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A review of defatting processes using sustainable solvents: parallels between seed and fish oil sources

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Conventional defatting and oil removal processes, employed for both oilseed and fish biomasses, are mainly based on petrochemical solvents like hexane. The noteworthy drawbacks of these efficient methods include the presence of residues in the products, environmental pollution, and high risks for the operators. This review aims at comparing conventional hexane extraction with alternative and sustainable defatting solvents, highlighting the parallel advantages, limitations, and research gaps identified in seed and fish oil processing. Current advances in clean and sustainable defatting solvents, including alcohols, terpenes, deep and natural deep eutectic solvents, ionic liquids, 2-methyloxolane, supercritical carbon dioxide, and vegetable oils, are covered, emphasising their chemical, physical, and safety properties. Actual constraints, mainly in terms of economic and technical feasibility and applicability, or industrial exploitation and scalability, are also mentioned, along with possible approaches for a more successful employment of these promising solvents. In this review, a parallel evaluation is also made in terms of main gaps and perspectives related to diverse pre-treatments for the optimisation of seed and fish defatting.

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Sustainability spotlight

Conventional defatting approaches for seed and fish oils are based on toxic, petroleum solvents such as hexane, causing environmental and safety concerns. This review critically compares sustainable solvent systems, such as alcohols, terpenes, deep and natural deep eutectic solvents, ionic liquids, 2-methyloxolane, supercritical carbon dioxide, and vegetable oils, highlighting how they enable efficient, low-impact lipid recovery from plant and marine resources. The work advances sustainability by encouraging solvent substitution strategies that limit volatile emissions, energy demand, and waste generation. It supports a circular approach to defatting, integrating green chemistry and process design principles. This review aligns with UN SDGs 9 (Industry, Innovation and Infrastructure), 12 (Responsible Consumption and Production), 13 (Climate Action), and 14 (Life Below Water), addressing innovation, responsible production, climate challenge, and sustainable use of aquatic resources.

Introduction

The market and consumer demand for oils and lipids of vegetal origin is currently rising worldwide due to their numerous uses and applications as renewable energy sources and as significant components in food items and other industrial products such as cosmetics, soaps, varnishes, lubricants, resins, greases, waxes, and adhesives.¹ Notably, consistent amounts of by-products containing valuable bioactives, like proteins, fibres, and minerals, are generated after the oil extraction and recovery.² In particular, plant proteins are playing a significant role in nutrition, and the removal of lipids from food products is required for healthy diets with low fat and high protein intake.³ Based on the quantity and quality of available resources, oilseeds definitely stand out among the plant protein sources the industry is focusing on. The possible lowest fat

content in oil matrices is recommended, since residual lipids can counteract the protein extraction and isolation process due to the formation of emulsions leading to reduced concentration and increased oxidation in the resulting isolates.⁴

Among the available sources, plant oils represent the predominant alternative to fish oil, because of production stability, cost reduction, and sustainability. However, fish oil possesses a unique composition, rich in omega-3 fatty acids, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and lower amounts of other fatty acids, such as linolenic acid.⁵ EPA and DHA are proven to be particularly beneficial against serious disorders like diabetes, asthma, cardiovascular and psychiatric diseases, and cancer. These polyunsaturated fatty acids cannot be produced by the human body and a daily intake of 0.65 g EPA and DHA is recommended for adults by the National Institute of Health (NIH).⁶ Efficient and sustainable defatting techniques can enrich and foster the nutritional features of other highly abundant compounds in the fish matrix, including proteins, vitamins, and minerals.

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The most traditional practices for oil extraction include mechanical pressing and chemical (solvent) extraction, usually performed by using hexane. The first process's main limitations are represented by the low yield and up-scaling possibilities, while hexane is flammable and has been categorised as a CMR 2, namely carcinogenic, mutagenic, and reprotoxic. Solvent extraction requires huge volumes and energy utilisation, and leads to losses in the solvent removal and recovery.⁷ Thus, the need to identify and apply greener, sustainable, and renewable options is significantly increasing.

In this perspective, greener solvents for oilseeds and fish defatting and oil removal are being developed aiming at reducing the strong negative impact of harmful solvent treatments on the health and environment.⁸ Among these, alternative solvents including alcohols and terpenes, deep and natural deep eutectic solvents (DESS, NADESS), ionic liquids (ILs), 2-methyloxolane (2-MeOx), supercritical carbon dioxide (CO₂), and vegetable oils are discussed in this review, highlighting their potential, applications, challenges, and future perspectives. In particular, it is worth considering the valuable opportunities given by such processes in terms of safer exploitation of defatted residual biomasses within

different sectors and for diverse purposes. Clean defatting methods can contribute to Agenda 2030 and Sustainable Development Goals (SDGs) by reducing wastes and supporting their utilisation, increasing productivity while improving environmental performance, and promoting efficient and less resource and cost-demanding processes.

Overview of traditional defatting and oil removal processes

Defatting and oil removal represent pivotal processes in several industries, including food production, cosmetics, and wastewater treatment. Defatting is among the primary steps in the valuable processing of oilseeds, such as peanuts, soy, and others, as well as fish sources, particularly the fatty ones like salmon.⁹ Fat and oil removal results in obtaining a low-fat product or defatted by-product, which would be rich in proteins and other valuable ingredients exploitable in the food industry. Thus, defatting processes produce extracted oils with interesting fatty acid profiles and health benefits, which find application in the pharmaceutical and cosmeceutical sectors, along with defatted meals or cakes to be used in food formulations due to their nutritional value.³

