Journal of Agriculture and Food Research 21 (2025) 101887

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Green extraction methods of fucoxanthin from brown macroalgae

Marcell Gyurkač [a,b], Taja Žitek Makoter [a], Miha Grilc [b], Blaž Likozar [b], Željko Knez [a,c], Maša Knez Marevci [a,c,*]

[a] Laboratory of Separation Processes and Product Design, Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova ulica 17, SI-2000, Maribor, Slovenia
[b] Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Hajdrihova 19, SI-1000, Ljubljana, Slovenia
[c] Faculty of Medicine, University of Maribor, Taborska 8, SI-2000, Maribor, Slovenia

ARTICLE INFO

Keywords:
Brown macroalgae
Fucoxanthin
Green solvents
Supercritical fluids
Ionic liquids

ABSTRACT

Brown macroalgae, harvested and processed at a scale of multimillion tons annually, contain fucoxanthin, a bioactive carotenoid with demonstrated anti-cancer and anti-inflammatory properties. While seaweed is primarily cultivated for food products, significant quantities of non-edible parts, still containing valuable compounds, are discarded during processing. This comprehensive review critically examines current approaches for upcycling macroalgal waste to food supplements and pharmaceuticals through the extraction of fucoxanthin, with particular emphasis on emerging green technologies and solvents, such as supercritical carbon dioxide and ionic liquids, applied individually or in combination. The article also evaluates these methods against conventional organic solvent extraction, considering extraction efficiency, solvent greenness and recyclability, process sustainability, and potential for industrial scale-up, while identifying current limitations and explore opportunities for process optimization. Despite demonstrating clear advantages over conventional organic solvents in extraction time, yield, recycling rate, and environmental safety, green solvents, especially ionic liquids, remain underutilized. This thorough analysis thus reveals critical gaps in current extraction strategies and provides evidence-based recommendations for future research directions in sustainable extraction technologies. Our findings suggest pathways for transforming macroalgal waste streams into high-value nutraceutical products, contributing to the development of circular bioeconomy approaches in marine biomass processing.

1. Introduction

Macroalgae are important and popular nutrients worldwide, exhibiting numerous health-promoting properties. As a result of globalization, these foods have become commercially available to consumers around the world [1]. Beyond their use for direct consumption, algae provide valuable bioactive compounds and antioxidants for pharmaceuticals and cosmetics. In the cosmetic industry, algal extracts are used to produce moisturizers, thickening agents and rejuvenating lotions, and their natural origin makes them increasingly attractive for industrial-scale cosmetic production [2]. Algae are widely utilized for a diverse range of applications, including wastewater treatment, biofertilizer production [3], sustainable biofuel manufacturing as well as in the textile industry, fisheries and various other industrial and environmental processes [4].

Algae are divided into microalgae and macroalgae according to their growth characteristics. While macroalgae are generally harvested from the sea, microalgae's ability to be cultivated enables their controlled production. Macroalgae can be further categorized into subgroups, with the three primary taxonomic classes being brown algae (Phaeophyceae), red algae (Rhodophyta), and green algae (Chlorophyta) [5].

Discussing the valorization of both micro- and macroalgae would however go beyond the scope of this article. Rather, the review focuses on the challenges associated with the significant quantities of macroalgae waste generated globally and explores the potential of utilizing these residues in green extraction processes to generate valuable products through extraction and separation. A detailed overview of fucoxanthin (Fx) extraction from brown macroalgae is provided, together with a critical evaluation of the greenness and recyclability of the solvents used in the extraction processes.

