and polarities of lipids.[55]

In addition, Smedes proposed a mixture of propan-2-ol–cyclohexane–water (8 + 10 + 11 v/v/v) as a safer alternative to the classical Bligh and Dyer method for total lipid extraction from marine species such as plaice (muscle), mussel, and herring (muscle). The extraction was compared to Bligh and Dyer where it was performed using the same procedure but with a different solvent system (propan-2-ol–cyclohexane–water). Results showed that the proposed method yielded slightly lower lipid yields for plaice (12.6 *vs.* 13.8 mg g^{-1}) and mussel (24.5 *vs.* 25.6 mg g^{-1}), while slightly higher values were yielded for herring (109.5 *vs.* 103.3 mg g^{-1}) compared to the traditional Bligh and Dyer method. Overall, the average difference in lipid yield between the two different methods is minimal (approximately 2%). Therefore, the proposed solvent system showed strong robustness as a safer and environmentally friendly

alternative to the official Bligh and Dyer method. However, further studies are needed to assess the fatty acids composition and oxidative stability of the obtained oil.[27]

2.1.2.2.4. Hara–Radin. In response to the high toxicity of the traditional Folch method (chloroform : methanol 1 : 2 v/v), Hara and Radin developed a safer alternative in 1978.[19] This technique utilizes a lower-toxicity solvent system composed of hexane : isopropanol (3 : 2, v/v) to recover lipids from various food matrices. The process begins by homogenizing the sample in the solvent mixture, which disrupts the cellular membranes and facilitates lipid release. Following homogenization, the mixture is filtered to separate the solid residue from the organic phase. This is followed by the addition of an aqueous sodium sulphate solution to further purify the extract by removing residual water and polar non-lipid components. The mixture is allowed to stir at room temperature for around 10 minutes and then allowed to rest to enable phase separation. Afterwards the upper organic layer, which is lipid-rich, is collected carefully and subjected to evaporation (*e.g.*, rota evaporation) to remove the solvent and recover the lipid fraction. Although it still relies on solvents, this method offers several advantages, including lower solvent toxicity, operational simplicity, and a non-thermal nature which helps in preserving thermolabile compounds.[7,19,56]

The Hara–Radin method has been successfully applied to recover lipids from fish tissues and by-products.[60] In this context, Rahimi *et al.* (2016) applied different extraction methods, including the Hara–Radin and Soxhlet techniques, to recover lipids from fish by-products (heads, tails, and bones of sardine). Soxhlet extraction was performed at 140 °C for 4 hours using 60 mL hexane, and the official Hara–Radin method was also applied as previously described. The results indicated that the Soxhlet method yielded significantly more oil (46.6 mg g^{-1}) than the Hara–Radin method (15.8 mg g^{-1}), suggesting that the higher temperature and extended extraction time employed in Soxhlet are needed to extract significant amount of lipids from the intracellular membranes of fish by-product tissues. However, the study didn't include further analysis of the oxidative status or the fatty acid composition of the oils extracted by the different methods.[56] Although hexane : isopropanol has been widely applied for the extraction of lipids from fish tissues and by-products—mainly due to its lower toxicity compared with other methods such as Folch—a comprehensive evaluation of the effect of the official Hara–Radin method on fish oil yield, oxidative stability, and fatty acid composition remains limited. Most available studies in the literature do not represent the original Hara–Radin method because they apply the hexane : isopropanol (3 : 2 v/v) solvent mixture but combine it with heat treatment, a condition which deviates from the original non-thermal protocol.[57–59]

The following table (Table 1) provides a comparative summary of the conventional fish oil extraction methods discussed above.

2.2. Green extraction methods

Given the aforementioned limitations of conventional extraction methods, particularly prolonged extraction times, high solvent consumption, and elevated temperatures that can contribute to the degradation of heat-sensitive compounds, there is a growing need for alternative, eco-friendly techniques capable of producing oil with high quality and purity.[60] In this context, green solvent extraction methods such as MAE, UAE, EAE, SFE, and PEF have emerged as promising, sustainable alternatives that offer several key advantages over conventional methods, including reduced solvent consumption, lower processing temperatures, and shorter extraction times, all of which contribute to the preservation of bioactive composition, especially heat-sensitive omega-3 fatty acids.[61]

However, the efficiency of these methods relies heavily on the optimization of process conditions, such as power intensity, extraction time and temperature, solvent type, and solid-to-solvent ratio. Careful optimization of these conditions is essential to achieve a final product with maximum yield, high quality, and acceptable oxidative stability. Overall, each method operates through a distinct mechanism, and thus presents its own advantages and limitations, which can indeed affect their industrial applicability in fish oil extraction.[61,62]

2.2.1. MAE. MAE has gained significant recognition in recent years as a sustainable, green, and eco-friendly method for the extraction of bioactive compounds, including lipophilic compounds and fish oil rich in omega-3 fatty acids. In contrast to conventional methods, MAE utilizes microwave radiation to create localized heating through ionic conduction and dipole rotation. As a result of these interactions, the movement of polar constituents and ions within the sample is accelerated, resulting in friction that causes rupture of the cell walls, thereby allowing the diffusion of intracellular compounds into the solvent.[63] Consequently, the extraction is followed by solvent evaporation, usually *via* rotary evaporation, to isolate the oil. This method has been commonly adopted over the past years to reduce both extraction time and solvent consumption, providing an eco-friendly and cost-effective alternative to traditional methods.[64]

However, the efficiency of MAE is influenced by different factors, particularly microwave power, type of solvent, extraction time and temperature, and the composition of the sample, including fat and moisture contents. For instance, solvents with high dielectric constants, such as methanol, ethanol, and acetonitrile, can effectively absorb microwave energy.[46,65] Yet, since oil is mostly non-polar, these polar solvents alone are not capable of efficiently solubilizing all lipid compounds; therefore, the use of binary solvent systems is often required to enhance cell disruption while ensuring efficient solubilization of the oil. Additionally, samples with high moisture content, while beneficial for heat generation, may limit extraction efficiency due to the absorption of microwave energy by water. Moreover, although high temperature and power intensity can enhance oil yield by promoting solvent penetration and cell disruption, they can also trigger hydrolysis and oxidation reactions, leading to degradation of omega-3 fatty acids and other thermolabile bioactive compounds, which raises concerns about the quality of the final product.[64,66]

With proper optimization, MAE has demonstrated high performance compared to conventional methods such as

Table 1 Comparative summary of conventional fish oil extraction methods[a]

Category	Method	Principle	Sample type	Oil yield (%) or (mg g^{-1})	Acidity and oxidation status (PV,-AV, TOTOX)	EPA + DHA (%)	Advantages	Limitations
Industrial (mechanical)	Wet pressing	Thermal + mechanical pressing	Tuna heads, *Tilapia* by-products, *Sardinella*, *Pangus*	5.37–19.25%	Acidity: 1.5; PV: 3.3; TOTOX: up to 39.96	0.21–1.15	Simple, solvent-free; scalable; moderate yield	High temperature causes oxidation and degradation of omega-3 fatty acids
	Cold pressing	Mechanical pressing without heat	Salmon heads, skin, viscera, and backbones	8.6–21.0% (up to 95% in skin)	PV significantly (four times) lower than hot methods	15.4	Preserves heat-sensitive compounds; low oxidation; energy-efficient	Extended pressing durations; higher risk of residual moisture/ emulsification
	Tricanter	Mechanical three phases separation (oil, water, solids) using centrifugation	Fish and fish by-products	NR	NR	NR	Continuous process; high efficiency	High energy cost
Laboratory-scale	Soxhlet	Continuous solvent reflux (*e.g.*, hexane, ether)	Pangus, mackerel, salmon by-products	13.5–77.0%	Acidity: 1.4–10.8; PV: 4.1	0.03–14.5 (species-dependent)	Official reference method; relatively high yield; reproducible	Long duration; high temperature; solvent toxicity; poor omega-3 fatty acids preservation
	Maceration	Solvent soaking at ambient temperature	*Anguilla marmorata* (eel), *Anguilla bicolor* (eel), *Chrysichthys nigrodigitatus*	3.7–5.8%	Acidity: 7.3–17.4; PV: 4.9–7.0; AV: 35.4	1.29–2.96	Simple; preserves heat-sensitive compounds	Long extraction time; low yield; oxidation risk from prolonged solvent exposure
	Folch	Chloroform : methanol (2 : 1 v/v), biphasic	By-products from farmed and wild salmon, red cusk-eel, and yellowtail kingfish	Raw: 5.6–20.2%; dried: 19.1–65.0%	Acidity: <1; PV: 0.68–2.56	2.83–24.6	Broad polarity range; preserves omega-3; mild conditions	Toxic solvents raise concerns about environmental and occupational risks
	Hara–Radin	Hexane : isopropanol (3 : 2 v/v), biphasic	Sardine by-products (heads, tails, and bones)	15.8 mg g^{-1}	NR	NR	Lower solvent toxicity; non-thermal; simple operation	Limited penetration to dense tissues; lower oil yield; understudied in fish matrices at ambient conditions

[a] NR: not reported.

Soxhlet extraction. For example, the potential of optimized MAE for extracting oil from a mixture of spoiled or shelf-life-expired fish parts (heads, fins, loins, fillets) from different marine species was investigated and compared to Soxhlet extraction using *n*-hexane at 80 °C for 6 hours. The results revealed that MAE achieved significant oil recovery between 60 and 100% of that obtained using Soxhlet, with extraction times under 19 minutes *versus* 6 hours in Soxhlet. Both methods produced oils rich in PUFAs (~29%), including EPA, DPA, DHA, and linoleic acid. Interestingly, the MAE-oil demonstrated superior antimicrobial, anti-inflammatory, and cytotoxic activities, particularly against breast and gastric adenocarcinoma cells. These findings highlight MAE's ability to preserve the lipid profile and enhance bioactive properties of the oil, possibly due to minimized oxidative degradation. Additionally, the combination of high yield, reduced solvent consumption, and shorter processing time suggests MAE as an efficient alternative to conventional methods.[40]

Similarly, a comparative study evaluated MAE (using ethyl acetate : methanol, 2 : 1, v/v, at 400 W, 54 °C for 16 minutes) for extracting oil from tuna (*Thunnus thynnus*), hake (*Cynoscion jamaicensis*), and pacu (*Piaractus mesopotamicus*), compared to the traditional Folch method. The MAE method was optimized using a design of experiment (DoE) and validated against a standard reference material (SRM). MAE yielded 10.1/100 g of oil, matching the SRM fish tissue certificate value (10.2 g/100 g). No significant difference was observed between the lipid yields from MAE and Folch. Scanning electron microscopy (SEM) revealed that MAE, despite using lower temperature and shorter time, achieved near-total tissue disruption. Fatty acid profile analysis showed no significant differences between oils extracted *via* both methods, indicating that MAE didn't induce degradation or loss of lipid constituents. Peroxide values were well below the Codex Alimentarius threshold of 10 meq O_2 per kg for Folch, further supporting MAE's effectiveness in preserving oil quality.[67]

On the contrary, another study reported that MAE yielded 21.5% and 20.75% oil from sea bass heads and sea bream heads, respectively, while Soxhlet extraction resulted in significantly higher values (39.14% and 41.58%). Yet, MAE was considered more efficient as it reduced the extraction time to around 11 minutes compared to 6 hours for Soxhlet and used a lower amount of solvent (50 mL *vs.* 250 mL), resulting in a 33-fold time reduction and a fivefold solvent savings. The extracted oils were rich in PUFAs, particularly DHA (11.02% to 13.61%), and EPA (4.33% to 4.60%), indicating MAE's ability to preserve lipid profiles. Moreover, the oils demonstrated low atherogenicity index (~0.3), low thrombogenicity index (~0.15), as well as high hypocholesterolemic index (4.11–4.22), suggesting potential cardiovascular benefits.[68]

Despite its advantages, the application of MAE for the extraction of fish oil encounters several limitations. The complex and variable composition of fish tissues can significantly influence oil yield. Industrial-scale applications might be challenging due to non-uniform heating, which is difficult to control in large systems. Furthermore, optimization of extraction conditions often requires comprehensive analysis and extensive trials, which can be time-consuming, expensive, and impractical for some industries. These challenges may limit the upscaling of MAE, highlighting the need for further improvements or alternative innovative methods.[66,69]

2.2.2. UAE. Another technology that has emerged as an innovative and sustainable green extraction method is UAE. This technique has been widely applied for the recovery of bioactive compounds, including fish oil rich in omega-3 fatty acids. The method utilizes mechanical sound waves that propagate through the solvent. The frequency of the ultrasound waves typically ranges from 20 kHz to 10 MHz, and the method is classified according to the selected frequency range. Low-intensity ultrasound (100 kHz to 1 MHz, power <1 W cm^{-2}) is mainly used for non-destructive analysis, whereas high-intensity ultrasound (16 to 100 kHz, power 10–1000 W cm^{-2}) is employed to enhance extraction efficiency by altering the physical and chemical properties of the sample.[70] The applied ultrasound waves induce acoustic cavitation, the core mechanism of UAE, in which rapidly collapsing microscopic bubbles generate localized pressure and heat. Eventually, this leads to cell wall perforation, enhanced solvent penetration, and consequently results in cell rupture and release of intracellular compounds.[71] The mechanism of action of UAE is illustrated in Fig. 6.