Oilseeds account for 20% of global grain production, and soybean, rapeseed, palm, and sunflower oils are the most produced oils worldwide.¹⁰ Generally, partial or total mechanical extraction or pressing is conducted as a first step for oil extraction after oilseed cleaning, drying, dehulling, and other conditioning processes. In case of required 2% or less residual oil in the meal, solvent extraction is carried out. If oilseeds have over 25% or 30% lipids, they are pressed totally or partially before solvent extraction, while solvent extraction is applicable straightaway for materials containing less than 20% oil.¹¹ Solvent extraction is traditionally and commonly performed by using continuous extractors or in batch by Soxhlet apparatuses, with petroleum-based solvents, typically hexane,^{12,13} petroleum ether¹⁴ or other solvents such as diethyl ether and chloroform.¹⁵ At the industrial level, the continuous counter current flow process is the most used for oil extraction, maximising its efficiency. Normally, after extraction, the solid phase, or meal, is separated from the liquid one, also called miscella, through filtration or centrifugation. The miscella is then briefly subjected to water vapour or noble gas injection for the separation of the oil and solvent recovery. In this phase, the boiling point of the solvent is reached, enabling its evaporation, followed by its recycling and reuse.¹⁶ The meal passes finally through a toaster for the complete removal of solvent residues. The crude oil can be treated in several ways, based on its following applications, and the defatted meal can be further processed for extraction of bioactives, such as proteins, and their concentration.¹⁷

Similarly, the production of fish oil is mostly achieved by rendering (wet, with added water, and dry), which consists of heating the fish for protein coagulation along with oil and bound water collection, followed by their removal, pressing of the mixture and oil separation in a decanter, and then by centrifugation. The water fraction undergoes concentration and



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is combined with the press cake, which is subsequently dried.¹⁸ Alternatively, solvent extraction methods again encompass Soxhlet and reflux extraction employing mainly hexane and other organic solvents like diethyl ether and chloroform.¹⁹ Nowadays, the processing industrial sector releases a fish waste quantity accounting for 20–50% of the overall fish weight and these by-products can contain up to 40% oil.⁶ Consequently, huge amounts of fish wastes could be exploited for oil removal and valorisation of the resulting biomass to recover valuable bioactive compounds, including collagen, gelatin, enzymes, proteins, and peptides. Fish oils are much more prone to oxidation than seed oils, due to their complex composition rich in omega-3 fatty acids, coexisting with phospholipids, waxes, cholesterol, and other lipids.²⁰ Moreover, proteins are often co-extracted with lipids, producing emulsions and making their separation and solvent removal more complicated.²¹ Thus, it is essential to take these aspects into consideration and conduct a deeper investigation for an adequate selection of fish defatting solvents. For instance, terpenes such as limonene could be themselves susceptible to oxidation, while other solvents like alcohols can exert antioxidant effects. Additionally, high temperature processes can facilitate lipid degradation, and the protein-lipid interactions typical of fish matrices may be hardly disrupted by low-polarity solvents.

Process limitations, and environmental and health impacts of solvent-based methods

The advantages of using hexane solvent for oil extraction encompass easy product recovery, a low boiling point of less than 70 °C, and very good solubilising capacity.²² Nevertheless, its use has raised significant concerns in terms of process quality and sustainability, as well as human and environmental safety. Indeed, being a non-polar solvent, it extracts neutral lipids effectively, but leaves a polar lipid-rich residue.⁴ Generally, the oil produced by solvent extraction possesses lower quality compared to pressing; moreover, solvent removal by evaporation is an energy-consuming step of the process.²³ In addition, meal toasting can lead to thermo-sensitive compound degradation, thus reducing its nutritional value.

Petroleum-based solvents make the oil extraction process costly and highly demanding in terms of transport, storage, and energy required for solvent recycling. A strong impact on the environment and high risks for operators' safety are also associated with hexane, as a solvent originating from non-renewable sources. It is highly flammable and when released into the atmosphere it can react with the other pollutants forming ozone and other oxidant photochemicals.²⁴ Hexane is also recognised as neurotoxic due to its solubility in neural lipids.²³ Nevertheless, its use is still permitted for food applications if the residue in the oil is lower than 1 mg kg⁻¹.²⁵

The above-mentioned environment, health, and safety drawbacks of these solvents have supported further investigation on alternative options for oil extraction, including the use of green solvents.

Clean and sustainable solvent extraction processes: advantages and limitations

Mechanical and thermal pressing extraction

Traditional mechanical extraction, or pressing, is a simple and safe oil extraction process, classified as extrusion and screw pressing, hot and cold pressing, and hydraulic pressing. Extrusion continuously works at high temperature for short times, making the retention of nutrients higher. It usually involves screw pressing, by the application of axial pressure through volumetric compression. A screw press consists of a horizontal screw in a perforated barrel.²⁶ Screw pressing combined with dry extrusion²⁷ and dry air roasting²⁸ was applied on peanuts without using any chemicals, resulting in high oil removal and oxidative stability. Traditionally, pressing is carried out in the industry employing continuous single-screw presses, but twin-screw extrusion has recently emerged, which allows valuable oil to be recovered, valorising at the same time the residual cake, as in the case of sunflower,²⁹ coriander,³⁰ and jatropha.³¹ The twin-screw extruder can be used in place of industrial extractors, where the biomass moves following the opposite current to the solvent. During cold pressing, pressure is applied to the material with temperature lower than 30 °C, which prevents compound loss, and without using solvents. Commercially, twin-screw presses and oil screw expellers are employed.^{32,33} Hot pressing utilises heat for oil extraction, inducing its oxidation.³⁴ Indeed, higher levels of carcinogenic compounds, mainly aflatoxins, were detected in peanut oil obtained by hot pressing than cold pressing.³⁵ Consequently, quality of oil from cold pressing is considered greater and this process is industrially preferred. However, cold pressing extraction yield is lower and it leaves generally higher content of residual oil in the biomass, with the consequent need for applying a second process, mostly solvent extraction.³⁶ Thus, in order to increase the oil yield and quality, several options have been industrially investigated, such as hydraulic pressing applied on groundnut kernels.³⁷ A particular improvement in the oil extraction from peanuts through hydraulic pressing was achieved by applying the 'Mechanical Expression Preserving Shape Integrity' (MEPSI) process. The innovation relies on the use of food grade specific materials for separation of the grains in the press unit, whose purpose was to reduce the material deformation and absorb the extracted oil.³⁸ The process parameters were optimised, limiting the oil lipid oxidation,³⁹ and preserving peanut structural features. Indeed, MEPSI was associated with a reconstitution step by Intensification of Vaporisation by Decompression to the Vacuum (IVDV), consisting of exposing the deformed material to 1.5 MPa steam pressure, and then releasing it into the vacuum, causing the expansion of the partially defatted peanuts.⁴⁰ This integrated technology allowed the development of an energy-effective process with a more than 50% defatting ratio, and able to maintain peanuts' structural and organoleptic features, improving consumers' acceptability and meeting their demands for healthy products.