Annually, more than 35,000,000 wet tons of algae are produced and harvested worldwide [6]. The brown alga *Undaria pinnatifida*,

* Corresponding author. Laboratory of Separation Processes and Product Design, Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova ulica 17, SI-2000, Maribor, Slovenia.
E-mail address: masa.knez@um.si (M. Knez Marevci).

https://doi.org/10.1016/j.jafr.2025.101887
Received 10 February 2025; Received in revised form 21 March 2025; Accepted 5 April 2025
Available online 6 April 2025

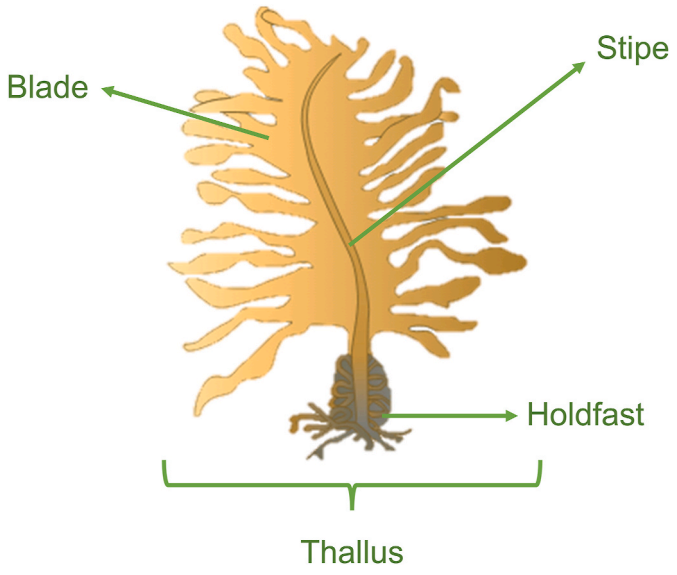

Fig. 1. *Structural features of Undaria pinnatifida* (own work).

commercially known as wakame, contributes over 3,000,000 wet tons to annual global production, ranking it as the fourth most cultivated seaweed worldwide [7]. Yin et al. [8] reports that more than 200,000 tons of wakame are dumped in Japan annually, which accounts for 8 % of the total worldwide wakame production discarded in one country. Several tons of Fx isolate could be recovered from the waste each year (assuming that 1 ton of dry wakame contains approximately 1–2 kg of Fx), if disposal were properly addressed [9].

Macroalgae waste accumulation is linked to their anatomy: holdfast (anchor against currents) and blade, connected by the stipe, all nutrient-rich components [10] (Fig. 1), while only the blade is commonly consumed. All other parts are discarded despite containing valuable pigments. Consequently, waste volumes exceed reported figures, considering both discarded seaweed parts and food waste [11]. Another example of waste generation from algae comes from the hydrocolloid industry, where it is estimated that even up to 50 wt percent (wt%) of waste is produced in the form of residual biomass after alginate or agar production [12]. An even larger market presence of *Saccharina japonica*, formerly *Laminaria japonica* (Phaeophyceae) (commercially available and labelled as Japanese kelp or kombu) of over 12,000,000 wet tons per year shows, that industrial opportunities for the extraction and isolation of Fx from these discarded seaweeds is feasible [13]. Kanazawa et al. [14] reports that more than 79,000 tons of *Saccharina japonica* are discarded yearly in Japan, which contain around 0.2 g of pure Fx per 1 kg of fresh algae. His research group has made big steps towards an industrialized scale of extraction, by extracting 10,000 kg of kombu waste parts with absolute ethanol (EtOH), yielding almost 1500 g of Fx with a decent purity and outstanding longtime stability.

Besides bioactive compounds, macroalgae comprise of even higher amounts of proteins, diverse carbohydrates, minerals, and moderate amounts of lipids, making them attractive for other applications, e.g. isolation of protein hydrolysates [15]. Even if less abundant than in microalgae, fats make macroalgae attractive for biofuel production [16], whereas the complex carbohydrates such as algin (or its salt, alginate), fucoidan, laminarin, mannitol, cellulose and agar, can be either hydrolyzed to produce fermentable sugars [17] or utilized to spin fibers which are fully biocompatible with the human body [18]. Rarely, but still in significant amounts, macroalgae contain lignin [19], as well as minor compounds such as trace minerals, and extractables [20,21].