Ciftci *et al.* (2025) used response surface methodology (RSM) to optimize UAE for extracting fish oil from Atlantic bonito (*Sarda sarda*) by-products. Under optimal conditions (16 mL g^{-1} hexane-to-solid ratio, 60 °C, 80 minutes), UAE yielded 45.11% oil from freeze-dried by-products—a yield 90.5% higher than that of Soxhlet. The UAE-oil exhibited significantly higher levels of PUFAs (45.21%) and omega-3 fatty acids (39.41%), along with lower acidity, PV, AV, and TOTOX values compared to Soxhlet, indicating both superior nutritional value and oxidative stability.[72]

Similarly, another study evaluated UAE for extracting oil from rainbow trout (*Oncorhynchus mykiss*) intestines. The

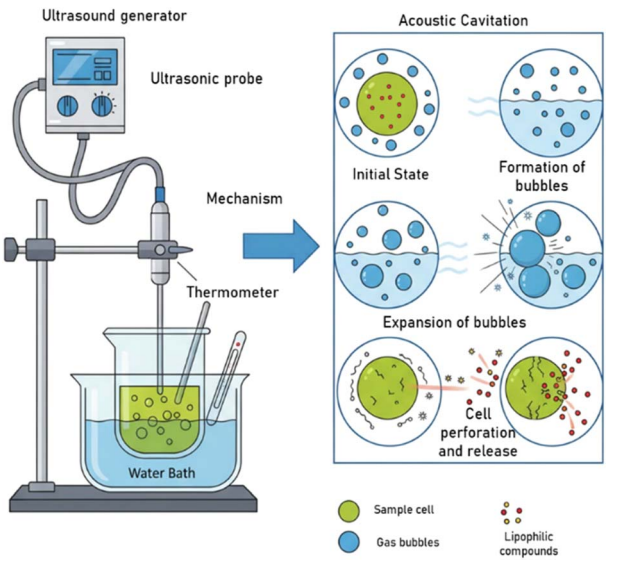

Fig. 6 UAE mechanism for oil recovery.

optimized extraction conditions (60 °C for 30 minutes) produced oil with acceptable acid value and free fatty acid (FFA) content according to Codex Alimentarius standards. However, the peroxide value reached 29 meq O_2 per kg, significantly exceeding the acceptable Codex Alimentarius limit (5 meq O_2 per kg). To improve oil quality, a purification step using adsorbents—silica gel (SG), aluminum oxide (Al_2O_3), and their mixture—was incorporated. SG achieved the highest peroxide removal after 60 minutes, reducing PVs to 12.0, 14.3, and 13.3 meq O_2 per kg, respectively. Despite this decrease, PVs remained above Codex Alimentarius limits. Therefore, antioxidants like tannic acid, gallic acid, and black chokeberry (*Aronia*) powder were added during extraction, followed by SG purification for 60 minutes. This significantly reduced PVs to 5.67, 9.00, and 8.00 meq O_2 per kg, respectively. Antioxidant addition also lightened oil color, reduced aldehyde levels as oxidation products, and increased MUFAs and PUFAs content, with no significant change in SFA. However, further enrichment with omega-3 fatty acids may still be required to enhance the oil's nutritional value.[73]

A further investigation aimed to optimize and compare the efficiency of MAE and UAE in obtaining edible oil from gilthead seabream (*Sparus aurata*), along with the traditional conventional Soxhlet extraction. Extraction parameters (time, temperature, solid-to-solvent ratio) were optimized using RSM. Under the optimal conditions (15.47 mL g^{-1}, 38 minutes, 42 °C), UAE yielded 38.40%, while MAE (15.84 mL g^{-1}, 18 minutes, 40 °C) yielded 36.84%. The longer extraction time in Soxhlet (240 minutes at 40 °C) contributed to the highest yield at 43.12%. In terms of oil quality, the extraction method had no significant effect on acidity. However, peroxide values varied: Soxhlet-extracted oil had the highest value (3.98 meq O_2 per kg), followed by MAE (1.89 meq O_2 per kg) and UAE (1.49 meq O_2 per kg). Similarly, AV were lowest in UAE (0.22), followed by MAE (0.34) and Soxhlet (0.35). TOTOX values followed the same trend: Soxhlet (8.31) > MAE (4.13) > UAE (3.20). These results suggest that prolonged exposure to heat and solvent during Soxhlet extraction accelerates oil oxidation. In contrast, UAE and MAE may enhance the release of intracellular antioxidants (*e.g.*, tocopherols), contributing to better oil stability. Furthermore, color analysis revealed that oils obtained *via* UAE and MAE were lighter in color compared to those from Soxhlet. Regarding fatty acid composition, regardless of the extraction technique, oleic acid predominated (31.21–31.40%), followed by linoleic acid (15.15–15.20%) and palmitic acid (14.99–15.24%). Oils were rich in unsaturated fatty acids, comprising ~75% of total fatty acids, with no statistically significant differences in fatty acid profiles ($p > 0.05$).[74]

Overall, the previously discussed studies highlight UAE as a promising green extraction method for fish oil recovery, offering high yield, excellent oxidative stability, and enhanced preservation of omega-3 fatty acids. Furthermore, this technique can improve and lighten oil color when combined with purification and antioxidant enrichment steps. While traditional extractions like Soxhlet resulted in higher oil yield under prolonged heating, from an industrial perspective, the production of oil with high nutritional value and oxidative quality is essential, thus UAE offers a promising choice.

2.2.3. EAE. Among the various green extractions developed as alternatives to conventional solvent-based extractions, EAE has emerged as a promising and advanced approach. This method utilizes exogenous enzymes (*e.g.* proteases, lipases, and cellulases) to selectively hydrolyze the components of cellular membranes and tissues, disrupting cell wall structural integrity and thereby facilitating the release of intracellular compounds.[75] The efficiency of EAE depends on several factors, including pH, temperature, enzyme concentration, solid-to-solvent ratio, and reaction time. These parameters must be optimized to ensure effective hydrolysis and the recovery of oil of acceptable quality. Upon completion of hydrolysis, enzymatic inactivation is usually achieved *via* mild heating. This is followed by a separation step, such as filtration or centrifugation, to separate the lipid-rich fraction from the aqueous phase and solid residues.[76]

In the context of fish oil extraction, proteases are particularly effective, as they target the protein-rich cellular membranes, creating perforations that allow intracellular lipophilic molecules to diffuse outward and be recovered as oil.[75]

A key advantage of EAE over other advanced extraction techniques, such as MAE and UAE, is its operation under mild thermal conditions and near-neutral pH, which helps preserve the composition of the oil, especially heat- and pH-sensitive compounds like omega-3 fatty acids (EPA, DPA, and DHA), which can easily degrade and oxidize under harsh extraction conditions. These EAE conditions are particularly essential for producing high-quality edible fish oil, where maintaining high nutritional quality is crucial. Additionally, EAE aligns with the principles of green chemistry, as it minimizes the use of toxic organic solvents, thereby reducing environmental impact and solvent residues in the final product.[71,77,78]

Overall, EAE presents a robust, scalable, and environmentally friendly approach for extracting oil from fish and their side-streams. However, the cost of high-purity enzymes can be unaffordable, especially when large volumes are required for industrial-scale applications. In addition, as with any extraction method, process parameters, including the type and concentration of enzyme, reaction time, pH, and temperature, must be carefully optimized to ensure efficient extraction and maximize yield. Insufficient control of any of these variables may result in low yield due to incomplete extraction, or degradation of the oil in the case of prolonged exposure to high enzymatic activity and thermal treatment.[77]

In this context, EAE was assessed as a green and sustainable method for the extraction of oil from whole Baltic herring (*Clupea harengus membras*) and its filleting by-products using three different commercial enzymes (Alcalase®, Neutrase®, and Protamex®) at two-time intervals (35 and 70 minutes). The results showed that extended extraction times generally improved oil yield, with the highest recovery from whole fish using Neutrase® at 70 minutes (6.25 g/100 g), and from by-products using Protamex® at 70 minutes (6.14 g/100 g). However, Alcalase® did not show a significant increase in yield with prolonged exposure, suggesting different enzyme kinetics.

Despite the improved yield, oil quality assessments revealed higher oxidation levels at 70 minutes, as indicated by elevated peroxide and AV, which exceeded the Codex Alimentarius limits across all enzymes and extraction times. The authors attributed this to the absence of refining steps and the lack of antioxidant additives. Fatty acid analysis showed high levels of omega-3 PUFAs (~28%), with the highest EPA and DHA levels obtained using Protamex® at 35 minutes. However, this sample also exhibited the highest oxidation level, which was explained by overestimated AV caused by increased absorbance from unsaturated aldehydes in omega-3-rich oils.[78]

Similarly, another study assessed the efficiency of aqueous extraction (AE) for recovering oil from Cobia (*Rachycentron canadum*) liver, optimized as follows: 15 minutes, 95 °C, and a 1 : 2 sample-to-water ratio. Under these conditions, the yield was 18.8%, which was considered unsatisfactory. To enhance recovery, enzyme-assisted aqueous extraction (EAAE), was applied using four enzymes—Alcalase®, papain, trypsin, and pepsin—for 2 hours at 95 °C. The results demonstrated a significant increase in yields upon enzyme incorporation. Papain achieved the highest recovery (38%), nearly double that of AE alone, highlighting its potential for enhancing oil recovery. However, Alcalase® remains more suitable for industrial use due to its microbial origin and large-scale production feasibility. Following papain's identification as the most effective enzyme, EAAE conditions were further optimized to pH 5, 30 °C, 0.5% enzyme concentration, and a 2-hour reaction time. Fatty acid analysis revealed oils rich in unsaturated fatty acids (55–60%), with no significant differences in SFA, MUFA, and PUFA contents across the different enzymes. However, EPA + DHA content was slightly lower in EAAE-extracted oil (17.55%) compared to AE (19.73%), likely due to enzymatic pretreatment which might have affected the stability of these PUFAs.[77]

To improve the efficiency of enzymatic extraction for fish oil recovery from *Labeo rohita* head, a comparative study explored the effects of coupling various pretreatments, particularly heating (HT), microwave irradiation, and ultrasound, prior to enzymatic extraction using Protamex®. These treatments were evaluated against a control group in which oil was enzymatically extracted without any pretreatment. Compared to the control yield (55.83%), both UAE and MAE pretreatments significantly enhanced oil recovery, where yields ranged from 58.74 to 67.48% for the former, and 68.45 to 69.75% for the latter, depending on extraction durations of 5, 10, or 15 minutes. The improved extraction was attributed to enhanced cell membrane disruption and the subsequent increased release of intracellular lipophilic compounds, triggered by dielectric heating (MAE) and cavitation (UAE). Additionally, the oxidative stability of the extracted oils was assessed. The highest PV was obtained in MAE–EAE (187.81 mg kg^{-1}), followed by the control (136.93 mg kg^{-1}), UAE (131.81 mg kg^{-1}), and HT-assisted (78.65 mg per kg oil) treatments. The elevated PV in MAE group was attributed to microwave-induced free radical formation, which accelerates lipid oxidation. Similarly, AV and thiobarbituric acid reactive substances (TBARS) followed a similar trend. AV ranged from 5.59 to 7.99, and TBARS from 10.06 to 10.61 mg MDA per kg. The highest values were observed in MAE treated samples (AV: 7.99; TBARS: 10.61 mg MDA per kg), significantly exceeding those of the control. In contrast, UAE showed no significant differences in PV or TBARS compared to the control, while HT-assisted extraction yielded slightly lower values for both markers than the control. Conjugated dienes levels, which typically accompany peroxide formation, were also highest in MAE samples (0.260). Similarly, FFA content, determined by the Lowry and Tinsley colorimetric method using a palmitic acid standard curve,[75] followed the same pattern, peaking in MAE (2.36 g/100 g), likely due to microwave-induced cleavage of esterified lipids. HT-assisted extraction resulted in lower FFA content than the control, possibly due to thermal deactivation of hydrolytic enzymes such as lipases and phospholipases at 90 °C. Fatty acid analysis revealed oils rich in PUFAs (37.51–39.28%) and MUFAs (24.83–26.46%). Linoleic acid (29.36–30.79%) and oleic acid (19.28–20.59%) predominated. Interestingly, the EAE–UAE increased PUFA content from 24.91% to 26.46% and MUFA content from 37.56% to 39.28%. In conclusion, this comprehensive study demonstrated that although EAE is a sustainable alternative to conventional extraction methods, its performance can be significantly enhanced through pretreatment. Among the tested strategies, UAE offered the most favourable balance between yield enhancement and oil stability, highlighting it as a promising candidate for integration with EAE.[79]

Similarly, ultra-high-pressure pretreatment (EHSUP) was applied prior to enzymatic hydrolysis of yellowfin tuna (*Thunnus albacares*) heads. Oil yield increased significantly to 67.97%, compared to 60% obtained EAE without pretreatment. Optimal conditions were identified as 200 MPa for 10 min or 100 MPa for 20 min, followed by hydrolysis using 1% papain for 60 minutes at 55 °C. Despite the improved yield, the extracted oil didn't meet Codex Alimentarius standards, due to elevated acid value (7.31 mg KOH per g), peroxide value (11.41 meq O_2 per kg), and AV (39.72). However, the authors attributed these results to cumulative oxidative degradation during pre-processing steps, including harvesting, cutting, transportation, and storage prior to pressure treatment. Furthermore, the fatty acid composition of EHSUP-extracted oil revealed high content of PUFAs (33.96%), with EPA + DHA comprising 27.92%, highlighting the potential of high-pressure pretreatment to enhance omega-3 recovery and produce oil with high nutritional value, despite challenges in oxidative stability.[80]

Although EAE can be used as a standalone method for extraction of fish oil, or applied after other pretreatments as discussed previously (*e.g.* ultra-high-pressure, UAE, MAE), there is a scarcity of studies that have explored the use of enzymatic hydrolysis as a pretreatment prior to a secondary extraction method specifically for fish oil recovery. One example of enzymatic pretreatment is provided by the PROBIS project, where different fish by-products (*e.g.*, head, viscera, frames) from anchovy, sea bream, sea bass were first pretreated with enzymatic mixtures with high endo- and exo-protease activity, such as papain, pepsin, trypsin, and microbial proteases, to disrupt cell walls structure and enhance lipid release. This was followed by a secondary extraction step using pressurized hot water (120–140 °C, 1.5–3 atm, 10–30 minutes) aiming to develop a green

and solvent-free multi-step process. Although the oxidation status of the obtained oil was not assessed, GC-MS and FTIR-ATR analyses confirmed a high omega-3 content, particularly EPA and DHA.[81] While such hybrid extraction techniques remain scarce in fish oil extraction, several studies have demonstrated the effectiveness of enzymatic pretreatment in other marine species, such as microalgae. For example, enzymatic pretreatment with different enzymes including cellulases prior to PLE of oil from *Nannochloropsis gaditana* enriched the recovered lipid fraction in omega-3 fatty acids.[82] Similar findings were reported for *Isochrysis galbana*, where enzymatic pretreatment with Viscozyme® and Celluclast® applied prior to UAE and PLE increased lipid recovery and improved omega-3 content, especially when combined with PLE.[83] Although the application of enzymatic pretreatment before secondary extraction has been shown to significantly enhance lipid recovery and omega-3 content in various marine species, this approach remains largely unexplored for fish oil extraction, highlighting a clear research gap in this context.