Table 1 Chemical and physical properties of green solvents employed for oilseed and fish defatting, compared to hexane

| Solvent | Molecular formula | Classification | Boiling point (°C) | Flash point (°C) | Density (g cm ⁻³ , 25 °C) | Viscosity (mPa s, 25 °C) | Water miscibility (g _{solvent} /100 g _{water}) | Green solvent rate ^a | | |
|--------------------|--|-------------------|--------------------|------------------|--------------------------------------|--------------------------|---|---------------------------------|--------|-------------|
| | | | | | | | | Health | Safety | Environment |
| Ethanol | C ₂ H ₅ OH | Amphiprotic | 78 | 13 | 0.789 | 1.074 | Infinitely | 3 | 4 | 3 |
| Isopropanol | C ₃ H ₈ O | Amphiprotic | 82 | 12 | 0.785 | 2.370 | Infinitely | 3 | 4 | 3 |
| Ethyl acetate | C ₄ H ₈ O ₂ | Polar aprotic | 77 | -4 | 0.902 | 0.423 | 8.4 | 3 | 5 | 3 |
| Butanol | C ₄ H ₉ OH | Amphiprotic | 118 | 35 | 0.810 | 2.573 | 7.7 | 4 | 3 | 3 |
| Acetone | C ₃ H ₆ O | Polar aprotic | 56 | -18 | 0.791 | 0.320 | Infinitely | 3 | 5 | 3 |
| <i>p</i> -Cymene | C ₁₀ H ₁₄ | Non-polar aprotic | 177 | 47 | 0.857 | 1.105 | 0.0023 | 5 | 4 | 5 |
| α -Pinene | C ₁₀ H ₁₆ | Non-polar aprotic | 155 | 33 | 0.858 | 1.303 | 0.0002 | 2 | 4 | 7 |
| Limonene | C ₁₀ H ₁₆ | Non-polar aprotic | 178 | 48 | 0.842 | 0.846 | 0.0008 | 2 | 4 | 7 |
| Pinane | C ₁₀ H ₁₈ | Non-polar aprotic | 169 | 42 | 0.847 | 1.3 | Insoluble | 2 | 4 | 7 |
| <i>p</i> -Menthane | C ₁₀ H ₂₀ | Non-polar aprotic | 168 | 44 | 0.797 | 3.4 | Insoluble | 2 | 4 | 7 |
| 2-Methylloxolane | C ₅ H ₁₀ O | Dipolar aprotic | 80 | -11 | 0.855 | 0.6 | 4.1 | 5 | 6 | 3 |
| Hexane | C ₆ H ₁₄ | Non-polar aprotic | 69 | -23 | 0.675 | 0.310 | 0.001 | 7 | 8 | 7 |

^a Values extrapolated from the CHEM21 selection guide of organic solvents (increasing values are associated with rising hazard/dousness).⁴⁹

Similarly, for fish oil recovery, screw and hydraulic presses are traditionally employed, preceded by a cooking step. As examples, hydraulic pressing was used for oil extraction from sardine wastes⁴¹ and tuna heads,⁴² and high treatment temperatures enhanced the oil yield, while increasing lipid oxidation and protein denaturation. For this reason, an accurate choice of heating temperature and time is crucial to preserve the fatty acids and the overall integrity of fish oil. Indeed, higher oil quality can be achieved by cold pressing, applied for instance on salmon waste,⁴³ but this process generally results in limited oil yield. An alternative extraction process consisting of biomass mincing, cooking, and oil separation in one step using a three-phase decanter and without including a pressing phase is conventionally applied to by-products from salmon, herring, and Rainbow trout.⁴⁴

Extraction with alternative and sustainable solvents

The above-mentioned limits and drawbacks of hexane and other petroleum solvents are supporting a more extensive use of less harmful and impactful solvents for defatting. The so-called green solvents meet these requirements, having limited toxicity and renewable origin.⁴⁵ For instance, ethanol can be derived from sugar-rich sources, and, along with CO₂ and bio-based ethyl acetate and acetone, has shown compatibility with lipid extraction and safe use for food components, ingredients, and raw biomass processing.⁴ Table 1 displays and compares solvents for oilseed and fish defatting, alternative to hexane, in terms of their main physicochemical properties and indicators of the safety profile and environmental sustainability. The latter can be more realistically and concretely evaluated on a life cycle basis, employing tools such as Life Cycle Assessment (LCA), and estimating the impacts attributed to solvent development and manufacturing, use, and disposal. For instance, the replacement of hexane with ethanol has been assessed for soybean oil extraction, resulting in a higher net present value for hexane, measuring the financial viability and profitability of the process. However, ethanol offered a lower global warming potential, reducing CO₂ emissions significantly at an industrial level.⁴⁶ Additionally, the environmental effects of limonene have been evaluated in comparison to hexane, employed for extraction of lipids for algae biodiesel production. The outcomes revealed that, despite being recognised as hazardous, hexane exerted the lowest environmental impacts in terms of climate change and primary energy demand, and overall, in 11 out of 19 categories. On the other hand, limonene was associated with the reduction of the impacts in most of the other categories, due to its much lower volatility than hexane (Table 1), non-hazardousness, and environmental production. Notably, the use of pre-treatments has been suggested to facilitate cell disruption, thereby reducing most of the environmental impacts and the primary energy demand.⁴⁷ Soybean oil production has again been subjected to LCA, comparing hexane and supercritical CO₂ extraction. At a laboratory scale, supercritical CO₂ extraction and separation processes were more energy-demanding, mainly due to the low solubility of the oil in the supercritical fluid. This serves as an example of a solvent which is itself environmentally



favourable, but the same cannot be necessarily stated for its application. This fact suggested implementations to make this technology more competitive and appealing for scale-up, namely the introduction of membrane techniques for improved CO₂ and oil separation, given the low viscosity and high diffusivity of CO₂.⁴⁸ From these insights, the need for further investigations is emerging in relation to LCA for sustainable and novel solvents, like 2-methyloxolane, particularly shedding light on their synthetic and manufacturing procedures.