The different extractives comprise a variety of bioactive compounds with health beneficial properties, such as phenolic compounds (phenolic acids, flavonoids, phlorotannins), carotenoids, chlorophylls, as well as minerals and vitamins [22–24]. Carotenoids (terpene-derived pigments) are further subdivided into carotenes (unsaturated hydrocarbons without an oxygen containing moiety) and xanthophylls (same as carotenes but containing at least one oxygen based moiety) [25]. Xanthophylls can be further subdivided, depending on the oxygen containing moiety present. These are alcohols (lutein, zeaxanthin), ketones (astaxanthin), esters (Fx), epoxides, and other minor representatives. All of them act as antioxidants due to their high number of conjugated pi-bonds [26].

An overview of these compounds found in brown macroalgae, together with their relative abundance is shown in Fig. 2. Neglecting water content (up to 90 wt%), ash and polysaccharides (alginate, laminarin and fucoidan) are the most abundant species found in brown seaweeds (both ranging even up to 40 wt% and beyond). The protein content is already significantly lower (around 10 wt%), while cellulose and lipid contents usually are lower than 10 and 5 wt% respectively. In the lower percent and percentile range extractives and trace minerals can be found, where Fx represents the most important and valuable extractive of interest [27–30].

2. Fucoxanthin availability in algae

Wakame, Japanese kelp and other brown macroalgae as well as some microalgae contain high amounts of Fx, even up to 2.5 wt% of the seaweeds total dry mass [31]. This compound is the most abundant of all

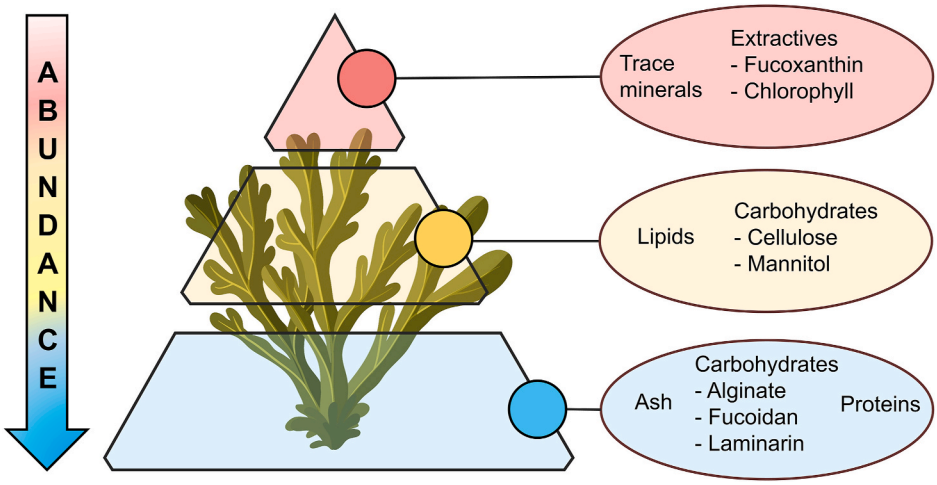

Fig. 2. Chemical composition of brown macroalgae with their respective relative abundance (own work).

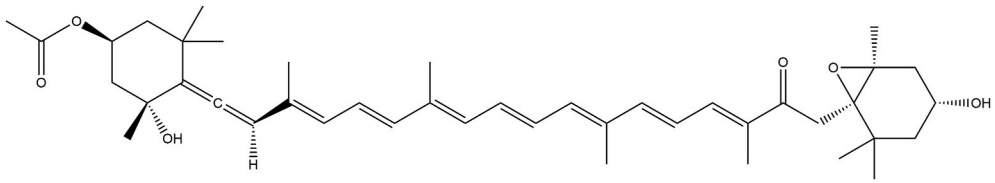

Fig. 3. Molecular formula of Fx.