In conclusion, these findings reinforce the potential of EAE as a sustainable and adaptable alternative to conventional extraction methods. When combined with pretreatments such as ultrasound, microwave irradiation, or high-pressure techniques, or used itself as a pretreatment prior to another extraction, EAE can enhance extraction efficiency and improve yields while preserving the nutritional integrity of the oil, particularly its PUFA and omega-3 content. However, the incorporation of antioxidants should also be considered to meet food-grade quality standards, particularly in terms of oxidative stability.

2.2.4. SFE. SFE is another green and advanced method that has emerged as an alternative to conventional solvent-based extraction techniques. This method utilizes supercritical fluids (SCFs) to extract different compounds, including lipophilic compounds, from complex samples. SCFs are substances that exhibit both liquid-like and gas-like properties when maintained above their critical temperature and pressure. This dual-phase characteristic provides low viscosity and high diffusivity, enabling SCFs to penetrate sample matrices more efficiently and enhance the release and recovery of target compounds.[84] Among available SCFs, supercritical carbon dioxide (CO_2) is the most widely used due to its low critical point, low toxicity, and environmental compatibility. The inert and oxygen-free atmosphere of this method helps preserve oil composition, particularly PUFAs, and minimizes oxidative degradation.[84,85] Despite these advantages, SFE presents various limitations. The high operational cost and technical complexity restrict its feasibility for small-scale applications. Moreover, efficient extraction requires precise control and optimization of parameters such as temperature, pressure, and flow rate, which require specialized expertise. Inappropriate selection of these conditions may lead to oxidation and degradation of sensitive fatty acids, especially omega-3s, thereby altering the nutritional quality and oxidative stability of the extracted oil.[86]

In this context, a comparative study was conducted to evaluate oil recovery from the fillets, caviar, and viscera of freshwater carp (*Cyprinus carpio* L.) using Soxhlet and SFE-CO_2. Soxhlet extraction was performed using three solvents—methylene chloride, petroleum ether, and hexane—with methylene chloride identified as the most effective. The optimized Soxhlet extraction involved continuous extraction for 140 minutes using 200 mL of methylene chloride with 20 g of lyophilized sample. SFE-CO_2 was conducted under varying conditions of temperature (40, 50 and 60 °C), pressure (200, 300, 350, and 400 bar), and extraction time (30, 60, 120 and 180 min) to determine optimal parameters. Although the best SFE-CO_2 yield was achieved at 60 °C and 400 bar, Soxhlet extraction produced higher oil yields overall, primarily due to its use of larger solvent volumes and possible elevated temperature and extended extraction duration. Soxhlet yielded approximately 80% from viscera, 70% from fillets, and 20% from caviar, while SFE-CO_2 yielded 50%, 30%, and 10%, respectively. Despite the lower yield, SFE-CO_2 resulted in oils with a higher PUFA content (34.10%) than Soxhlet (33.88%). Although oxidative stability was not assessed, the study concluded that SFE-CO_2 is effective for obtaining nutritionally valuable oils with enhanced PUFA content.[87]

Similarly, another study compared Soxhlet and SFE-CO_2 for oil recovery from various parts of Indian mackerel (*Rastrelliger kanagurta*), particularly the head, flesh, skin, and viscera. Under optimized SFE-CO_2 conditions (35 MPa, 60 °C, 2 mL min^{-1}), several techniques were evaluated: continuous extraction with pure CO_2, cosolvent-assisted extraction using ethanol, soaking in CO_2 for 10 hours prior to extraction, and pressure swing technique, which involved altering pressure levels during the extraction to enhance mass transfer and tissue disruption. Soxhlet yielded 53.6%, 17.3%, 13.6%, and 11.4% oil from skin, flesh, heads, and viscera, respectively. In contrast, all SFE-CO_2 techniques produced higher yields across all tissues, although differences among the tested techniques were negligible. Fatty acid analysis revealed no significant differences in SFA and MUFA content across methods. PUFA levels ranged from 56% to 75%, with the highest concentrations found in oils from skin and flesh, regardless of the extraction method. EPA, DPA, and DHA levels ranged between 9–12%, 2–3%, and 10–14%, respectively, with slightly higher values observed in oils extracted *via* soaking and pressure swing SFE-CO_2 methods. These findings suggest that SFE-CO_2, particularly when combined with soaking and pressure swing, can significantly enhance oil yield and produce oil with high nutritional value. However, oxidative stability was not assessed, which remains a crucial consideration for future applications.[88]

Nonetheless, SFE-CO_2 (25 MPa, 313 K) was conducted to recover oil from various fish by-products, including hake, orange rough, salmon, and liver offcuts, each from a different fish species, and compared the results with conventional methods such as cold extraction, wet reduction, and enzymatic extraction. SFE yielded the highest oil recovery, particularly from salmon offcuts (51%). In contrast, cold and wet reduction methods produced significantly lower yields, especially from lean tissues such as hake and squid liver, where oil recovery was minimal or not possible. Notably, SFE recovered 18% and 17% oil from hake and squid liver, respectively. Although enzymatic extraction was more efficient than cold and wet methods, it still

yielded less oil than SFE. Fatty acid profiles were similar across methods, with PUFAs predominating over MUFAs and SFAs. For example, SFE oil from salmon offcuts contained 309 mg per g PUFA, 250 mg per g MUFA, and 230 mg per g SFA. In addition, EPA, DPA, and DHA were particularly high in SFE oils, reaching 79 mg g^{-1}, 38.4 mg g^{-1}, and 63 mg g^{-1}, respectively. Acidity analysis revealed that SFE-extracted oil from salmon had lower acidity than oils obtained *via* non-SFE methods, reflecting minimal hydrolysis of triacylglycerols. On the contrary, SFE oil from orange roughy exhibited higher acidity despite its low free fatty acid content, likely due to the co-extraction of certain volatile acidic compounds such as acetic acid, which could be extracted in the closed SFE system, but lost in the open-vessel methods. Oxidative stability, evaluated *via* TOTOX values, revealed that salmon oil had the highest value regardless of the extraction method, attributed to its high PUFA content. However, other oils extracted using SFE remained within recommended TOTOX limits, whereas enzymatically extracted oils sometimes exceeded acceptable thresholds (*e.g.*, ~35 for salmon offcuts). The superior oxidative stability of SFE oils is attributed to the mild, oxygen-free conditions of the process, which help preserve sensitive fatty acids.[89]

These studies collectively highlight the effectiveness of SFE, particularly with supercritical CO_2, as a sustainable and oxygen-free alternative for fish oil recovery. While SFE may yield less oil in some cases, it can produce oil with high nutritional value, improved fatty acid profiles, and superior oxidative stability. The species of fish and their inherent lipid composition significantly influence extraction outcomes, regardless of the method employed. Despite its clear advantages, the high cost and technical complexity required for the implementation of SFE limit its application, especially in small-scale settings.

2.2.5. Other emerging techniques. Lately, various other innovative technologies have emerged as promising green techniques for the extraction of fish oil, including PEF, deep eutectic solvents (DES), and pressurized liquid extraction (PLE). While these techniques align well with the principles of green chemistry and environmental sustainability, the current literature remains limited regarding their application. Most available studies highlight their effectiveness in extracting bioactive compounds from plant and animal matrices, with only a few studies focusing specifically on fish oil extraction and its impact on oil yield and quality compared to conventional methods.

2.2.5.1. PEF. PEF is an emerging non-thermal technology that utilizes brief pulses of high-voltage electricity to induce electroporation in tissue cells. These pulses generate an electric field across the cell membrane, triggering the formation of temporary pores, a phenomenon known as electroporation phenomenon. Consequently, the membrane integrity is disrupted, resulting in the release of intracellular compounds such as lipids, and facilitating oil recovery.[90,91] A typical PES system consists of a treatment chamber, electrodes, and a pulse generator (Fig. 7). Key parameters such as pulse strength, duration, and frequency can be optimized to enhance extraction yield and minimize degradation.[92] This technique has recently gained significant attention in meat and fish processing due to its rapid, energy efficient, and non-thermal characteristics,

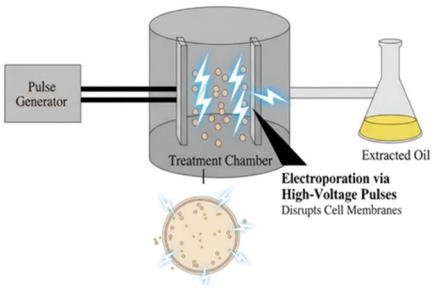

Fig. 7 PEF technology for oil recovery.

which help preserve the final product's quality and oxidative stability. Unlike conventional methods that rely on heat, which often degrade heat-sensitive compounds and alter nutritional composition, PEF offers a gentler alternative.[93]

In a study, PEF was applied at different intensities (0.62, 1.25, and 1.875 kV cm^{-1}) and frequencies (25, 50, and 100 Hz), with temperature increases monitored with increasing pulses but not exceeding 55 °C, to evaluate the impact of increasing PEF input on lipid extraction from hoki roe. The results revealed that increasing PEF intensity led to higher oil yields and enhanced recovery of bioactive compounds. The maximum oil yield (16.2%), highest phospholipid content (46 μmol g^{-1}), and n-3 fatty acid concentration (32%) were achieved at 112 kJ kg^{-1}, 1.875 kV cm^{-1}, and 100 Hz. Although these high-input conditions were effective in maximizing oil yield and composition, the high-energy conditions disrupted phospholipid structure, causing sn-2-bound EPA and DHA to shift to sn-1,3 positions, which might alter their bioavailability and nutritional benefits. Interestingly, no significant differences were observed in TBARS and PVs between samples subjected to varying PEF intensities and frequencies, compared to both the untreated control and heat-treated control incubated at 55 °C. The average PV was around 4 meq O_2 per kg, which falls within the acceptable limit for edible fish oil, indicating minimal primary lipid oxidation. Similarly, TBARS values showed no significant variation, suggesting that even the highest energy inputs didn't compromise the oils' oxidative stability.[94]

Furthermore, a similar related study applied PEF to hoki fish co-products (male gonad) using the same intensities and frequencies mentioned above to compare PEF treatment with traditional ethanol–hexane-based (ETHEX) extraction. PEF treatment increased oil yield from 4.1% to 6.7% at a mild intensity of 1.25 kV cm^{-1} and frequency of 50 Hz. Additionally, EPA, DPA, and DHA concentrations slightly increased from 8.0% to 8.2%, 2.5% to 2.7%, and 35.2% to 35.7% in the unheated control. Under heat-treated conditions EPA, DPA, and DHA contents were 7.9%, 2.5%, and 34%, respectively.[95]

Another study assessed the combined effect of PEF (10 and 20 A) and brine salting (5% and 10% NaCl) on the oxidative stability of oil extracted from sea bass (*Dicentrarchus labrax*). The study revealed that PEF significantly increased both primary and secondary oxidation products, especially at the higher intensity of 20 A. However, even with this increase, oxidation values remained within acceptable limits.[96]

These studies highlight PEF as an effective non-thermal method capable of enhancing oil recovery and yield while preserving the nutritional composition and oxidative stability of fish oil. More studies are needed to better understand how different PEF parameters influence both the recovered oil yield and quality. Recent literature has shown that the influence of PEF treatment is highly dependent on the characteristics of the sample matrix as well as the specific PEF conditions applied. Various factors including tissue conductivity, moisture content, the presence of salts, and endogenous pro-oxidants can significantly influence electroporation efficiency and oxidative behavior of the extracted oil. This helps explain the previously mentioned findings where PEF improved lipid recovery from hoki roe without inducing oxidation, whereas in sea bass (*Dicentrarchus labrax*), the combination of PEF with brine salting promoted higher levels of primary and secondary oxidation products. These results highlight the need to further explore the effect of matrix composition and method conditions influencing oxidation status during PEF processing.[93]

In general, the current research remains limited in terms of in-depth and comprehensive studies applying PEF specifically for fish oil extraction, and several important gaps persist. An overview of PEF applications in meat and fish processing highlighted that most studies have focused on microbial inactivation, enhanced mass transfer, and textural modifications, while its targeted application for lipid recovery remains limited. The review also mentioned that PEF can alter the structure of cellular membranes and muscle proteins, which may consequently affect lipid release and susceptibility to oxidation; however, these mechanisms have not yet been confirmed through applied studies on fish oil extraction. Furthermore, the study highlighted the complexity of PEF optimization due to the high variability in the outcomes depending on different factors including electrode design and pulse intensity. Comparative studies evaluating PEF against other extraction methods such as UAE, MAE, and EAE are also scarce.[97,98]

Overall, while PEF shows promising potential as a green, advanced, and non-thermal extraction method for fish oil, further research is still needed to provide stronger evidence of its effectiveness in recovering oil from different fish species and by-products, while understanding how process parameters can affect the quality of the obtained oil.