Extraction with alcohols. In relation to oilseeds, alcohols such as ethanol and isopropanol have been employed and compared for oil extraction from crushed rapeseed⁵⁰ and ground soybean collects.⁵¹ Ethanol or other polar solvents are able to better extract polar lipids, and water presence can even further limit lipids' dissolving capacity with respect to hexane. Moreover, ethanol's higher latent heat of vaporisation makes the recycling of this solvent more energy demanding. On the other hand, these aspects can be exploited to simplify the separation between the oil and solvent without fully evaporating it but by simply cooling down the mixture.⁵² Ethanol was compared to hexane for lipid extraction from macadamia nuts, showing similar performances and yields higher than 50%.⁵³ The same two solvents have been used for oil extraction from peanut press cake, giving comparable results with respect to cold pressing, and suggesting ethanol as an alternative option to hexane.⁵⁴ Another alcohol, isopropanol, along with hexane and ethyl acetate, was employed to obtain oil from macauba, giving the highest yield and greater levels of β-carotene and free glycerol, while ethyl acetate could extract flavonoids at higher concentrations⁵⁵ (Table 2).

Regarding fish oil, ethanol has proven to be the most effective among the tested solvents in providing high oil yield (49%) with abundant oleic acid content, obtained by Soxhlet extraction of mixed freshwater fish wastes. Moreover, a mixture of ethanol : hexane in a 4 : 6 ratio was used on krill shrimps and the oil obtained from the ethanol layer showed significant phospholipid and n-3 polyunsaturated fatty acid content (both higher than 45%), and the lowest fluorine amount, which can lead to health issues if contained at high levels⁵⁶ (Table 2). Overall, the positive defatting performance of ethanol and its capacity to retain high-quality lipid components and fatty acids in the extracted oils can be attributed to minimal lipid degradation and oxidation, probably due to simultaneous extraction of antioxidant and protective compounds, such as phenolics. On the other hand, ethanol's lower volatility and affinity for non-polar lipids can require greater solvent amount, extraction time, and temperature, compromising the economic viability and scalability of the process.⁵⁴

Extraction with terpenes. Terpenes represent a class of natural products consisting of isoprene units (C₅H₈) originated from agricultural residues, such as D-limonene from citrus peels, and α-pinene from mint, pine trees, and ginger. They can be employed as green extraction solvents and they are endowed with anti-inflammatory, antimicrobial, antiviral, and antifungal properties.⁵⁷ Terpenes have been reported to possess similar solubility characteristics to hexane, and a safer profile because

of a higher flash point.⁵⁸ The main limitation of using terpenes as solvents can be the process scalability due to their high boiling point, density, and viscosity. This could be overcome by performing terpene extraction at low temperature and pressure by Clevenger systems, allowing consistent recycling rates to be achieved.⁵⁹

Concerning oilseeds, few representatives of the terpene class (*i.e.* D-limonene, α-pinene, and *p*-cymene) were used in comparison to hexane, ethanol, and isopropanol for the recovery of rapeseed oil. Interestingly, the results showed *p*-cymene as the most promising substitute for hexane, due to the remarkable lipid extraction yield and selectivity, even though the detected tocopherol, tocotrienol, and micronutrient levels were relatively low.⁶⁰ α-Pinene also showed better extraction performances than hexane for soybean and sunflower oils.⁶¹ Other studies on rapeseed oil have highlighted for D-limonene and *p*-cymene an equal or higher capability than hexane for dissolving triacylglycerols or triglycerides, sterols, and tocopherols.⁵⁹ The oil from the same biomass, obtained using the saturated and more stable pinane (*cis/trans* 7 : 3)⁶² and *p*-menthane,⁶³ derivatives of pinenes and D-limonene, respectively, possessed a comparable yield to hexane (Table 2).

As for fish oil, limonene and *p*-cymene were used to extract oil from salmon, containing higher amounts of di-glycerides and lower levels of triglycerides compared to the other tested solvents. Additionally, these terpenes provided extracts with reduced content of certain unsaturated fatty acids, probably because of compound degradation due to high temperatures and susceptibility of the terpenes themselves, mainly limonene, to oxidation.⁶⁴ D-Limonene was also employed on anchovy fish wastes, yielding an omega-3-rich oil⁶⁵ through an economically and environmentally advantageous process. Additionally, the same terpene was used in combination with ultrasounds for the oil extraction from milkfish by-products, providing more than 21 and 12% yield and omega-3 level, respectively, under the optimised conditions.⁶⁶ D-Limonene-based extraction was also applied for shrimp head and carapace, to produce an oil rich in the carotenoid astaxanthin and omega-3 lipids, which possess synergistic antioxidant activity⁶⁷ (Table 2).

Extraction with DESs and NADESS. Deep eutectic solvents (DESs) and natural deep eutectic solvents (NADESS) are emerging green solvents, with the latter being produced with natural substances such as amino acids, sugars, and organic acids, endowed with safety, biodegradability, and low environmental impact.⁸⁰ These solvents present numerous advantages in terms of cost-effective and relatively simple development, and lower cost with respect to ionic liquids.⁸¹ Their polarity, density, and viscosity can be adjusted by adding water or modifying the composition of the hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) constituents, allowing targeted compound extraction.⁸² However, their industrial adoption is still challenging,⁸¹ due to commonly high viscosity, especially for sugar-based DESs, which complicates the process but can be reduced by adding water or increasing the temperature.⁸³ Additionally, despite the biodegradability, long-term environmental effects and toxicity necessitate additional exploration.⁸⁰ Even if recyclable, the solvent recovery processes