carotenoids in aquatic life and is present in the chloroplast of the algal cell by complexing with chlorophyll [32]. It is a polyunsaturated xanthophyll with many oxygen-based functional groups, such as hydroxyl, ester and oxo groups and a rare allene (C=C=C) bond as well (Fig. 3). Fx concentration in brown seaweed varies depending on the harvesting season and are at the highest level early in the year till spring when temperatures and exposure to light are at their lowest level [33,34]. However, there are also some outliers, where the Fx content in the algae was the highest in the summer season [35]. Furthermore, Fx content varies depending on the body part of the macroalgae used for extraction. The blade of fresh *Undaria pinnatifida* contains up to twice the amount of Fx than the sporophyll. A similar trend is observed for other macroalgal species as well, where the leaves contain more pigment than the other parts of the macroalgae [33,36,37]. Moreover, more than 50 wt% of the Fx from fresh algae is decomposed during commercial end-product preparation. For preservative and mild processing, freeze-drying of the fresh algae is recommended to yield a maximum of Fx in the end-product [36]. Furthermore, Fx content may also vary depending on the depth of growth as well as the temperature of the water and its exposure to light [38].

Although the review focuses on the pretreatment, extraction, isolation, and quantification of Fx, the findings are not limited to this target molecule, but rather serve as a good anchor point for the extraction and isolation of other extractives, especially other xanthophylls found in algae.

For instance, astaxanthin can be found in Rhodophyta and in microalgae (*i.e.* in *Haematococcus lacustris* – formerly *Haematococcus pluvialis* - Chlorophyta), which is produced only at around 200 tons scale annually [39]. The advantage of microalgal cultures in comparison to macroalgae lies in their growth properties, which makes it possible to produce them artificially and thus despite their higher production costs, they are of great industrial interest for the manufacture of high-quality products [5,40]. Furthermore, both lutein and zeaxanthin can be found in Rhodophyta and microalgae (e.g. in *Chlorella* - Chlorophyta*)* and are readily absorbed in the human body through nutrition [5]. In contrast to Fx, which is almost exclusively found in marine environment (algae, diatoms and corals) [41], the above-mentioned carotenoids can be found in other feed sources as well. Zeaxanthin and lutein are present in salads like kale and spinach [42], whereby shrimp shells and crab shells contain astaxanthin [43].

The main motivation for the green extraction of Fx from algae consists of two key elements. First Fx has many health benefiting properties for the human body, ranging from anti-oxidative, -cancer, -inflammatory and -adiposity features, which are already well investigated and discussed [44], hence this review omits the biochemical and nutritional discussion. Secondly, employing green extraction methods and solvents to isolate Fx can drastically reduce the carbon footprint by reducing solvent consumption and overcoming issues of non-recyclability. This is in direct coherence with the European green deal [45]. In the following section, environmentally benign pretreatment methods, as well as green extraction media for Fx extraction from algae are thoroughly discussed and moreover, the greenness and recyclability of these solvents are critically evaluated.

3. Pretreatment and extraction methods for Fx from macroalgae

The extraction of biologically active compounds from waste biomass is a vital step in waste valorization, pharmaceutical drug production and the improvement of human health through nutritional supplementation. By employing an economically efficient and environmentally friendly process for extraction that follows the zero-waste principle, we can successfully reduce environmental impact while added value is generated. The ability to pretreat waste biomass with the right ionic liquids (ILs) and a further extraction step of the heterogeneous mixture with supercritical carbon dioxide ($scCO_2$) provides a green and sustainable two-step process to achieve these goals. This innovative two-step process was previously described by Kornpointner and co-workers [46], where cannabinoids from IL pretreated hemp were extracted by $scCO_2$, improving the purity of the analytes many times without compromising yields.