2.2.5.2. DES. DES are formed by combining two or more solid components that interact through hydrogen bonding, resulting in a liquid with a melting point significantly lower than that of the individual components. These solvents exhibit various unique characteristics, including tunable polarity, ionic characteristics, and are considered environmentally friendly alternatives to conventional organic solvents. Their green chemical profile supports the selective extraction of valuable compounds, such as fatty acids, making DES a promising option for eco-friendly lipid recovery.[99] Recent studies have assessed the application of DES for extracting lipids and omega-3 fatty acids from fish by-products. For example, DES was applied to extract omega-3, 6, and 9 fatty acids from Vietnamese Basa (*Pangasius bocourti*) fish processing by-products. DES was synthesized using choline chloride and urea in molar ratios of 1 : 1 and 2 : 1. Initially, the concentration of these fatty acids was approximately 57% of the total content, which increased to 91% following DES application. Optimal extraction conditions were achieved using a mixture of 20 g methyl ester, 200 g methanol, and 15–20 g DES. These findings suggest that DES-based extraction significantly increased omega-3 fatty acid concentration by facilitating the removal of undesirable components, thereby improving selectivity and isolation.[100]

Furthermore, a methanol-based DES composed of methanol : lidocaine (1 : 1 M) was applied as a green solvent for isolating EPA and DHA from New Zealand green-lipped mussels (*Perna canaliculus*). This DES yielded significantly higher amounts of EPA and DHA (172.04 μg per g DW and 602.79 μg per g DW), compared to ethanol (95.65 μg per g DW and 311.49 μg per g DW, respectively). Further optimization of extraction conditions (1 : 2 M methanol : lidocaine, 65 °C, 2.12 hours, and 5 w/v%) enhanced yields resulting in 267.59 μg per g DW of EPA and 1014.84 μg per g DW of DHA. These findings highlight the potential of methanol-based DES as a sustainable and green solvent for extracting omega-3 fatty acids from marine by-products.[101]

Despite these promising results, the current literature available on DES for fish oil extraction remains limited, and a clear research gap exists regarding their application for lipid recovery from fish. Unlike the previously mentioned techniques that mainly rely on physical disruption of cell walls, DES are strongly influenced by solvent chemistry. In DES, key parameters such as polarity, viscosity, hydrogen-bonding capacity, and water content can significantly influence the ability of lipid release from the sample and the stability of PUFAs (*e.g.*, omega-3) during extraction. According to the available literature, high viscosity is a common characteristic of DES that has been a priority to be optimized, as DES viscosity can inhibit mass transfer and slow extraction kinetics. Moderate heating and controlled addition of water can reduce viscosity, but these conditions need to be carefully optimized as increased temperatures or excessive dilution can disrupt hydrogen-bond formation and alter solvent efficiency, thereby affecting lipid integrity and quality.[102]

While limited studies exist on the application of DES to fish and its by-products, studies on other marine species have shown its outperforming effectiveness in extracting lipids and carotenoids from crustacean shells, microalgae, and krill, compared to conventional solvents, especially in terms of PUFA selectivity.[102] For example, choline-chloride-based DES tend to show higher affinity toward polar lipids and phospholipid-bound omega-3s, whereas hydrophobic DES (*e.g.*, terpene-based) are more effective for neutral lipids extraction.[103,104] Due to their low volatility and reduced oxygen solubility, DES can also offer a protective medium for highly unsaturated fatty acids, although this potential needs to be further confirmed through fish oil studies.[102] One other major advantage of DES is the possibility of recycling, which enables their application over multiple extraction cycles with minimal loss of efficiency, highlighting them as economically and environmentally friendly for large-scale applications.

Despite the available data, most DES-based studies don't assess the oxidative status (*e.g.*, peroxide value, TBARS) or complete fatty acid profile of the obtained lipid fraction, leaving uncertainty regarding the real influence of DES on lipid quality and composition. Furthermore, comparative studies with other extraction techniques such as UAE and MAE are also scarce. Therefore, these points need to be prioritized in future studies on fish oil extraction.

2.2.5.3. PLE. PLE, also known as accelerated solvent extraction (ASE), is a modern green technology developed for the recovery of bioactive compounds from various matrices, including marine products and by-products. This technique combines elevated pressure (usually 10–20 MPa) with elevated temperature (ranging from 50–200 °C), allowing the solvent to remain in its liquid state below its critical point. Under these conditions, surface tension and viscosity are reduced, enhancing solvent penetration into the sample matrix (Fig. 8). This results in improved solubility of target compounds and increased mass transfer, both of which contribute to higher extraction yield. Compared to conventional methods, PLE offers several advantages: shorter processing time (usually 15–30 minutes), greater efficiency, and enhanced sustainability, especially when combined with green solvents such as ethanol or water. However, the high cost of the required equipment, along with the complexity of sample preparation and system setup, may limit its application.[105]

To date, no studies have directly applied PLE to fish tissues. However, its application to other marine products, particularly algae, has been explored. For example, PLE was employed to extract fatty acids from the brown alga *Laminaria ochroleuca* under a pressure of 100 MPa and temperatures of 80 °C, 120 °C, and 160 °C, using solvents of varying polarities (hexane, ethyl acetate, ethanol, and ethanol : water 1 : 1). The highest lipid yield (52%) was obtained using ethanol : water at 160 °C. Ethyl acetate and ethanol yielded the highest concentrations of unsaturated fatty acids, including linolenic, palmitoleic, linoleic, oleic, arachidonic, and EPA, accounting for around 55% of the total fatty acids. Furthermore, the ω-6/ω-3 ratio was lowest with ethanol, indicating superior nutritional quality.[106] Similarly, another study applied PLE to *Nannochloropsis oculata*, a microalga known for its high omega-3 content, especially EPA and DHA. Extractions were performed at 60 °C and 10–12 MPa using hexane, hexane : propanol (2 : 1), and ethanol (96%). Consistent with the previously discussed findings, ethanol yielded the highest extraction efficiency (36%), while hexane produced the lowest yield (6.1%). The total fatty acid yield reached 16.7% with ethanol, with EPA comprising 3.7%.[107]

Although PLE has not been applied to fish tissues, these findings reinforce the potential of PLE, especially ethanol-based PLE, as an innovative technique for recovering omega-3 fatty acids from marine samples. PLE is strongly influenced by the interplay between temperature, pressure, and solvent polarity. Elevated temperatures help increase lipid solubility and disrupt cell walls, while pressure plays a dual role ensuring that the solvent remains in its liquid state and enhancing its penetration into complex cell walls of the sample, especially those of marine species.[108] Together, these conditions can maximize the release of omega-3 fatty acids, but if uncontrolled, they may also accelerate oxidation of PUFAs and affect their concentrations.[109] Moreover, solvent polarity is another major factor to be considered, where the choice of solvent is important based on the targeted compounds of interest. For example, ethanol and ethanol : water mixtures primarily target the extraction of PUFA-rich fractions, whereas non-polar solvents can favor the recovery of neutral lipids with lower content of omega-3 content.[109] Therefore, optimizing PLE conditions requires complex study and careful control to enhance the release of compounds of interest while avoiding thermal degradation. When optimized, PLE can achieve higher yields and better selectivity compared to conventional solvent extraction or other advanced techniques.[105,106,109] However, further investigations are needed to assess its application for lipid recovery from fish products and by-products.

The following figure (Fig. 9) summarizes all the previously discussed green extraction methods and emerging techniques applied for fish oil recovery.

2.2.6. Industrial feasibility, cost considerations, and technological readiness. Green extraction technologies offer significant advantages over conventional extractions, including reduced solvent usage, lowered extraction temperature, and shortened processing time. These conditions consequently help in the preservation of thermolabile compounds such as EPA, DPA, and DHA. However, their scale up from laboratory to

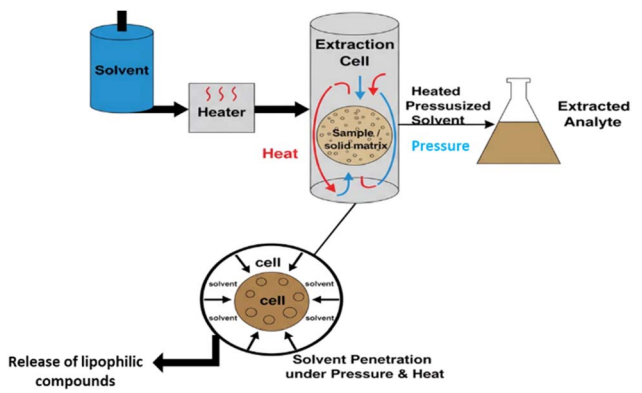

Fig. 8 PLE for fish oil recovery.

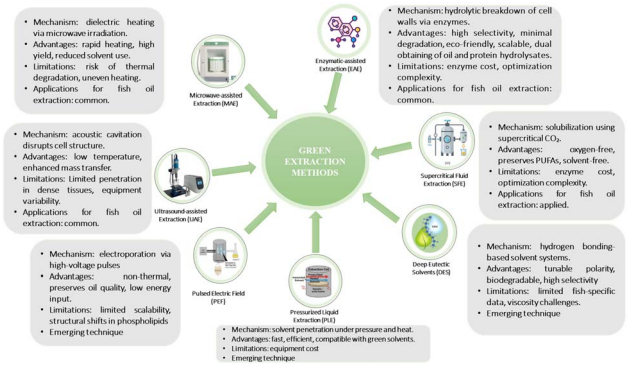

Fig. 9 Comparative summary of green extraction methods for fish oil recovery.

industrial level remains limited due to several economic, operational, and technological constraints.

Conventional extraction methods (*e.g.*, wet pressing, cold pressing, tricanter centrifugation) remain widely applied in the industries owing to their robust scalability, continuous operation, and compatibility with regulatory standards and industrial infrastructure. These techniques allow high-operational processing of diverse fish matrices and fish by-products without the need for sophisticated equipment and complex process control. However, their major limitation lies in the harsh thermal and mechanical conditions, which trigger oxidation and degradation of omega-3 PUFAs, thereby altering the overall oxidative and nutritional quality of the final product. For this reason, most industries often address this issue through subsequent refining steps.

In contrast, green extraction technologies (*e.g.*, MAE, UAE, EAE, SFE, and other emerging approaches) offer clear advantages in terms of milder extraction conditions (*e.g.*, time, temperature, solvent use), but their scale-up faces some challenges. For example, MAE requires specialized microwave reactors and can experience non-uniform heating at larger volumes due to limited in-depth penetration. UAE, on the other hand, presents a better potential for scalability because powerful ultrasonic systems can be incorporated into flow-through reactors and their energy can be distributed more uniformly. Although cavitation efficiency might decrease as volume increases, UAE remains easier to scale than MAE because its reactor design is more flexible and the system can be expanded to ensure effective energy distribution. EAE has a good scalability profile since enzymatic processes are already widely applied in the industrial sector.[110,111] However, enzyme cost, stability, and the need for a subsequent separation step remain limiting factors. In addition, SFE and other emerging technologies face limited industrial implementation in fish oil extraction due to high cost of equipment and the need for precise monitoring and optimization of the process conditions.

In terms of cost considerations, the continued dominant use of conventional extraction methods such as wet and cold pressing is related to their moderate equipment cost and relatively low operational expenses, as their required energy relies either on heating or mechanical force, both of which are manageable at the industrial level. In contrast, conventional solvent-based laboratory techniques (*e.g.*, Folch, Soxhlet) are not suitable for industrial use due to their reliance on large amounts of toxic solvents and long extraction times, both of which can pose significant labor and safety risks. Although green extraction methods are environmentally friendly, they require high investment in terms of specialized equipment, process optimization, training and maintenance costs, and costly enzymes (EAE). While some emerging techniques such as DES may be less expensive and offer the advantage of being recyclable, challenges such as high viscosity and slow mass transfer still limit their application. In this context, both economic feasibility and environmental consequences should be evaluated through Life Cycle Costing (LCC) and Life Cycle Assessment (LCA), which allow a comprehensive assessment of investment costs, operational expenses, and long-terms sustainability and environmental impacts. By integrating LCC and LCA, a more realistic understanding of the overall industrial profitability can be achieved.[112,113]

Overall, while conventional mechanical extractions can operate at an industrial scale, green technologies remain at a laboratory or pilot scale. Recent studies highlight UAE and EAE as the most promising methods for industrial scale-up, particularly as pretreatments for already existing workflows that can enhance the yield and the quality of fish oil. In contrast, other methods like MAE, SFE, DES, PLE, PEF still require further optimization and pilot-scale assessment in terms of more practical demonstrations, cost–benefit assessments, and evaluation of environmental and economic impacts through LCA and LCC analyses.[114]

3. Omega-3 fatty acids

After the discussion of conventional extraction, as well as green extraction techniques which are increasingly favored for their reduced reliance on high temperatures and toxic solvents, it is essential to address the next critical step: enhancing the nutritional profile of extracted fish oil, particularly through omega-3 enrichment. While the milder conditions of green extraction methods aim to preserve bioactive compounds such as omega-3 PUFAs, some losses may still occur during processing and extraction.[115] Furthermore, the availability of omega-3 fatty acids in the initial sample depends on several factors. To better understand this topic, the following section will explore the structural characteristics, dietary sources, synthesis, and health benefits of omega-3 fatty acids. This will provide a comprehensive overview of these fatty acids before diving into the next section on omega-3 enrichment strategies to address the growing consumer demands for natural, high-purity sources of omega-3 fatty acids.