Table 2 Conditions and efficiency of seed and fish oil extraction by sustainable solvents, compared to conventional solvent extraction

| Biomass | Extraction solvent – conditions | Lipid/oil yield | Comparative conventional extraction – oil yield | References |
|---------------------|---|--|---|------------|
| Oilseed crop | Alcohol extraction | | | |
| Rapeseed | Ethanol (92 wt% or 96 wt%), isopropanol (84 wt% or 88 wt%) – four stage cross-current extraction (solvent/seed ratio: 1500 g/100 g per stage, 10 min per stage, stirring at 42 rpm and 50 °C) | 83–84% with 96 wt% ethanol and 88 wt% isopropanol; 46% with 92 wt% ethanol and 66% with 84 wt% isopropanol | Hexane – 91% | 50 |
| Soybean collects | Absolute and aqueous ethanol with 6 and 12 mass% of water – stainless steel isothermal cylindrical reactor (solvent to solid mass ratio of 3, 7 temperature points from 40 to 90 °C) | 19% (average, wet basis) with absolute ethanol > aqueous ethanol, except for 6% water/ethanol at 90 °C | — | 51 |
| Macadamia nut | Ethanol – Soxhlet extraction (4 and 6 h) | >50% | Hexane – comparable yield >50% | 53 |
| Peanut press cake | Absolute ethanol – stainless-steel batch extractor (180 rpm, 1 h, 3 temperature points from 60 to 90 °C) | 87 ± 4% with ethanol, 75 °C, solid/solvent ratio 1/5, 3 contact stages | Hexane, 2 stages, 55 °C, solid/solvent mass ratio 1/4 – 86 ± 2% | 54 |
| Macauba | Isopropanol, ethyl acetate – orbital shaker (40 °C, 40 rpm, 4 h, biomass weight : solvent volume 1 : 8) | 27.43 ± 0.33% with isopropanol; 22.97 ± 0.33% with ethyl acetate | <i>n</i> -Hexane – 23.39 ± 0.15% | 55 |
| Oilseed crop | Terpene extraction | | | |
| Rapeseed | <i>p</i> -Cymene, α -pinene, limonene, ethanol, isopropanol, butanol – Soxhlet extraction (6 h, biomass weight : solvent volume 1 : 20) | 38.22% (dry mass) with <i>p</i> -cymene; 28.18% (dry mass) with α -pinene; 37.74% (dry mass) with limonene; 9.79% (dry mass) with ethanol; 35.74% (dry mass) with isopropanol; 33.67% (dry mass) with butanol | <i>n</i> -Hexane – 25.02% | 60 |
| Rapeseed | <i>p</i> -Cymene, <i>D</i> -limonene – Soxhlet extraction (under reflux, 8 h, biomass weight : solvent volume 1 : 10) | 39.71 ± 3.03% dry matter with <i>p</i> -cymene; 36.94 ± 0.82% dry matter with <i>D</i> -limonene | Hexane – 46.71 ± 0.75% dry matter | 59 |
| Rapeseed | Pinane (<i>cis/trans</i> 7 : 3) – mechanical stirring and maceration (biomass weight : solvent volume 1 : 10, 4000 rpm, 1 h, room temperature) | 42.5% dry matter | <i>n</i> -Hexane – 43.2% dry matter | 62 |
| Rapeseed | <i>p</i> -Menthane – Soxhlet extraction (biomass weight : solvent volume 1 : 10, 4000 rpm, 60 min, 25 °C) | 37.1 ± 0.07% dry matter | <i>n</i> -Hexane – 38.38 ± 0.05% dry matter | 63 |
| Soya | α -Pinene – Soxhlet extraction (8 h, biomass weight : solvent volume 1 : 10) | 21.1 ± 0.2% (dry mass) | <i>n</i> -Hexane – 19.5 ± 0.3% (dry mass) | 61 |
| Sunflower | α -Pinene – Soxhlet extraction (8 h, biomass weight : solvent volume 1 : 10) | 67.2 ± 0.2% (dry mass) | <i>n</i> -Hexane – 52.6 ± 0.2% (dry mass) | 61 |



Table 2 (Contd.)

| Biomass | Extraction solvent – conditions | Lipid/oil yield | Comparative conventional extraction – oil yield | References |
|--|---|--|--|------------|
| Oilseed crop Cottonseed | DES and NADES extraction Choline chloride : oxalic acid (1 : 1)–ethyl acetate–water mix (20% NADES, 20.4% water, 59.6% ethyl acetate) – homogenisation and ultrasound extraction (solvent volume : biomass weight 5 : 1, 40 kHz, 200 W ultrasound, 120 rpm, 30 °C, 60 min) | 90.68% | <i>n</i> -Hexane – 29.0% | 68 |
| Oilseed crop Soybean | Ionic liquid extraction DBU amidine/ethanol switchable polarity solvent – sealed reaction vessel (biomass weight : solvent volume 1 : 5, 25 °C and 70 °C); morpholine secondary amide switchable polarity solvent – sealed reaction vessel (biomass weight : solvent volume 1 : 5, 25 °C) | 10.5% with DBU/ethanol after 60 min at 25 °C, 20% increase at 70 °C; 22.9% with morpholine after 60 min | Hexane, 60 °C – 23.3% after 60 min | 7 |
| Oilseed crop Rapeseed | 2-Methyloxolane extraction 2-MeOx – Soxhlet extraction (biomass weight : solvent volume 1 : 10, under reflux, 8 h) | 45.96 ± 0.80% dry matter | <i>n</i> -Hexane – 46.34 ± 0.48% dry matter | 69 |
| Soybean | 2-MeOx and 2-MeOx 95.5% – automatic Soxhlet extraction (8 h) | 23.5 ± 0.1% dry matter with 2-MeOx; 23.7 ± 0.1% dry matter with 2-MeOx 95.5% | Hexane – 18.8 ± 0.1% dry matter | 70 |
| Oilseed crop Soybean residue | Supercritical CO₂ extraction High-pressure stainless steel CO ₂ extractor vessel (300 bar, 50 °C, 450 min, 3 L min ⁻¹ flow rate) | 18.5% ± 0.8% wt% | — | 71 |
| Macadamia nut | — | 68.5% | Petroleum ether – 74.6% | 53 |
| Fish Squid by-products | Alcohol extraction Ethanol, acetone, ethyl acetate, 1/1 ethanol/acetone, 1/1 ethanol/ethyl acetate, 1/1 acetone/ethyl acetate – stirring extraction (biomass weight : solvent volume 1 : 2.9, 1 min, 4 °C) | 65.6 ± 4.2 g kg ⁻¹ by-products with ethanol; 36.3 ± 0.4 g kg ⁻¹ by-products with acetone; 46.8 ± 1.6 g kg ⁻¹ by-products with ethyl acetate; 73.6 ± 2.7 g kg ⁻¹ by-products with ethanol/acetone; 71.1 ± 1.2 g kg ⁻¹ by-products with ethanol/ethyl acetate; 41.6 ± 2.1 g kg ⁻¹ by-products with acetone/ethyl acetate | 1/1 chloroform/methanol – 103.8 ± 1.8 g kg ⁻¹ by-products | 72 |
| Krill shrimp | Ethanol/hexane 4 : 6 | 4.38 ± 0.45% in the ethanol layer | 3.77 ± 0.32% in the hexane layer | 56 |