3.1. Pretreatment of brown macroalgae

The brown macroalgae cell wall is built-up by different proteins and polysaccharides, such as fucoidan interlinked with cellulose and salts of alginic acid binding to phenol-containing moieties [47], which can reduce or prevent efficient diffusion of solvents into the inner parts of the cells. To effectively extract carotenoids from biomass samples with strong cellular structure, effective penetration of the matrix is fundamental. Using physical and physicochemical methods to accelerate the cell wall disruption facilitates extraction rates manifold. These methods include the use of ultrasound, microwaves, enzymes, electric field and high pressure, which can replace or modify traditional organic treatments. Organic solvents can be replaced by green alternatives such as deep eutectic solvents (DES), ILs and supercritical fluids [48]. ILs, which are usually referred to as green alternatives for volatile and hazardous organic solvents, are gaining increasing recognition in the biomass sector. They have interesting properties such as negligible vapor pressure and high thermal stability. Interestingly, ILs are organic salts, but in a liquid state, even at ambient temperature, so they could be called salty solvents. This makes them attractive for various applications in most areas of chemistry and other disciplines such as pharmacy, metallurgy, and materials science [49]. Due to the wide variety of anions and cations that can be used for their synthesis, ILs are tunable solvents in terms of their polarity, acid-base properties and viscosity [50]. However, not all ILs are green, especially when their preparation process is examined. Even if the IL itself is non-toxic and environmentally friendly as an end product, the steps involved in its synthesis and purification can indeed be environmentally harmful and even hazardous. Some time ago, researchers successfully developed a method for the halogen-free production of ILs using a methanolic solution of dimethyl carbonate as an alkylating reagent for the precursors, which enables the halogen-free production of many different ILs even on a ton scale [51].

Novel pretreatment steps using pure or aqueous ILs or bio-based ILs, such as 2-hydroxyethyl-trimethylammonium lysinate (Chol Lys) or 2-hydroxyethyl-trimethylammonium lactate (Chol Lac) could ease the extraction of value-added chemicals from seaweeds by allowing faster extraction times, milder conditions compared to conventional pretreatment steps and extraction methods, as well as allowing the reusability/recyclability of the solvent used and thus converting

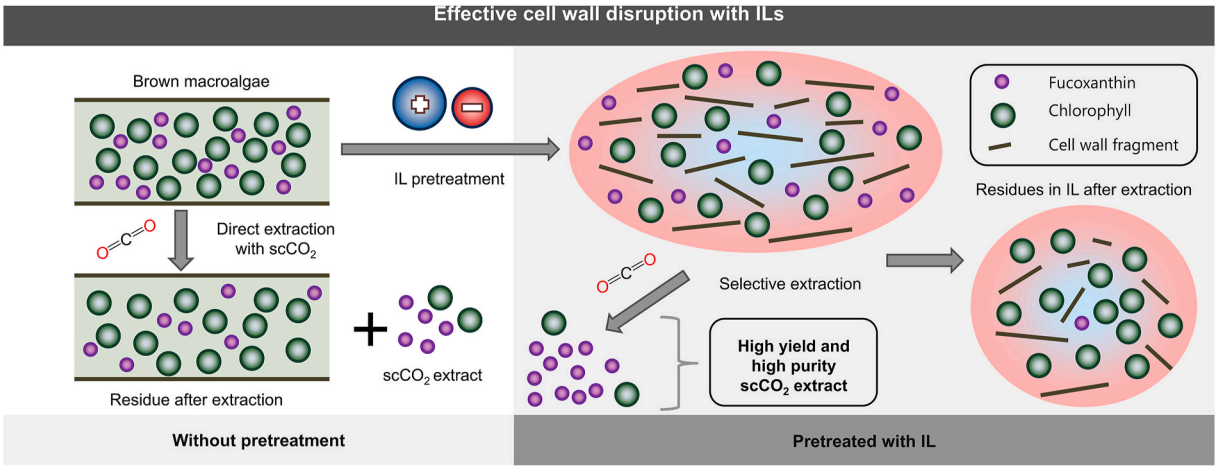

Fig. 4. Difference in $scCO_2$ extraction yield of Fx comparing non-pretreated brown macroalgae and IL pretreated brown macroalgae (own work).