3.1. Structural classifications

Omega 3 fatty acids are considered among the most well-known PUFAs for their significant health benefits. Structurally, omega-3 fatty acids are characterized by the presence of a double bond between the third and fourth carbon atoms in their hydrocarbon chain, counting from the methyl ($-CH_3$) end. This structural feature explains why they are referred to as "omega-3" (ω-3) fatty acids. The *cis* configuration of their double bonds allows the chain to be highly flexible, which contributes to their biological activity.[116] ω-3 PUFAs include different types that can be classified according to their chemical structure. The simplest form is ALA, which consists of an 18-carbon chain and three double bonds (18:3). When consumed from food sources, only a small fraction of ALA undergoes a multi-step metabolic pathway to form EPA and DHA. The process begins with the desaturation of ALA into stearidonic acid (SDA; 18:4 n-3) *via* the delta-6 desaturase enzyme. This is followed by the elongation to eicosatetraenoic acid (ETA; 20:4 n-3) with the aid of elongase. ETA is then desaturated by delta-5 desaturase to form eicosapentaenoic acid (EPA; 20:5 n-3). EPA is further elongated to docosapentaenoic acid (DPA; 22:5 n-3) which is then converted

to docosahexaenoic acid (DHA; 22:6 n-3) through a complex process that involves elongation, desaturation, and β-oxidation in peroxisomes.[117] This pathway requires specific enzymes responsible for the formation of *cis* double bonds and elongating the carbon chain. However, these enzymes are not sufficiently expressed in the human body, resulting in a limited and inefficient conversion of ALA to EPA and DHA.[118,119] Given this limitation, the recommended combined intake of EPA and DHA is 250–500 mg per day.[11]

3.2. Sources

Omega-3 fatty acids are considered essential nutrients that must be obtained from dietary sources to meet physiological needs. These sources vary depending on the form of omega-3 present. For example, plant-based sources, such as flaxseeds, chia seeds, walnuts, green leafy vegetables, canola oil, and soybean oil, are rich in ALA.[116] However, because ALA endogenous conversion to EPA and DHA is highly limited, plant sources alone are generally insufficient to meet recommended intake. In contrast, marine organisms are rich in EPA, DPA, and DHA, making them the most reliable dietary sources of omega-3s.[120]

The lipid content and omega-3 composition of marine species can vary significantly due to internal and external factors. Internal biological factors, including age, developmental stage, reproductive status, metabolic rate, and tissue-specific lipid deposition, strongly influence PUFA levels.[121,122] For instance, many fish increase lipid reservation prior to spawning in order to support the elevated energy demands, which consequently alters omega-3 composition of muscle and liver tissues.[123] Furthermore, younger fish usually contain lower lipid stores, whereas larger and mature fish tend to accumulate higher lipid concentrations.[124] In addition, species-specific differences are also major influencers. For example, Atlantic herring, with a total lipid content of 10.5–16%, can provide between 700–1100 mg EPA and 900–1400 mg DHA per 100 g. On the other side, lean species such as cod, with only 1–2% lipids, provide up to 150 mg EPA and 200 mg DPA per 100 g.[125–127]

Additionally, external factors such as feeding pattern, water temperature, salinity, depth, and seasonal changes can affect omega-3 profiles.[128,129] Diet and trophic levels are among the most influential factors. Since fish do not synthesize omega-3 fatty acids *de novo*, but rather obtain them from the consumption of marine microalgae (*e.g.*, *Schizochytrium* and *Nannochloropsis*), species feeding on PUFA-rich zooplankton (*e.g.*, copepods) exhibit profiles rich in EPA and DHA, and species feeding on them (*e.g.*, sardines, anchovies, herring) tend to hold higher omega-3 PUFA levels.[122,130] In addition, cold-water species generally accumulate higher PUFA levels to maintain membrane flexibility and function in low-temperature conditions. This explains why fish species such as salmon and mackerel present in colder regions have higher EPA and DHA levels compared with warm-water species.[131] To increase omega-3 intake among populations with low fish consumption, many food products such as milk, yogurt, juice, cereals, and vegetable oils, are now fortified with omega-3. In addition, omega-3 supplements, primarily derived from fish oil, are widely available and serve as a concentrated source of EPA and DHA. These supplements typically contain omega-3s in the form of natural triacylglycerols, ethyl esters, free fatty acids, or phospholipids, depending on the formulation.[132] For individuals with limited dietary intake, such as those with fish allergies or following vegan or vegetarian diets, supplementation is essential. In other cases, a combination of dietary sources and supplements is ideal for achieving optimal health outcomes.

In addition to edible species, fish by-products represent a promising valuable source of omega-3 PUFAs. By-products, including heads, skin, viscera, liver, and trimmings, can account for 40–60% of total fish biomass depending on the species and processing method. These residual products often contain lipid profiles comparable to, or even exceeding, those of fillets. In particular, viscera and liver accumulate substantial amounts of long-chain PUFAs as they are considered the primary sites of lipid storage.[133,134] As discussed in the previous sections, several studies have successfully extracted high-quality oils from fish by-products, highlighting their potential as sustainable and cost-effective sources of omega-3.[110,111,135–140]

4. Post-extraction purification and stabilization of fish oil

Given the significant health benefits of omega-3 fatty acids, especially EPA and DHA, their preservation is essential to maintaining the nutritional value and health benefits of fish oil. While the extraction process aims to recover these bioactive compounds, crude fish oil often contains undesirable substances and impurities, such as insoluble phospholipids, trace metals, pigments, residual solvents, and organic pollutants, which may alter its quality and safety for human consumption. Hence, to ensure that the oil is suitable for nutritional and pharmaceutical applications, a series of post-extraction purification and stabilization steps are typically employed. These steps are crucial for removing contaminants and off-flavors while preserving the oil's nutritional integrity and oxidative stability, especially the sensitive omega-3 PUFAs.[30]

The initial purification step involves degumming, which targets the removal of undesirable compounds like phospholipids and trace metals that contribute to emulsification, instability, and reduced shelf life.[14] Traditionally, chemical degumming starts with the addition of water, resulting in the swelling and separation of phospholipids from the oil. Non-hydratable phospholipids that remain bound to oil require an additional step of acid treatment, typically using a small amount (0.1–0.3%) of an 85% solution of citric or phosphoric acid neutralized with sodium hydroxide to precipitate these impurities. The process continues with separation of the precipitated phospholipids using centrifugation or decantation, leaving behind a high-purity oil. However, the addition of water and acids, even in small amount, may cause partial hydrolysis of some lipids, compromising the lipid integrity of the oil.[141,142] Therefore, green alternatives have been proposed and applied. These include enzymatic degumming using phospholipases

such as Lecitase Ultra® and LysoMax®, which can selectively hydrolyze phospholipids into lysophospholipids and other small fragments that usually dissolve in the aqueous phase and can be separated later.[14,143] These enzymes operate under mild temperatures and controlled pH, allowing effective purification without the need for harsh conditions (*e.g.*, acid) and resulting in minimal impact on omega-3 concentrations, thus offering a gentler approach aligned with natural product preservation.[144,145]

Following degumming, deacidification then is usually applied as it targets FFAs, which often contribute to rancidity and off flavours. The conventional process utilizes alkali neutralization with a base such as sodium hydroxide to react with FFAs, converting them into soap (fatty-acid salts), which can be further separated by centrifugation and/or washing. However, this saponification reaction can generate an aqueous soap stock, where the soap emulsifies with water and can retain with it some oil, raising concerns about the possible risk of partial hydrolysis of PUFAs. For this reason, molecular distillation or vacuum steam application can be applied to remove volatile FFAs without the need for alkali treatment. However, these methods require high temperature control and long processing time which can degrade the thermolabile oil compounds and volatile antioxidants. However, nowadays, other approaches such as enzyme-assisted deacidification using Novozym® 435 (lipase from *Candida antarctica*) have shown promising results by offering milder alternatives that can work under moderate temperatures, where they can re-esterify and remove FFAs, thereby lowering the acidity value while better preserving EPA and DHA. However, the high cost of enzymes and the need for enzyme immobilization and process optimization limit their application. Additionally, selective separation approaches, such as membrane-based fractionation and deep eutectic solvents (DES) (*e.g.*, betaine or choline-based solvent systems), allow selective removal or FFAs and polar contaminants while preserving antioxidants and omega-3s, offering greener options and milder alternatives to conventional alkali-based processes which might alter the extracted fish oil integrity. However, these methods still require process optimization and are quite costly.[144]

After deacidification removes FFAs and polar impurities, the bleaching step follows to improve color and stability by removing pigments, oxidation–prone compounds, and certain trace metals that can alter the oil color. Adsorbents that are commonly used include acid-activated carbon, natural clays, silica, and magnesium silicate (Magnesol). Each adsorbent is characterized by its own affinity for pigments and other oxidation compounds and metal ions. When applied under optimized conditions, such as under vacuum treatment or inert atmosphere and under controlled temperatures, bleaching minimizes exposure to oxygen and thermal degradation, representing an acceptable step for removing pigments and heavy metals with minimal impact on omega-3 EPA and DHA.[146] Process variables such as type of adsorbent, concentration, contact time, temperature, and stirring speed can significantly influence the effectiveness of pigment removal and the oxidation status of the oil (*e.g.*, peroxide and AV). For example, prolonged exposure to high temperatures or overuse of adsorbents can lead to oxidation, remove fat soluble antioxidants, and adsorb some bioactive substances, thereby reducing the nutritional quality and altering the oxidation status of the oil. Therefore, the conditions must be carefully controlled to ensure producing a high-quality, stable, and nutritious fish oil.

The purification process continues with deodorization, which is applied to remove volatile compounds (*e.g.*, aldehydes and ketones) that might be formed during lipid oxidation and are responsible for the unpleasant odors of the oil. While vacuum steam distillation has been traditionally used, the elevated temperatures and prolonged times can degrade sensitive compounds such as omega-3s and volatile antioxidants.[144] To address this issue, researchers have shifted towards gentler alternatives, such as nanofiltration, which separates molecules based on size and polarity, and short-path or wiped-film vacuum distillation that operate under lower and controlled temperatures. These milder conditions were shown to effectively remove volatile odorants while better preserving EPA and DHA concentrations, aligning with the goal of producing natural and high-quality fish oil.[147]

Studies have shown that these purification and stabilization steps, when optimized, can significantly improve the concentration of PUFAs and enhance oxidative stability, as measured by peroxide and AV. Importantly, these methods support the production of fish oil that is safe, stable, and suitable for enrichment, without compromising its natural composition.[14,146,148]

5. Omega-3 enrichment strategies

While post-extraction purification of fish oil is essential for removing impurities such as phospholipids, pigments, and trace metals, thereby improving its oxidative stability and sensory quality, it doesn't sufficiently enhance the oil's nutritional profile. In particular, the concentration of long-chain PUFAs, especially EPA and DHA, often remains below the recommended levels required for achieving therapeutic benefit. To meet the growing demand for omega-3 rich products, the incorporation of an additional enrichment step is necessary to increase the EPA and DHA content and improve their bioavailability. In fact, the addition of this step boosts the health benefits of the oil and meets both regulatory standards and consumer expectations for fish oil quality. Different techniques have been proposed for this aim, including Urea Complexation (UC), Low Temperature Crystallization (LTC), Molecular Distillation (MD), membrane-based enrichment, silica gel column chromatography, and Enzymatic purification. The following section outlines the different techniques that have been suggested and developed for this aim, highlighting the distinct advantages and limitations of each method.

5.1. UC

UC is one of the most widely used methods for enriching omega-3 PUFAs such as EPA and DHA in fish oil.[148] This technique utilizes urea due to its ability to form crystalline

complexes when mixed with SFAs and MUFAs, leaving PUFAs unbound due to their complex structure and multiple *cis* double bonds, which prevent them from crystallization with urea. In this method, fish oil is first converted to its ethyl ester form, then mixed with urea and an organic solvent (*e.g.*, ethanol or methanol) to enhance solubility and promote interaction. After the mixture is cooled to a specific temperature that allows crystallization, the urea–fatty acids complex forms crystals, isolating PUFAs in the remaining liquid fraction, which can be subsequently obtained through filtration or nitrogen blowing.[148]

Although it is considered a conventional method, UC offers several benefits as it has proven its effectiveness in selectively concentrating EPA and DHA PUFAs. In this context, different studies have assessed the effect of UC application with the aim of increasing the concentrations of omega-3 in fish oil. For example, optimized UC conditions using RSM and reported that under optimal parameters: urea : fatty acid ratio of 6.0, crystallization temperature of −18 °C, duration of 14.8 hours, and stirring speed of 500 rpm, the total omega-3 content in salmon oil significantly increased from 13.78% to 80.51%, with EPA increasing from 7.53% to 31.2% (a 4.1-fold increase).[149] Similarly, another study demonstrated that under optimized conditions (urea : fatty acid ratio of 4.21, –15 °C, 24 hours and 1000 rpm), 71.52% of EPA and DHA were obtained, with EPA reaching 20.50%.[150]

5.2. LTC

LTC is another widely used strategy for enriching omega-3 PUFAs, particularly EPA and DHA, in fish oil. The principle of this technique is conceptually similar to urea complexation, utilizing the differential crystallization behavior of fatty acids under controlled cooling process. During LTC, saturated fatty acids are separated through a cooling process that results in their crystallization into the solid phase, while PUFAs are retained in the liquid phase. Solvents such as hexane, acetone, and methanol are commonly employed to enhance crystallization efficiency and purity. Additionally, antioxidants such as α-tocopherol can be added to prevent oxidative degradation during the process and further improve the quality of the oil.