Table 2 (Contd.)

| Biomass | Extraction solvent – conditions | Lipid/oil yield | Comparative conventional extraction – oil yield | References |
|-------------------------------------|---|---|---|------------|
| Fish | Terpene extraction | | | |
| Salmon | Limonene, <i>p</i> -cymene – Soxhlet extraction (biomass weight : solvent volume 1 : 12, under reflux, 8 h) | 33.5% dry matter with limonene and <i>p</i> -cymene | Hexane – 32.0% dry matter | 64 |
| Anchovy by-products | <i>D</i> -Limonene – stirring extraction (700 rpm, 21 h, biomass weight : solvent weight 1 : 1) | 6% fresh matter | — | 65 |
| Milkfish by-products | <i>D</i> -Limonene – ultrasonic chamber (25 kHz, 200 W, 68 min, 84 °C, biomass weight : solvent volume 1 : 3) | 21.95% | — | 66 |
| Shrimp by-products | <i>D</i> -Limonene – stirring extraction (24 h, room temperature, biomass weight : solvent weight 1 : 2) | 4.18% | — | 67 |
| Fish | 2-Methyloxolane extraction | | | |
| Salmon | 2-MeOx – Soxhlet extraction (biomass weight : solvent volume 1 : 12, under reflux, 8 h) | 32.5% dry matter | Hexane – 32.0% dry matter | 64 |
| Fish | Supercritical CO₂ extraction | | | |
| Yellowtail fish waste | Laboratory-scale semi-batch CO ₂ process (30 MPa, 50 °C, 27 g min ⁻¹ flow rate, 180 min) | 40.87% | <i>n</i> -Hexane – 48.48 ± 1.62%; ethanol – 56.13 ± 2.19% | 73 |
| Mackerel muscle | Laboratory-scale equipment (biomass : solvent weight 1 : 5, 25 MPa, 45 °C, 120 min, 27 g min ⁻¹ flow rate) | 20.00 ± 0.54% | Hexane – 25.62 ± 0.62% | 74 |
| Tuna by-products (head) | Supercritical fluid extractor (biomass : solvent weight 1 : 2, 40 MPa, 65 °C, 120 min, 3 g min ⁻¹ flow rate) | 35.6 ± 2.4% dry matter | <i>n</i> -Hexane – 36.2 ± 1.8% dry matter | 75 |
| Salmon by-products (trimmed muscle) | Laboratory scale CO ₂ extraction process (biomass : solvent weight 1 : 1, 25 MPa, 45 °C, 180 min, 27 g min ⁻¹ flow rate) | 86.99 ± 1.14% dry matter | <i>n</i> -Hexane – 45.46 ± 0.57% dry matter | 76 |
| Shrimp waste | Fixed bed supercritical extraction unit (biomass : solvent weight 1 : 20, 30 MPa, 50 °C, 100 min, 3000 g min ⁻¹ flow rate, 15% wt. of ethanol) | 93.8% | <i>n</i> -Hexane – 67% | 77 |
| Sardine waste | Lab-scale semi-batch CO ₂ apparatus (25 MPa, 40 °C, 15 g min ⁻¹ flow rate, separation at 50 bar, 50 °C) | 20.3 ± 0.2 g/100 g raw material | — | 78 |
| Hake by-products | Semi-pilot supercritical fluid extraction plant (25 MPa, 40 °C, 10 kg h ⁻¹ flow rate, 3 h) | 96.4% | — | 79 |

appear to be energy-intensive, countering upscaled commercialisation.⁸⁴ Notably, the polarity of these solvents is highly adaptable, leading to the development of hydrophilic and

hydrophobic DESs/NADESs. The latter, including menthol and fatty acid-based solvents, possess high affinity for oils and lipophilic constituents.⁸⁵ Viscosity is reported to widely vary;



however, fatty acid-based hydrophobic DESs have shown relatively low viscosity.⁸⁵ These solvents are generally well recyclable and recovered with high efficiency by anti-solvent precipitation, back-extraction, or adsorption with resins. In certain cases, the recovery is complicated by the solvent's low volatility.⁸⁴ The food-grade potential is considered high, especially for choline chloride, urea, organic acids, and sugars, classified as GRAS (generally recognised as safe). Despite this, novel mixtures should be assessed for safety and eventual synergistic toxicity.⁸⁶

When applied to oilseeds defatting, NADESs enabled effective oil removal. The two-phase NADES choline chloride : glycerol/menthol : thymol was employed for simultaneous co-extraction of protein and oil from hemp flour, producing a protein isolate with lower fat residue than hexane.⁸⁷ High oil recovery was achieved using a choline chloride : oxalic acid-ethyl acetate-water mix on cottonseed, giving better performance than the Soxhlet hexane extraction.⁶⁸

As for fish and seafood biomasses, while the use of NADESs and DESs has been carried out to obtain ingredients such as proteins from sardines,⁸⁸ astaxanthin from shrimp waste,⁸⁹ chitin from crustacean shells, and collagen peptides from cod skins,⁹⁰ the oil extraction/removal by means of these solvents has not been documented. Literature insights still suggest the great potential of hydrophobic low viscous fatty acid-based DESs for fish defatting, which till now have been solely employed to remove micro-pollutants⁸⁵ and volatile fatty acids from aqueous solutions.⁹¹ Natural molecules forming NADESs can display antioxidant and protective effects on lipids,⁹² which would be highly beneficial for preserving and enhancing the fish oil quality.

Extraction with ILs. Ionic liquids (ILs) consist of salt solutions of an organic cation and organic or inorganic anion, and they can be easily kept in the liquid form due to their low charge density and reduced melting point.⁹³ Generally, these green solvents possess low flammability and vapour pressure, as well as high selectivity, stability, and solvation capability.⁹⁴ They are versatile and suitable for designing solvents with particular and unique characteristics in terms of polarity, conductivity, and hydrophobicity.⁹⁵ ILs include switchable solvents that are able to switch from an ionic liquid under a CO₂ atmosphere to a non-ionic one under an N₂ atmosphere.