conventional processes into greener and environmentally friendlier ones. Considering the literature which investigated imidazolium and ammonium based ILs and the similar acid-base properties of 1-ethyl-3-methylimidazolium acetate (EMIM OAc) and Chol Lys (which can be derived from cellulose dissolution experiments [52,53]), there is a very high probability that novel ILs and bio-based ILs would enhance the extraction procedure [54–59]. A great example of cell wall disruption was investigated by Orr et al. [60] during lipid extraction from *Chlorella vulgaris* (Chlorophyta) by room-temperature ILs ranging from imidazolium, ammonium, phosphonium, and pyridinium based ones. Samples were extracted with pure n-hexane and compared to the extraction by IL/n-hexane mixture. The yields were significantly higher for almost all ILs tested. The environmentally friendly and cheap EMIM ethyl sulfate ($EtSO_4$) gave 5-times higher oil yield than the n-hexane. Nevertheless, the ILs were used only for cell wall disruption and the extraction of the oil was still carried out with n-hexane, which makes the process more efficient but does not make the process greener overall. Furthermore, the recycling of the EMIM $EtSO_4$ was achieved by adding methanol (MeOH) as an antisolvent. Especially n-hexane is an undesirable solvent for process intensification due to its environmental concerns, while MeOH is toxic and flammable as well. Replacing these co-solvents after IL pretreatment with $scCO_2$ could overcome the issue of toxicity and hazardousness, yielding solvent-free and high purity extracts, without the need for the removal of the extraction solvent from the high-value extract (Fig. 4).

3.2. Extraction methods for Fx

3.2.1. Conventional maceration extraction

Most common extraction method used. The solid sample is extracted with a liquid solvent under different extraction parameters (time, temperature, solid loading, mixing efficiency, pH). Solvents used range from methanol, EtOH, acetone, dichloromethane (DCM), chloroform ($CHCl_3$) as well as hexane, heptane, and diethyl ether. The main drawback of the solvent extraction procedure is the uneconomical use of large quantities of hazardous solvents with compromised extraction efficiencies due to the rigid cell wall structure of the seaweeds [61,62]. The maceration extraction can be modified by adding a stirring equipment to the extractor and thus increasing the mixing efficiency [63].

3.2.2. Conventional soxhlet extraction

The Soxhlet apparatus allows a semi-continuous solid/liquid extraction under reflux conditions [64]. The main advantage of this technique is the recyclability of the solvent used and no need to separate solids from the liquid stream. Furthermore, the amount of solvent used is kept minimal due to the recirculation after each cycle. The main drawback, however, are the long extraction times, and the high temperatures used as well as high energy consumption to vaporize the extraction medium, thus generating lots of energy losses [65]. The high temperatures and the prolonged heating of the extract could lead to analyte decomposition.

The following subsections describe advanced extraction methods that are commonly used for pigment extraction. The review does not include emerging advanced technologies based on electrical current, such as pulsed electric field assisted extraction, moderate electric field assisted extraction and high voltage electric discharge assisted extraction, which are well described elsewhere [48,66].

3.2.3. Ultrasound assisted extraction (UAE)

High-frequency ultrasonic vibrations create cavitation in liquids, and consequently a high-intensity burst (implosion) of the gas bubbles leading to a temperature increase and disruption of the algal cell wall, thus facilitating the mass transfer between the algae's solid matrix and solvent used [67,68]. Carreira-Casais et al. [65] investigated different parameters during extraction (time, temperature, solid loading, extraction media, and ultrasound energy) on their effect on extraction efficiency of bioactive compounds from marine seaweeds. It was concluded that higher-energy waves, generated by lower-frequency vibrations, improve the extraction yields and reduce the time necessary for extraction. Increasing temperature does not always improve extraction yields, thus this parameter must be optimized for each biomass sample and analyte of interest separately. Additionally, the shape of the horn and the reactor itself are of great importance since the reflectance of the generated waves is different depending on the spatial distribution and wall thickness of the setup. Furthermore, combining UAE with conventional solvents/methods or with modern green solvents like ILs can improve extraction yields. Cikos et al. [67] investigated the Fx extraction yield from three macroalgal species found in the Adriatic Sea and improved the extraction efficiency for each species ~5-times compared to conventional solid/liquid extraction with methanol (50 °C, 30 min, S/L = 20 mL/g). Not only that the extraction yields improved, but ultrasound-treatment also allows the use of less solvent, decreased extraction times and reduced operating costs as well as the dimensioning of continuous flow processes compared to conventional maceration extractions [65,67–69]. Lourenço-Lopes et al. [70] described the UAE of Fx from *Undaria pinnatifida* with EtOH and obtained a yield of 20.91 mg/g algae, significantly higher than the extraction yield of 3.80 mg/g algae obtained by Soxhlet extraction with pure EtOH. Nie et al. [71] used renewable green solvents (ethyl lactate, limonene and vegetable oil) combined with UAE to extract Fx from *Sargassum fusiforme* (Phaeophyceae) and proved that using sustainable approaches, can improve extraction capacity on the one hand (ethyl lactate) and selectivity on the