Several key parameters can influence the effectiveness of LTC, including oil composition, solvent type, oil-to-solvent ratio, crystallization temperature, and cooling rate. Generally, lower crystallization temperatures favor PUFAs retention in the liquid phase. For example, applying LTC to fish oil derived from herring and sprat by-products from the Baltic Sea at −70 °C for 18 hours using acetone significantly increased PUFA content from 35.62% to 61.72%, with the addition of α-tocopherol in methanol effectively preventing oxidation during enrichment.[151] Similarly, LTC applied to sardine oil in hexane at varying crystallization temperatures (−55, −65, −75, and −85 °C) and durations (1–24 hours) allowed the identification of optimal process conditions at −85 °C for 24 hours, where PUFAs concentration increased from 45.5% to 83.4%, with EPA reaching 38.6%.[152] Despite its proven efficacy and cost-effectiveness, various challenges limit the application of LTC. These include the requirement for specialized equipment to maintain ultra-low temperatures and the potential for residual solvent contamination, especially since they are often used in large volumes.[148]

5.3. MD

MD is an advanced separation technique that utilizes high vacuum and elevated temperatures applied over a short duration. The extremely low distillation pressure in this technique allows sensitive compounds to evaporate at significantly lower temperatures, due to their decreased boiling point compared to the standard conditions. Hence, thermal stress is minimized, which eventually helps in preserving heat-sensitive compounds such as EPA and DHA during the separation process. In fact, a distinctive characteristic of MD is that the distance between the evaporation surface and the condensation surface is less than or equal to the mean free path of the molecules. This means that as compounds escape from the evaporation surface, they can rapidly move to the condenser without interacting with other compounds, thereby allowing effective and rapid condensation. Subsequently, the molecules are condensed with minimal thermal degradation.[148,153]

In the context of fish oil enrichment, the different fatty acids present in fish oil with varying lengths of carbon chain and degrees of unsaturation affect their characteristics, particularly their boiling points under high vacuum. Hence, different stages of molecular distillation are performed, with adjustments to temperature, pressure, and flow rate across each stage to ensure that specific types of PUFAs, such as EPA and DHA, are selectively separated. For this aim, commercial standards of EPA and DHA are often used to validate the separation process. Various optimization methods have been employed for the optimization of molecular distillation technique, including RSM and other predictive models to simulate optimal conditions of MD.[154]

There is a scarcity of literature on the isolation and purification of omega-3 using molecular distillation, with only limited studies addressing this strategy. For instance, an optimized separation process of omega-3 fatty acids was achieved using two stages: the first stage operating between 100–140 °C and the second stage between 120–140 °C, where a validated mass transfer model was employed to explain the dynamics of evaporation. Subsequently, different predictive models were created to optimize MD parameters and simulate its complex behaviors.[155]

Overall, the MD technique offers a promising method for PUFAs enrichment due to its efficiency in the selective removal of MUFAs, which are challenging to be removed using other methods. Furthermore, MD offers other superior advantages as it is solvent-free, scalable, and suitable for high-boiling compounds. However, the high operational costs along with sophisticated heating and vacuum systems limit its application in small-scale industries.[148,156]

5.4. Membrane-based enrichment

Among other emerging omega-3 enrichment strategies, membrane-based filtration has gained significant attention as

a promising and environmentally friendly technique. This method utilizes membranes with defined pore size, enabling selective permeability based on molecular size and weight. In this context, the selective isolation of long-chain PUFAs, namely EPA and DHA, can be achieved, separating them from other lipid fractions.[154,157]

Membrane filtration operates without the use of organic solvents, under mild temperatures and pressures, and is considered a low-energy non-complex process. These characteristics favor its application. The use of dense polymeric membranes, especially when coupled with enzymatic transesterification, has shown significant enrichment effectiveness. For example, when applied to fish oil, the omega-3 content increased from 31.65% up to 54% (a 1.7-fold increase), where the process was applied and evaluated in a pilot-scale membrane-based process, which was shown to be technically feasible and cost-effective.[154] Despite its proven effectiveness, the method presents several limitations where the membrane could be blocked overtime due to the accumulation of lipids, proteins, and other macromolecules, which reduces permeability and necessitates frequent cleaning. The membrane cleaning conditions and chemicals used can eventually degrade its material, resulting in a shorter operational lifespan. In addition, membrane systems are generally expensive, and their integration at an industrial scale requires high costs, especially when considering the additional costs for pretreatment steps, such as degumming and deacidification discussed earlier, which are necessary in this case to ensure compatibility with membrane performance.[148,157]

5.5. Silica gel column chromatography

Silica gel column chromatography offers an effective strategy for enriching EPA and DHA omega-3 PUFAs. The method is based on the differences in polarity among the various fatty acids present in fish oil. In this technique, the oil is passed through a column based packed with silica gel, where MUFAs and SFAs are retained due to their lower degree of unsaturation and simpler molecular structure, allowing closer packing and stronger van der Waals interactions with the silica surface. On the contrary, due to their more complex structure and high degree of unsaturation, EPA and DHA interact weakly with the silica surface, resulting in their faster elution and effective separation from other fatty acids. This selective isolation can be enhanced when combined with the gradient elution chromatography method, which allows separation based on polarity shifts in the mobile phase. Studies have demonstrated the effectiveness of this technique in enriching EPA and DHA, especially within specific lipid subclasses such as phosphatidylglycerol (PG) and phosphatidylethanolamine (PE), which are known for their higher bioavailability and functional relevance. However, silica gel chromatography presents several limitations, as the method requires high operational costs, extensive optimization, and the use of large volumes of organic solvents. Despite these challenges, it can be considered a complementary method to other enrichment methods such as urea complexation or molecular distillation, helping to achieve better purification of omega-3.[158]

5.6. Enzymatic purification

The use of enzymes offers a green, selective, and cost-effective strategy for the enrichment of omega-3 PUFAs. Due to its simplified processing, low equipment requirements, and solvent-free operation under moderate temperatures, this approach has been a preferred alternative to the more complex strategies discussed earlier. Various enzymes have been evaluated for this aim, including protease, exopeptidase, endopeptidases, and lipases.

Among these, lipases are particularly valued for their high selectivity toward omega-3 PUFAs. However, their hydrolytic activity against EPA and DHA is often limited particularly due to steric hindrance caused by the high number of double bonds in these fatty acids, which create a barrier that prevents enzymatic access and hydrolysis. This selective blockage allows lipases to hydrolyze other fatty acids, resulting in the formation of free-fatty acids, monoacylglycerols, diacylglycerols, and triacyclglycerols, while leaving the intact forms of EPA and DHA, which can be subsequently isolated.[159]

Lipases from different microbial and fungal sources exhibit variable selectivity. For example, *Thermomyces lanuginosus* lipase has demonstrated enhanced selectivity toward EPA during ethyl esterification of fish oil.[160] Similarly, OUC-lipase 6, an alkaline and organic solvent-resistant lipase, has demonstrated its regioselectivity favoring EPA enrichment.[161] Moreover, a comparative analysis of three different lipases revealed that lipases from *T. thermophila* and *Rhizomucor miehei* were more EPA-selective than *Candida antarctica* B, highlighting the importance of considering enzyme source and specificity before application.[162]

Therefore, despite being environmentally friendly, nearly solvent-free, and requiring simple operational conditions, the application of the enzymatic purification method faces several challenges. These include the favorable EPA-selective enrichment over DHA, and the susceptibility of enzymes to denaturation and degradation, which raises concerns about their stability, reusability, and lifespan, increasing overall process costs.[148]

5.6.1. Marine lipases in omega-3 enrichment. To address some of these limitations, particularly low enzymatic stability and substrate selectivity, marine-derived lipases have emerged as promising biocatalysts. These enzymes, sourced from marine fungi, bacteria, actinomycetes, yeasts, and other extremophilic microorganisms, have been shown to possess enhanced thermal and pH stability, as well as higher growth rates. Their ease of production at laboratory scale and feasibility for industrial application make them ideal candidates for omega-3 enrichment.[163]

Among marine lipases, yeast-derived enzymes such as those from *Candida antartica* and *Candida rugosa* are commonly used and are classified as Generally Regarded as Safe (GRAS), making them suitable for food-grade applications, including fish oil. Studies have shown that marine microbes display high

adaptability to environmental conditions, with each strain having its own optimal working range. For example, yeast strains such as *Yarrowia lipolytica* and *Candida parapsilosis* produce stable lipases at 35–40 °C and pH 6.0–8.5, while lipases from *Oceanobacillus caeni* remained active at a wider pH range (3–11) and temperatures from 10 to 70 °C.[164]

To further enhance the stability and performance of marine lipases, enzyme immobilization has been applied. This technique involves attaching enzymes to solid matrices (*e.g.*, agarose, chitosan, cellulose, silica) to improve stability and facilitate recovery for reuse. In this context, immobilization of *Candida antarctica* lipase B on resin resulted in increased selectivity and stability.[165]

Despite the effectiveness of the current omega-3 enrichment strategies, the low recovery of DHA compared to EPA remains a significant and persisting challenge. Recent strategies are therefore focused on developing approaches to overcome this limitation and address the selectivity gap. One promising approach is enzyme engineering, where the structure of lipases is modified to improve how their active sites target DHA, whose high degree of unsaturation makes it more difficult to access during hydrolysis. Specifically, marine-derived lipases are interesting in this context, as they naturally tolerate extreme conditions and have shown flexible substrate preferences. Studies also indicate that these enzymes' performance improves when they are immobilized. In particular, covalent immobilization, where the enzyme is attached to solid support through covalent bonds, allows for better control of the reaction by increasing stability and preventing leaching, all of which can enhance DHA selectivity. Additional emerging techniques include the use of multi-enzyme systems and hybrid enrichment methods, in which enzymatic hydrolysis is coupled with another separation step (*e.g.*, membrane filtration, urea complexation, or molecular distillation). By pairing different techniques, these hybrid systems can enhance DHA recovery compared to single methods alone. Together, these emerging approaches represent promising candidates for achieving a more balanced enrichment of both EPA and DHA, although further research is still needed to optimize these methods for industrial applications.[163]

5.7. Industrial feasibility and applications of omega-3 enrichment

The continuously growing demand from consumers for high-quality, high-purity EPA and DHA-rich fish oil, especially due to the increasing spotlight on its nutraceutical benefits, has placed significant pressure on the nutraceutical industry. Companies are required to produce and deliver enriched fish oil products that meet nutritional and quality standards while also aligning with cost-efficiency benchmarks, a balance that is often challenging.[1]

In fact, when considering again the previously discussed methods, molecular distillation remains the most widely adopted method at the commercial scale due to its proven efficiency in producing highly concentrated omega-3 fractions that meet regulatory standards. However, its high energy and cost demands, mainly due to the need for high temperatures and complex vacuum systems, make it less feasible for smaller industries or those operating with a limited budget.[156]

In contrast, when viewed from a green chemistry perspective, especially with the rising awareness and shift toward environmental sustainability, membrane-based filtration and enzymatic purification methods stand out as more eco-friendly and energy-efficient alternatives. In particular, membrane-based filtration has shown promising results at the pilot scale, where economic evaluations have suggested that it can be competitive with other industrial techniques, particularly when coupled with enzymatic transesterification.[154] In addition, enzymatic approaches, mainly those involving immobilized marine lipases, are gaining more attention for their selectivity and mild processing conditions, although enzyme degradation and lower DHA selectivity remain limiting factors.[153,159]

Despite ongoing efforts to improve these techniques, industries continue to face persistent challenges. The main persistent issues lie in process optimization, especially in identifying optimal conditions that enhance DHA selectivity, which remains underrepresented in most methods despite various advancement trials. Moreover, membrane fouling and degradation, enzyme denaturation, and concerns about long-term stability raise questions about the practicality of applying these methods at an industrial scale. On top of that, regulatory constraints, such as solvent use and GRAS certification further add complexity and limit flexibility.[154]

Current efforts to overcome these challenges and improve the enrichment process include exploring hybrid techniques and AI-driven process control systems. These approaches aim to enhance yield, reduce losses, and improve product quality, ultimately helping to develop method systems that are scalable, sustainable, and optimized both nutritionally and economically.

6. Conclusions

The continuous market demand for high-quality, omega-3-rich fish oil—driven by its remarkable nutraceutical benefits—poses a significant challenge for industries striving to balance between high product quality, cost-efficiency, and high profitability, while also complying with sustainability and green chemistry regulations. Although significant advanced have been achieved in extracting fish oil and enriching it with omega-3, several critical research gaps persist. A major challenge is scaling up these advanced and greener extraction methods. Most of these techniques have shown outstanding effectiveness at laboratory scale, but there is still limited evidence on their real performance at an industrial level. Therefore, future work should include robust pilot-scale studies and economic (LCC) and sustainability assessments (LCA). Another research gap is related to DHA enrichment, where most recent methods have shown a clear selectivity for EPA over DHA, which remains difficult to target due to its structure. Therefore, careful optimization and comprehensive understanding of all process-influencing parameters and their variability ranges are needed. In this context, implementing a multivariate design of

experiments is crucial to systematically evaluate these factors and identify the optimal conditions. Such an approach enables the best possible compromise between competing variables, ultimately aiming to maximize both the extraction yields and the enrichment step.[166]

Moreover, the rising research interest in sustainability and circular economy approach is crucial, but future work needs to explore how diverse mixtures of fish by-products can be reliably upcycled and converted into high-value omega-3 oils, especially considering the natural heterogeneity of by-products, their complex composition, and the added costs of pre-treatment steps such as sorting, which might be challenging for small-scale industries. The European EcoeFISHent project focuses precisely on this aspect of utilizing 'non-separated' fish biomass in order to overcome one of the most expensive steps in the value chain. By addressing these gaps, the field can move forward toward extraction and enrichment methods that enable the production of high-quality omega-3-rich fish oil using methods that are not just efficient, but also scalable and environmentally friendly.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included, and no new data were generated or analysed as part of this review.

Acknowledgements

The present work stems from the EcoeFISHent project ("Demonstrable and replicable cluster implementing systemic solutions through multilevel circular value chains for eco-efficient valorisation of fishing and fish industry side-streams"), funded under the Horizon 2020 Green Deal – Innovation Action (Grant Agreement ID: 101036428). AI-powered tools, particularly Microsoft Copilot and Google Gemini, were employed during manuscript preparation—Copilot supported language editing and refinement, while Gemini facilitated the creation of illustrative figures.