For instance, regarding oilseeds, amidine/alcohol and secondary amine switchable solvents (switchable-polarity solvents) were employed to extract oil from flaked soy seeds⁷ (Table 2). Such solvents show miscibility with lipids in their low polarity form, and immiscibility after switching to the high polarity one. The first mentioned solvent allowed achieving a positive separation; nevertheless, its use was unsuccessful due to the occurrence of side transesterification and interference with water. The second switchable-polarity solvent provided slow separation, along with significant contamination levels and health issues, leading again to rejection. In the same work, an amidine with excess water (switchable-hydrophilicity solvent) was also studied, giving better extraction and separation that were achieved in the solvent's hydrophobic and hydrophilic forms, respectively.⁷ Subsequently, the solvent can be isolated from water by making it return to its hydrophobic nature. This last phase can be particularly challenging; thus, further studies examining the economic and

technical feasibility and applicability of ionic liquids for oils extraction are needed.⁸

Moreover, in relation to fish oil, a lack of studies on the application of ILs on fish biomass to recover lipids and oils can be observed. Indeed, some published works focused on the enrichment of fish oil in beneficial poly-unsaturated fatty acids by employing ILs, not on their use directly on the fish material for oil extraction. This topic may represent an interesting research area to uncover and explore in the near future.

Extraction with 2-MeOx or 2-MeTHF. 2-Methyloxolane (2-MeOx) or 2-methyltetrahydrofuran (2-MeTHF) is a solvent originating from renewable sources, including bagasse and corncobs, with a boiling point of 80 °C allowing good lipid extraction efficiency as well as easy removal and recycling. Moreover, it possesses similar density and viscosity to hexane. Despite being flammable, 2-MeOx has a higher explosivity limit, higher electrical conductivity than hexane, and partial miscibility with water, contributing to a better safety profile. Particular attention should be paid to handling the solvent due to the possible formation of peroxides. Nevertheless, the safer toxicological profile and reduced impact on the environment with respect to hexane, along with the compatibility with up-scaled industrial exploitation, make 2-MeOx a valid alternative to petrochemicals as a defatting solvent.²³ Its exploitation in industry is currently constrained by higher price and energy for solvent removal, with respect to hexane. These limitations can be overcome by reducing losses and optimising recycling.²

Considering oilseeds, 2-MeOx was used in comparison with hexane for lab and pilot scale Soxhlet extraction of rapeseed oil, providing equal and higher yields, respectively.⁶⁹ 2-MeOx and its form saturated with water were able to extract soybean oil with increased yield compared to hexane, because of an enhanced recovery of polar lipids and isoflavones, and a similar lipid profile and fatty acid composition⁷⁰ (Table 2).

As for fish oil recovery, this solvent was reported to efficiently solubilise triglycerides, di-glycerides, free fatty acids, and ergosterol and give more than 30% oil yield from Atlantic salmon⁶⁴ (Table 2). Research on the use of 2-MeOx for fish oil extraction is very limited. The high potentialities of this green solvent surely support a more extensive utilisation for the above purpose, given the European Food Safety Authority (EFSA)'s declaration of its safety if used in compliance with the intended conditions and the indicated maximum residue limits in food ingredients.⁹⁶

Extraction with supercritical CO₂. The valorisation of biological residues from oilseed and fishing industries can also be achieved through supercritical carbon dioxide (CO₂) extraction as an advantageous and sustainable alternative to conventional organic solvents.⁹⁷ It is recognised as an environmentally benign solvent, easily available, non-toxic, non-flammable, and with limited cost. Supercritical CO₂ has been classified as GRAS by the Food and Drug Administration (FDA) and the EFSA.⁹⁸ This solvent can be readily removed from the extracts, due to the easy transition back to the gaseous state determined by depressurisation, providing solvent-free products.⁹⁹ One of its main advantages is the adaptable selectivity, permitting the extraction of specific compounds by temperature and pressure regulation.⁷³ On the other hand, the main limitation of this



high-pressure technology is the consistent installation cost and capital investment, as well as the energy required for solvent compression and recycling. Notably, the energy used for supercritical CO₂ extraction of oil seed rape by the pilot plant accounted for 684 kJ kg⁻¹ solvent,¹⁰⁰ higher than the energy needed for the distillation of *n*-hexane (around 420 kJ kg⁻¹ solvent)¹⁰¹ and 2-MeOx (greater than 460 kJ kg⁻¹ solvent), but lower than the energy requirement for ethanol recycling (almost 970 kJ kg⁻¹).²³ Moreover, adequate sample preparation is essential, especially for reducing moisture content below 20% and avoiding excessively low particle size, to improve the contact with CO₂ and enhance the extraction efficiency.⁹⁸

For oilseeds, supercritical CO₂ has been successfully employed to extract soybean residue oil⁷¹ or produce defatted soy meal rich in protein, with minimal denaturation and maintaining their functional properties better than hexane.¹⁰² On the other hand, supercritical CO₂ has limited capacity to extract polar compounds, resulting in the need to add a co-solvent like ethanol,¹⁰³ making the process more complex and less sustainable.⁷¹

Regarding fish biomasses, numerous studies report the use of supercritical CO₂ to obtain highly stable and nutritionally valuable fish oil from yellowtail fish viscera,⁷³ salmon⁷⁶ and hake⁷⁹ by-products, tuna⁷⁵ and sardine⁷⁸ waste, mackerel muscle,⁷⁴ and shrimp waste.⁷⁷ Moreover, supercritical CO₂ has been optimised to obtain defatted catfish with high protease activity and low fat residue, confirming the ability of this technique to preserve proteins and enzymes.⁹⁷ Conversely, overall lower oil yields than those obtained by conventional methods can be observed, again due to limited affinity with polar compounds.¹