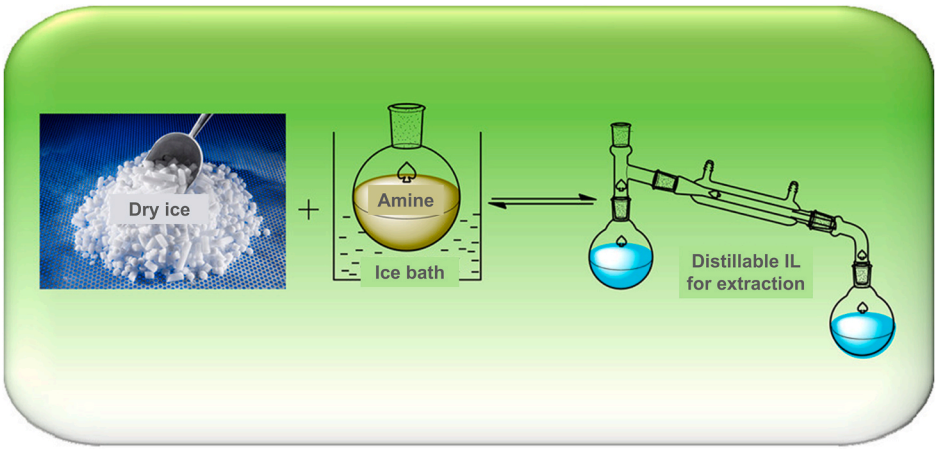

Fig. 5. Synthesis of a carbamate based DIL (amine + CO_2) and its recycling by distillation - Reprinted with permission from Ref. [86]. Copyright © 2014, American Chemical Society.

other hand (limonene and vegetable oil).

3.2.4. *Microwave-assisted extraction (MAE)*

Analogous to UAE, high-frequency microwaves facilitate extraction of bioactive compounds from algae by reducing extraction media consumption and allowing faster extraction with less energy consumption by manifold. Mechanistically, the waves generate vibrations and thus energy which manifests in the form of heat in the molecules. The molecules must be susceptible to microwaves, that is, they must have a constant dipole moment [62]. The energy waves readily deconstruct cell walls and thus facilitate biomass wetting. Lourenço-Lopes et al. [70] was able to extract up to 10.01 mg Fx per one g dry *Undaria pinnatifida* with EtOH as solvent, showing that MAE is superior to Soxhlet extraction but inferior to UAE using the same solvent (previous subsection). However, the extraction time of 3 min is vastly better than the 30 min used for UAE and 4 h for the Soxhlet extraction, making MAE a better option to extract Fx faster. Similarly, Xiao et al. [72] extracted Fx from *Undaria pinnatifida*, *Saccharina japonica,* and *Sargassum fusiforme* by MAE in EtOH. The extraction yields were again comparable to conventional methods used and the extraction was much faster (10 min per batch).

3.2.5. *Pressurized liquid extraction*

High-temperature and high-pressure processes also facilitate the extraction of bioactive compounds by reducing extraction time and solvent consumption, allowing the use of environmentally friendly solvents like water and EtOH to increase their leaching power due to the extreme conditions [61]. The main drawbacks, however, are the high investment costs for pressurized vessels and the nature of pigments which are thermolabile molecules. Therefore, for the extraction of bioactive compounds with reduced stability under extreme conditions other extraction methods are recommended [73].