References

1 M. T. Baker, P. Lu, J. A. Parrella and H. R. Leggette, *Int. J. Environ. Res. Public Health*, 2022, **19**, 1217.
2 S. U. Khan, A. N. Lone, M. S. Khan, S. S. Virani, R. S. Blumenthal, K. Nasir, M. Miller, E. D. Michos, C. M. Ballantyne, W. E. Boden and D. L. Bhatt, *eClinicalMedicine*, 2021, **38**, 100997.
3 Y. Song, Q. Wang and L. Jia, *J. Clin. Hypertens.*, 2024, **26**, 382–390.
4 M. Banaszak, M. Dobrzyńska, A. Kawka, I. Górna, D. Woźniak, J. Przysławski and S. Drzymała-Czyż, *Clin. Nutr. ESPEN*, 2024, **63**, 240–258.
5 R. K. Saini and Y.-S. Keum, *Life Sci.*, 2018, **203**, 255–267.
6 R. K. Saini, P. Prasad, X. Shang and Y.-S. Keum, *Int. J. Mol. Sci.*, 2021, **22**, 13643.
7 C. Picot-Allain, M. F. Mahomoodally, G. Ak and G. Zengin, *Curr. Opin. Food Sci.*, 2021, **40**, 144–156.
8 I. Hamed, F. Özogul, Y. Özogul and J. M. Regenstein, *Compr. Rev. Food Sci. Food Saf.*, 2015, **14**, 446–465.
9 M. Pateiro, P. E. S. Munekata, R. Domínguez, M. Wang, F. J. Barba, R. Bermúdez and J. M. Lorenzo, *Mar. Drugs*, 2020, **18**, 101.
10 S. Alijani, A. Hahn, W. S. Harris and J. P. Schuchardt, *Prog. Lipid Res.*, 2025, **97**, 101318.
11 D. S. Siscovick, T. A. Barringer, A. M. Fretts, J. H. Y. Wu, A. H. Lichtenstein, R. B. Costello, P. M. Kris-Etherton, T. A. Jacobson, M. B. Engler, H. M. Alger, L. J. Appel and D. Mozaffarian, *Circulation*, 2017, **135**, e867–e884.
12 M. Pateiro, R. Domínguez, T. Varzakas, P. E. S. Munekata, E. Movilla Fierro and J. M. Lorenzo, *Mar. Drugs*, 2021, **19**, 233.
13 M. Rodrigues, A. Rosa, A. Almeida, R. Martins, T. ânia Ribeiro, M. Pintado, R. F. S. Gonçalves, A. C. Pinheiro, A. J. M. Fonseca, M. R. G. Maia, A. R. J. Cabrita, L. Barros and C. Caleja, *Food Bioprod. Process.*, 2024, **145**, 32–41.
14 M. Yi, Y. You, Y. Zhang, G. Wu, E. Karrar, L. Zhang, H. Zhang, Q. Jin and X. Wang, *Molecules*, 2023, **28**, 672.
15 International Fish Oil Standards (IFOS), *Codex Standard for Fish Oils (CODEX STAN 329-2017)*, IFFO, London, 2017, accessed 26 October 2025.
16 A. Mathews, A. V. Arbal, A. Kaarunya, P. K. Jha, A. Le-Bail and A. Rawson, in *Extraction Processes in the Food Industry*, ed. S. M. Jafari and S. Akhavan-Mahdavi, Woodhead Publishing, 2024, pp. 97–146.
17 J. Folch, I. Ascoli, M. Lees, J. A. Meath and F. N. LeBaron, *J. Biol. Chem.*, 1951, **191**, 833–841.
18 J. Folch, M. Lees and G. H. S. Stanley, *J. Biol. Chem.*, 1957, **226**, 497–509.
19 A. Hara and N. S. Radin, *Anal. Biochem.*, 1978, **90**, 420–426.
20 B. de la Fuente, J. Pinela, F. Mandim, S. A. Heleno, I. C. F. R. Ferreira, F. J. Barba, H. Berrada, C. Caleja and L. Barros, *Food Chem.*, 2022, **386**, 132778.
21 M. Usman, M. Nakagawa and S. Cheng, *Processes*, 2023, **11**, 3444.
22 E. Imamoglu, *Bioresour. Technol. Rep.*, 2024, **27**, 101952.
23 K. Chakraborty and D. Joseph, *Eur. J. Lipid Sci. Technol.*, 2015, **117**, 837–850.
24 T. Fouda, *J. Zool. Res.*, 2020, **2**, 14–19.
25 M. D. Luque de Castro and F. Priego-Capote, *J. Chromatogr. A*, 2010, **1217**, 2383–2389.
26 E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.*, 1959, **37**, 911–917.
27 F. Smedes, *Analyst*, 1999, **124**, 1711–1718.
28 A. P. Bimbo, *Marine Oils – AOCS*, accessed 30 October 2025.
29 FAO, *The Production of Fish Meal and Oil – Contents*, 1986, accessed 30 October 2025.
30 J. R. Bonilla and J. L. H. Concha, *Corpoica Cienc. Tecnol. Agropecu.*, 2018, **19**, 621–644.

31 K. P. C. Lakmini, S. T. Gonapinuwala, H. P. S. Senarath, C. A. N. Fernando, I. Wijesekara and M. D. S. T. de Croos, *Sri Lanka J. Aquat. Sci.*, 2022, **27**, 44–62.
32 S. H. Suseno, N. Nurjanah, Y. Yoshiara and S. Saraswati, *KnE Life Sci.*, 2015, **1**, 125–135.
33 N. Rahman, S. Hashem, S. Akther and J. S. Jothi, *Food Sci. Nutr.*, 2023, **11**, 4688–4699.
34 B. Çakaloğlu, V. H. Özyurt and S. Ötleş, *Ukr. Food J.*, 2018, **7**, 640–654.
35 A. Głowacz-Różyńska, M. Tynek, E. Malinowska-Pańczyk, D. Martysiak-Żurowska, R. Pawłowicz and I. Kołodziejska, *Eur. J. Lipid Sci. Technol.*, 2016, **118**, 1759–1767.
36 C. Neagle, A. Chouljenko, G. Bolton, S. Mirtalebi, M. O. Frinsko, S. G. Hall, B. J. Reading and M. Joseph, *Processes*, 2025, **13**, 511.
37 SEPARATECH, *3-phase Decanter Centrifuge for Fish Oil Processing*, https://www.separatech.com/3-phase-decanter-centrifuge-for-fish-oil-processing/, accessed 6 November 2025.
38 B. Zhuang, G. Ramanauskaite, Z. Y. Koa and Z.-G. Wang, *Sci. Adv.*, 2021, **7**, eabe7275.
39 Z. A. Fitri, F. Ahmadi, M. A. Islam, E. N. Ponnampalam, F. R. Dunshea and H. A. R. Suleria, *Food Sci. Nutr.*, 2025, **13**, e70138.
40 V. C. Roy, J.-S. Park, T. C. Ho and B.-S. Chun, *Mar. Drugs*, 2022, **20**, 70.
41 J. Jamaluddin, C. Rusli, Y. Yuyun and A. Widodo, *J. Pharm. Nutr. Sci.*, 2019, **9**, 125–129.
42 H. Sasongko, N. R. Efendi, A. Budihardjo, Y. Farida, T. Amartiwi, A. A. Rahmawati, A. Wicaksono and Sugiyarto, *J. Phys.: Conf. Ser.*, 2017, **795**, 012021.
43 M. R. Simplice, W. H. Macaire, N. N. F. Hervé, T. D. Fabrice, D. Dj. Justin, T. François and K. Jules-Roger, *Lipids Health Dis.*, 2018, **17**, 45.
44 M. Á. Rincón-Cervera, M. B. Villarreal-Rubio, R. Valenzuela and A. Valenzuela, *Eur. J. Lipid Sci. Technol.*, 2017, **119**, 1600516.
45 C. Breil, M. Abert Vian, T. Zemb, W. Kunz and F. Chemat, *Int. J. Mol. Sci.*, 2017, **18**, 708.
46 S. Pati, B. Nie, R. D. Arnold and B. S. Cummings, *Biomed. Chromatogr.*, 2016, **30**, 695–709.
47 C. Z. Ulmer, C. M. Jones, R. A. Yost, T. J. Garrett and J. A. Bowden, *Anal. Chim. Acta*, 2018, **1037**, 351–357.
48 S. J. Iverson, S. L. C. Lang and M. H. Cooper, *Lipids*, 2001, **36**, 1283–1287.
49 J. Zhou, M. Wang, J. A. Saraiva, A. P. Martins, C. A. Pinto, M. A. Prieto, J. Simal-Gandara, H. Cao, J. Xiao and F. J. Barba, *Food Chem.*, 2022, **384**, 132236.
50 E. M. Grima, A. R. Medina, A. G. Giménez, J. A. Sánchez Pérez, F. G. Camacho and J. L. García Sánchez, *J. Am. Oil Chem. Soc.*, 1994, **71**, 955–959.
51 J. Sheng, R. Vannela and B. E. Rittmann, *Bioresour. Technol.*, 2011, **102**, 1697–1703.
52 G. Caprioli, F. Giusti, R. Ballini, G. Sagratini, P. Vila-Donat, S. Vittori and D. Fiorini, *Food Chem.*, 2016, **192**, 965–971.
53 S. J. Lee, B.-D. Yoon and H.-M. Oh, *Biotechnol. Tech.*, 1998, **12**, 553–556.
54 J.-H. Lin, L.-Y. Liu, M.-H. Yang and M.-H. Lee, *J. Agric. Food Chem.*, 2004, **52**, 4984–4986.
55 Y. Wang, K.-E. Eilertsen, E. O. Elvevoll and M. J. Walquist, *J. Am. Oil Chem. Soc.*, 2025, **102**, 871–883.
56 M. A. Rahimi, R. Omar, S. Ethaib, M. K. Siti Mazlina, D. R. Awang Biak and R. Nor Aisyah, *IOP Conf. Ser.: Mater. Sci. Eng.*, 2017, **206**, 012096.
57 X. Bian, W. Jin, Q. Gu, X. Zhou, Y. Xi, R. Tu, S.-F. Han, G.-J. Xie, S.-H. Gao and Q. Wang, *World J. Microbiol. Biotechnol.*, 2018, **34**, 39.
58 N. I. Iberahim and B. C. Tan, *IOP Conf. Ser.: Mater. Sci. Eng.*, 2020, **932**, 012038.
59 N. C. Shahi, S. Goutam, R. R. Thakur, A. Singh and U. C. Lohani, *Curr. J. Appl. Sci. Technol.*, 2017, **25**, 1–12.
60 C. P. Mungwari, C. K. King'ondu, P. Sigauke and B. A. Obadele, *Sci. Afr.*, 2025, **27**, e02509.
61 R. K. Gaikwad, I. H. Mondal, K. K. Dash, A. M. shaikh and K. Béla, *J. Agric. Food Res.*, 2025, **19**, 101546.
62 P. W. Mwaurah, S. Kumar, N. Kumar, A. K. Attkan, A. Panghal, V. K. Singh and M. K. Garg, *Compr. Rev. Food Sci. Food Saf.*, 2020, **19**, 3–20.
63 L. Gomez, B. Tiwari and M. Garcia-Vaquero, in *Sustainable Seaweed Technologies*, ed. M. D. Torres, S. Kraan and H. Dominguez, Elsevier, 2020, pp. 207–224.
64 J. Pinela, B. de la Fuente, M. Rodrigues, T. C. S. P. Pires, F. Mandim, A. Almeida, M. I. Dias, C. Caleja and L. Barros, *Biomolecules*, 2022, **13**, 1.
65 A. Singh, G. R. Nair, P. Liplap, Y. Gariepy, V. Orsat and V. Raghavan, *Antioxidants*, 2014, **3**, 99–113.
66 M. Melikoglu, *Sustainable Chem. Clim. Action*, 2025, **7**, 100122.
67 D. dos, S. V. Costa and N. Bragagnolo, *Eur. J. Lipid Sci. Technol.*, 2017, **119**, 1600108.
68 B. de la Fuente, J. Pinela, R. C. Calhelha, S. A. Heleno, I. C. F. R. Ferreira, F. J. Barba, H. Berrada, C. Caleja and L. Barros, *Food Bioprod. Process.*, 2022, **136**, 97–105.
69 B. V. Cheriyan, K. K. Karunakar, R. Anandakumar, A. Murugathirumal and A. S. Kumar, *Sustainable Chem. Clim. Action*, 2025, **6**, 100054.
70 K. Kumar, S. Srivastav and V. S. Sharanagat, *Ultrason. Sonochem.*, 2021, **70**, 105325.
71 F. Chemat, N. Rombaut, A.-G. Sicaire, A. Meullemiestre, A.-S. Fabiano-Tixier and M. Abert-Vian, *Ultrason. Sonochem.*, 2017, **34**, 540–560.
72 O. Ciftci and H. Keskin Cavdar, *Eur. J. Lipid Sci. Technol.*, 2025, **127**, e202400131.
73 T. T. H. Mai, Y. Choi, H. Park, J. L. Cheon, J.-S. Choi, D. Park and H. Kim, *Appl. Biol. Chem.*, 2023, **66**, 80.
74 H. Keskin Çavdar, H. Bilgin, S. Fadıloğlu and F. M. Yılmaz, *Eur. J. Lipid Sci. Technol.*, 2023, **125**, 2200089.
75 A. Łubek-Nguyen, W. Ziemichód and M. Olech, *Appl. Sci.*, 2022, **12**, 3232.
76 F. S. Almeida, F. F. G. Dias, A. C. K. Sato and J. M. L. N. De Moura Bell, *Food Bioprocess Technol.*, 2022, **15**, 1794–1809.
77 Y.-H. Wang, C.-H. Kuo, C.-L. Lee, W.-C. Kuo, M.-L. Tsai and P.-P. Sun, *Catalysts*, 2020, **10**, 1323.