Extraction with vegetable oils. Vegetable oils have the potential to be valid substitutes of petrochemical solvents due to their good dissolving capability, oxidative stability if enriched with antioxidant constituents, and organoleptic quality. These bio-based solvents possess the advantages of being non-toxic and biodegradable, and not emitting volatile organic compounds. The vegetable oils' selectivity is variable, as well as their extraction efficiency.¹⁰⁴ The literature reports the application of some vegetable oils, in particular seed oils, as extraction solvents for astaxanthin and carotenoid recovery. Astaxanthin-rich oil has been obtained using soy, palm, and flaxseed oil on crawfish waste, giant tiger shrimp, and other shrimp by-products, respectively.^{105,106} Carotenoids were also extracted from shrimp waste employing several vegetable oils, including sunflower, mustard, soybean, coconut, and rice bran oils, with the first one giving greater carotenoid yield.¹⁰⁷ Other oils were also used on other biomasses for the same purpose, as in the case of carrot waste and pumpkin by-products treated with cottonseed and canola oil, respectively.^{108,109}

Interestingly, the use of vegetable oils for lipids extraction and enrichment from oilseed and fish biomasses has not been documented. Considering the similar non-polar nature of both carotenoids and lipids and their affinity towards oils as solvents, the applications of the latter could be more widely explored and investigated especially for lipid recovery and potential synergism and enhancement of the fatty acid profile in the obtained products.

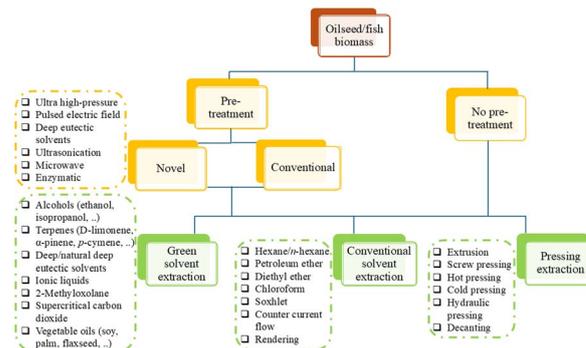


Fig. 1 Overview of pre-treatments, green, and non-green solvent extraction for oilseed and fish defatting.

Biomass pre-treatments prior to defatting. Pre-treatment processes can facilitate biomass's cell disruption and ensure faster extraction and higher yields of the compounds of interest. The currently available literature provides numerous insights into the positive effects of biomass pre-treatments followed by lipid extraction and defatting processes.¹¹⁰ In this regard, it is worth considering that pre-treatments are often associated with extraction by conventional solvents or other methods, and not by novel green solvents. For instance, fish oil has been obtained from yellowfin tuna heads by enzymatic hydrolysis with papain, firstly applying ultra high-pressure pre-treatment, with the improvement of the oil recovery through sequential pressurisation and depressurisation phases.¹¹¹ Moreover, pulsed electric field-assisted pre-treatment combined with porcine lipase hydrolysis was used on seabass skin to effectively remove lipids and fishy smell prior to collagen extraction.¹¹²

Notably, most of the published reports on pre-treatments before defatting are not related to oilseed crops and fish, but to other biomasses, mainly microalgae, mostly due to their rigid, complex, and highly resistant cell walls. For instance, *Chlorella* sp. was treated with aqueous DESs in combination with ultrasonication and homogenisation, before proceeding with lipid extraction by using ethyl acetate and ethanol.¹¹³ Similarly, DES treatment and ultrasonic homogenisation, followed by lipid extraction in a methanol-*n*-butanol mixture, provided consistent lipid yield from *Chlorella pyrenoidosa*.¹¹⁴ Ultrasound-assisted extraction was also associated with pre-treatment with commercial enzymes for *Isochrysis galbana* microalga, giving a significant recovery of polar lipids, EPA, and DHA.¹¹⁵ In another case, fresh microalgae were pre-treated by pulsed microwave as an efficient, energetically sustainable, and clean processing strategy to be applied before lipid extraction with ethanol and hexane.¹¹⁶

Overall, these numerous and promising insights support the application of the above pre-treatments on fish and oilseed biomasses, followed by lipid extraction and defatting by sustainable solvents (Fig. 1), providing potential beneficial impacts in terms of reduction of the extraction time, solvent use, temperature, and nutrient losses, and increase of the extraction yield and protein utilisation from the residue. Techniques such as ultrasound and pulsed electric fields can



contribute to improving the mass transfer, which is limited in case of viscous solvents, like certain terpenes and DESs.

Conclusions

The comparative investigation of seed and fish oil removal and defatting methods highlights the common predominance of conventional solvent-based approaches, mostly utilising hexane and other petroleum solvents. Despite their recognised high yield and efficacy, they are facing increasing disapproval due to health and environmental risks, as well as regulatory issues, supporting the exploration of alternative options. Emerging sustainable solvent solutions, including alcohols, terpenes, deep and natural deep eutectic solvents, ionic liquids, 2-methylloxolane, supercritical carbon dioxide, and vegetable oils possess great potential to limit the ecological impact and increase safety in the defatting processes. However, their main challenges include scalability, costs, inconstant performance, and additional optimisation requirements. In linking oil removal solvent methods for the investigated sources, along with clear process parallelisms, some discrepancies between seed and fish matrices can be evidenced, highlighting research gaps mainly in fish oil defatting and in the use of vegetable oils for both biomasses. In addition, based on literature insights on diverse sources, innovative pre-treatment techniques prior to sustainable solvent defatting can be adapted to seed and fish, aiming at improving extraction yield, environmental sustainability, and use of the residues, while minimising nutrient loss and process duration. In conclusion, as green solvents for defatting provide promising avenues to align with sustainability targets and reduce environmental impact, additional research and industrial advancements are vital to promote broad adoption, addressing actual limitations. Notably, the implementation of solvent removal and recycling, the combination of green solvents such as natural deep eutectic solvents with mechanical and advanced extraction technologies, and the development of novel food-grade hydrophobic deep eutectic solvents deserve to be further explored. Active cooperation among academic research, industry stakeholders, and regulatory institutions appears essential to advance sustainable solvent defatting methods and ensure efficient and safe valorisation of oilseed and fish resources.

Author contributions

Eugenia Mazzara: conceptualisation, investigation, visualisation, writing – original draft, writing – review & editing. Bri-jesh K. Tiwari: conceptualisation, supervision, funding acquisition, writing – review & editing.

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.

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