78 E. Aitta, A. Damerau, A. Marsol-Vall, M. Fabritius, L. Pajunen, M. Kortesniemi and B. Yang, *Food Chem.*, 2023, **424**, 136381.
79 S. F. Bruno, T. G. Kudre and N. Bhaskar, *J. Food Process Eng.*, 2019, **42**, e12990.
80 Y. Zhang, Q. Sun, S. Liu, S. Wei, Q. Xia, H. Ji, C. Deng and J. Hao, *Innovative Food Sci. Emerg. Technol.*, 2021, **70**, 102670.
81 M. Lucarini, A. Zuorro, G. D. Lena, R. Lavecchia, A. Durazzo, B. Benedetti and G. Lombardi-Boccia, *Sustainability*, 2020, **12**, 8997.
82 N. Castejón and F. J. Señoráns, *Algal Res.*, 2019, **37**, 74–82.
83 M. Señoráns, N. Castejón and F. J. Señoráns, *Molecules*, 2020, **25**, 3310.
84 S. Singh, D. K. Verma, M. Thakur, S. Tripathy, A. R. Patel, N. Shah, G. L. Utama, P. P. Srivastav, J. R. Benavente-Valdés, M. L. Chávez-González and C. N. Aguilar, *Food Res. Int.*, 2021, **150**, 110746.
85 D. M. J. Möck, L. M. Kühn and J. Appelt, *Holzforschung*, 2025, **79**, 434–444.
86 K. K. Anas, L. R. G. Kumar, C. S. Tejpal and S. Mathew, *Int. J. Curr. Microbiol. Appl. Sci.*, 2020, **9**, 730–735.
87 S. Kuvendziev, K. Lisichkov, Z. Zeković, M. Marinkovski and Z. H. Musliu, *J. Supercrit. Fluids*, 2018, **133**, 528–534.
88 F. Sahena, I. S. M. Zaidul, S. Jinap, A. M. Yazid, A. Khatib and N. A. N. Norulaini, *Food Chem.*, 2010, **120**, 879–885.
89 N. Rubio-Rodríguez, S. M. de Diego, S. Beltrán, I. Jaime, M. T. Sanz and J. Rovira, *J. Food Eng.*, 2012, **109**, 238–248.
90 M. M. A. N. Ranjha, R. Kanwal, B. Shafique, R. N. Arshad, S. Irfan, M. Kieliszek, P. Ł. Kowalczewski, M. Irfan, M. Z. Khalid, U. Roobab and R. M. Aadil, *Molecules*, 2021, **26**, 4893.
91 E. Puértolas, E. Luengo, I. Álvarez and J. Raso, *Annu. Rev. Food Sci. Technol.*, 2012, **3**, 263–282.
92 K. Nowosad, M. Sujka, U. Pankiewicz and R. Kowalski, *J. Food Sci. Technol.*, 2021, **58**, 397–411.
93 G. Ghoshal, *Heliyon*, 2023, **9**, e17532.
94 M. K. Ahmmed, A. Carne, H. (Sabrina) Tian and A. E.-D. A. Bekhit, *Food Chem.*, 2022, **384**, 132476.
95 A. Burnett, M. K. Ahmmed, A. Carne, H. (Sabrina) Tian, I. A. M. Ahmed, F. Y. Al-Juhaimi and A. E.-D. A. Bekhit, *Foods*, 2022, **11**, 610.
96 J. Cropotova, S. Tappi, J. Genovese, P. Rocculi, M. D. Rosa and T. Rustad, *Heliyon*, 2021, **7**, e05947.
97 B. Gómez, P. E. S. Munekata, M. Gavahian, F. J. Barba, F. J. Martí-Quijal, T. Bolumar, P. C. B. Campagnol, I. Tomasevic and J. M. Lorenzo, *Food Res. Int.*, 2019, **123**, 95–105.
98 Y. R. Shams and K. K. Dash, *Food Bioprocess Technol.*, 2025, **18**, 2218–2235.
99 S. Shafique, A. S. Belousov, R. Rashid, I. Shafiq, K. H. H. Aziz, N. Riaz, M. S. Khan, A. Shaheen, M. Ishaq, P. Akhter and M. Hussain, *J. Mol. Liq.*, 2025, **419**, 126769.
100 T. X. Le Thi, H. L. Tran, T. S. Cu and S. L. Ho, *J. Chem.*, 2018, **2018**, 6276832.
101 L. Anstiss, C. C. Weber, S. Baroutian and K. Shahbaz, *J. Chem. Technol. Biotechnol.*, 2023, **98**, 1791–1802.
102 C. McReynolds, A. Adrien, N. Castejon and S. C. M. Fernandes, *Green Chem. Lett. Rev.*, 2022, **15**, 383–404.
103 D. J. G. P. van Osch, L. F. Zubeir, A. van den Bruinhorst, M. A. A. Rocha and M. C. Kroon, *Green Chem.*, 2015, **17**, 4518–4521.
104 B. D. Ribeiro, C. Florindo, L. C. Iff, M. A. Z. Coelho and I. M. Marrucho, *ACS Sustainable Chem. Eng.*, 2015, **3**, 2469–2477.
105 L. Barp, A. M. Višnjevec and S. Moret, *Foods*, 2023, **12**, 2017.
106 P. Otero, M. I. López-Martínez and M. R. García-Risco, *J. Pharm. Biomed. Anal.*, 2019, **164**, 86–92.
107 S. Pieber, S. Schober and M. Mittelbach, *Biomass Bioenergy*, 2012, **47**, 474–482.
108 A. Mustafa and C. Turner, *Anal. Chim. Acta*, 2011, **703**, 8–18.
109 P. Otero, S. E. Quintana, G. Reglero, T. Fornari and M. R. García-Risco, *Mar. Drugs*, 2018, **16**, 156.
110 F. Grasso, F. Turrini, M. Jenssen, V. Orlandi, A. Swaidan, F. Falco, K. Lian and R. Boggia, *Sustainable Food Technol.*, 2026, **4**, 1475–1491.
111 M. Jenssen, I. Sone, F. Grasso, F. Turrini, F. Tardanico, G. D. N. Atanasio, D. M. Paz, R. V. Sobrado, M. A. Martínez, R. Boggia, E. Grasselli and K. Lian, *Front. Nutr.*, 2025, **12**, 1663294.
112 A. Hublin, H. Malbaša, D. Stanec Svedrović and M. Jerman Vranić, *Waste Biomass Valorization*, 2024, **15**, 4487–4499.
113 B. Bashiri, J. Cropotova, K. Kvangarsnes, O. Gavrilova and R. Vilu, *Resources*, 2024, **13**, 61.
114 E. Díaz-de-Cerio and E. Trigueros, *Agriculture*, 2025, **15**, 2100.
115 R. Martins, A. Barbosa, B. Advinha, H. Sales, R. Pontes and J. Nunes, *Processes*, 2023, **11**, 2255.
116 M. Cholewski, M. Tomczykowa and M. Tomczyk, *Nutrients*, 2018, **10**, 1662.
117 S. Wang, Y. Pan, J. Li, H. Chen, H. Zhang, W. Chen, Z. Gu and Y. Q. Chen, *RSC Adv.*, 2017, **7**, 40946–40951.
118 R. K. Saini, P. Prasad, R. V. Sreedhar, K. Akhilender Naidu, X. Shang and Y.-S. Keum, *Antioxidants*, 2021, **10**, 1627.
119 Y. Fu, Y. Wang, H. Gao, D. Li, R. Jiang, L. Ge, C. Tong and K. Xu, *Mediators Inflammation*, 2021, **2021**, 8879227.
120 S. C. Dyall, *Front. Aging Neurosci.*, 2015, **7**, 52.
121 M. Durmuş, *Food Sci. Technol.*, 2018, **38**, 1.
122 M. I. Gladyshev, N. N. Sushchik and O. N. Makhutova, *Prostaglandins Other Lipid Mediators*, 2013, **107**, 117–126.
123 E. Slesinger, O. P. Jensen and G. Saba, *ICES J. Mar. Sci.*, 2021, **78**, 1010–1022.
124 M. Arts and C. Kohler, in *Lipids in Aquatic Ecosystems*, 2009, pp. 237–256.
125 P. Literáková, T. Zavřel, D. Búzová, P. Kaštánek and J. Červený, *Front. Nutr.*, 2024, **11**, 1290701.
126 M. V. Esteves, D. M. C. Marques, J. D. de Almeida, N. T. Faria and F. C. Ferreira, *Foods*, 2025, **14**, 1522.
127 H. Wu, B. Forghani, M. Abdollahi and I. Undeland, *Food Chem.: X*, 2022, **16**, 100488.
128 N. Swetha and S. K. Mathanghi, *Food Chem. Adv.*, 2024, **4**, 100603.

129 F. Ben Rebah, A. Abdelmouleh, W. Kammoun and A. Yezza, *J. Mar. Biol. Assoc. U. K.*, 2010, **90**, 569–573.
130 C. C. Parrish, in *Lipids in Aquatic Ecosystems*, ed. M. Kainz, M. T. Brett and M. T. Arts, Springer, New York, NY, 2009, pp. 309–326.
131 M. Moustaka, R. D. Evans, G. A. Kendrick, G. A. Hyndes, M. V. W. Cuttler, T. J. Bassett, M. J. O'Leary and S. K. Wilson, *Landscape Ecol.*, 2024, **39**, 28.
132 J. Qin, E. Kurt, T. LBassi, L. Sa and D. Xie, *Front. Microbiol.*, 2023, **14**, 1280296.
133 F. A. Fornaro, A. Zarli, C. Serangeli, A. Salladini, V. Piemonte, G. Iaquaniello and L. Di Paola, *Waste Biomass Valorization*, 2024, **15**, 3953–3967.
134 V. G. Alfio, C. Manzo and R. Micillo, *Molecules*, 2021, **26**, 1002.
135 *The Project – ecoeFISHent*, EcoeFISHent, accessed 26 February 2026.
136 V. Orlandi, L. Dondero, F. Turrini, G. D. N. Atanasio, F. Grasso, E. Grasselli and R. Boggia, *Molecules*, 2023, **28**, 7637.
137 F. Grasso, D. Méndez-Paz, R. Vázquez Sobrado, V. Orlandi, F. Turrini, G. De Negri Atanasio, E. Grasselli, M. Tiso and R. Boggia, *Gels*, 2023, **9**, 760.
138 F. Grasso, D. M. Paz, R. V. Sobrado, V. Orlandi, F. Turrini, L. Agostinis, A. Morandini, M. Jenssen, K. Lian and R. Boggia, *Gels*, 2024, **10**, 246.
139 F. Grasso, M. M. A. Martínez, F. Turrini, D. Méndez Paz, R. Vázquez Sobrado, V. Orlandi, M. Jenssen, K. Lian, J. Rombi, M. Tiso, E. Razzuoli, C. Costas and R. Boggia, *Antioxidants*, 2024, **13**, 1011.
140 L. Dondero, G. De Negri Atanasio, F. Tardanico, E. Lertora, R. Boggia, V. Capra, A. Cometto, M. Costamagna, L. S. E. Fi, M. Feletti, F. Garibaldi, F. Grasso, M. Jenssen, L. Lanteri, K. Lian, M. Monti, M. Perucca, C. Pinto, I. Poncini, F. Robino, J. V. Rombi, S. S. Ahsan, N. Shirmohammadi, M. Tiso, F. Turrini, M. Zaccone, M. Zanotti-Russo, I. Demori, P. F. Ferrari and E. Grasselli, *Mar. Biotechnol.*, 2025, **27**, 63.
141 S. Gharby, *Sci. World J.*, 2022, **2022**, 6627013.
142 *Chemical Degumming – AOCS*, AOCS, accessed 29 October 2025.
143 *Enzymatic Degumming – AOCS*, AOCS, accessed 29 October 2025.
144 C. Vaisali, S. Charanyaa, P. D. Belur and I. Regupathi, *Int. J. Food Sci. Technol.*, 2015, **50**, 13–23.
145 D. L. Lamas, *Appl. Food Res.*, 2022, **2**, 100170.
146 B. Soldo, V. Šimat, J. Vlahović, D. Skroza, I. Ljubenkov and I. Generalić Mekinić, *Eur. J. Lipid Sci. Technol.*, 2019, **121**, 1800513.
147 Y. Fang, S. Gu, J. Zhang, S. Liu, Y. Ding and J. Liu, *Int. J. Food Sci. Technol.*, 2018, **53**, 692–699.
148 M. Yi, Y. You, Y. Zhang, G. Wu, E. Karrar, L. Zhang, H. Zhang, Q. Jin and X. Wang, *Molecules*, 2023, **28**, 672.
149 G. Dovale-Rosabal, A. Rodríguez, E. Contreras, J. Ortiz-Viedma, M. Muñoz, M. Trigo, S. P. Aubourg and A. Espinosa, *Molecules*, 2019, **24**, 1642.
150 M. E. Pando, A. Rodríguez, A. Galdames, M. M. Berríos, M. Rivera, N. Romero, M. A. Valenzuela, J. Ortiz and S. P. Aubourg, *Eur. Food Res. Technol.*, 2018, **244**, 937–948.
151 R. Bodkowski, K. Czyż, E. Sokoła-Wysoczańska, M. Janczak, P. Cholewińska and A. Wyrostek, *Animals*, 2020, **10**, 1834.
152 R. Morales-Medina, G. De León, M. Munio, A. Guadix and E. Guadix, *J. Food Eng.*, 2016, **183**, 16–23.
153 A. Alkhudhiri, N. Darwish and N. Hilal, *Desalination*, 2012, **287**, 2–18.
154 M. Ongis, G. Di Marcoberardino, D. Ormerod, F. Gallucci and M. Binotti, *Sep. Purif. Technol.*, 2025, **362**, 131190.
155 P. C. Rossi, M. d. C. Pramparo, M. C. Gaich, N. R. Grosso and V. Nepote, *J. Sci. Food Agric.*, 2011, **91**, 1452–1458.
156 E. A. Mahrous and M. A. Farag, *Sep. Purif. Rev.*, 2022, **51**, 300–317.
157 S. T. Minton, A. L. Almada, J. L. Evans, M. Laidlaw and J. Opheim, *PLoS One*, 2023, **18**, e0265462.
158 N. Rubio-Rodríguez, S. Beltrán, I. Jaime, S. M. de Diego, M. T. Sanz and J. R. Carballido, *Innovative Food Sci. Emerg. Technol.*, 2010, **11**, 1–12.
159 D. G. Filho, A. G. Silva and C. Z. Guidini, *Appl. Microbiol. Biotechnol.*, 2019, **103**, 7399–7423.
160 T. O. Akanbi, J. L. Adcock and C. J. Barrow, *Food Chem.*, 2013, **138**, 615–620.
161 K. Gao, W. Chu, J. Sun and X. Mao, *Food Chem.*, 2020, **330**, 127225.
162 S. Moreno-Perez, D. F. M. Turati, J. P. Borges, P. Luna, F. J. Señorans, J. M. Guisan and G. Fernandez-Lorente, *J. Agric. Food Chem.*, 2017, **65**, 117–122.
163 M. Karia, M. Kaspal, M. Alhattab and M. Puri, *Mar. Drugs*, 2024, **22**, 301.
164 L. Wang, Z. Chi, X. Wang, Z. Liu and J. Li, *Ann. Microbiol.*, 2007, **57**, 495–501.
165 G. Coşkun, Z. Çıplak, N. Yıldız and Ü. Mehmetoğlu, *Appl. Biochem. Biotechnol.*, 2021, **193**, 430–445.
166 R. Leardi, *Anal. Chim. Acta*, 2009, **652**, 161–172.