3.2.6. *Enzyme-assisted extraction (EAE)*

Enzymes can also affect the algal cell wall by hydrolyzing the barriers of the seaweed samples allowing easier solvent penetration and mild analyte extraction [61,74]. The main drawback of using biocatalysts lies in their high production cost. For example, Billakanti et al. [75] reports that with an enzymatic pretreatment (0.05 wt% of alginate lyase and 99.95 wt% of fresh wakame, extracted at 37 °C, for 2 h, with an overhead mixer) and subsequent follow-up extraction with dimethyl ether and EtOH, an extraction efficiency of 94 % of Fx is achieved. The 0.05 wt% of enzyme loading however would require an amount of 0.5 kg alginate lyase on a ton-scale process, which would cost over 1,000,000 (€) per batch only for the enzyme, if we estimate its cost from Sigma-Aldrich [76], therefore enzyme recycling is crucial to reduce production costs. Furthermore, it would be desirable to replace ether solvents with less flammable and non-explosive ones, to minimize equipment requirements being compliant with the explosive atmospheres (ATEX) directives, which in turn can skyrocket extractor and separator equipment costs.

3.2.7. *Supercritical fluid extraction (SFE)*

SFE is one of the most prominent extraction techniques in the field of bioactive molecules obtained from biomass samples. Although the generation of supercritical fluids (for instance $scCO_2$) is cost-intensive, the advantages outweigh this drawback. The solvent allows fast mass transfer due to its low viscosity, tunable polarity with changing pressure and temperature in the reaction vessel, as well as mild and selective extraction of target molecules due to parameter optimization. Especially the high selectivity towards less polar compounds can yield very pure extracts of carotenoids, without the need for a downstream washing or purification step [77]. It is also possible to use co-solvents in the reactor system to enhance and fine-tune the polarity of the extraction media, which makes the diversity of this technique a real frontrunner. An additional benefit is the solvent waste free extraction with supercritical fluids, simplifying the extraction process [78,79]. Moreover, $scCO_2$ after expansion is completely recyclable when the plants are operating at a sufficiently high capacity [80].

Macías-Sánchez et al. [68] showed that by applying $scCO_2$ with MeOH as co-solvent one can achieve a similar extraction efficiency of Fx from *Dunaliella salina* (microalgae - Chlorophyta) than with UAE using MeOH. Recently, Honda et al. [81] extracted Fx from *Undaria pinnatifida* by applying high temperature in the extraction cell and EtOH as a co-solvent. They claim that at higher temperatures a *Z*-isomerization occurs, which makes the Fx more prone to cellular uptake. The extraction efficiencies however, are moderate, below 31 wt%, and the probability of Fx degradation at these elevated temperatures is very high.

3.2.8. *Subcritical water extraction (SWE)*

The extreme conditions of SWE at high temperatures and pressures can reduce the dielectric constant of water, changing its polarity to mimic common organic solvents used for pigment extraction. This allows faster and easier extraction of less polar and water-insoluble analytes from plants, with the greenest solvent to date, water. The process offers advantages such as shorter extraction time, lower solvent usage, and enhanced selectivity. However, a major drawback of SWE is the high temperatures required to reach water's subcritical state, making the process energy-intensive and unsuitable for extracting the thermolabile Fx from brown macroalgae. Nevertheless, SWE is an emerging green processing technology, suitable for the extraction of more robust analytes, such as polyphenols or carbohydrates while avoiding the use of toxic organic solvents [77,82].

3.2.9. IL-assisted extraction

ILs have already been used to extract valuable macromolecules as well as pigments from algae. Furthermore, IL assisted pretreatment of seaweed showed promising results in biofuel production [83]. For instance, the extraction of proteins from *Ulva lactuca* (Chlorophyta) with 1-ethyl-3-methylimidazolium dibutyl phosphate (EMIM DBP) showed excellent yields and selectivity at mild extraction conditions. Over 80 % of the proteins present in the seaweed were extracted at 25 °C using a 40 % EMIM DBP solution. The recycling recovery of the IL after extraction however, was only near 85 % [84].

Khoo et al. [85] extracted Fx from *Chaetoceros calcitrans* (Mediophyceae, microalga) utilizing distillable ILs, more specifically alkyl carbamate ones. An extraction yield of 17.51 mg/g algae was achieved at 25 °C, while extracting only for 3 min with aqueous diallyl ammonium diallyl carbamate (90/10 v/v). This approach is promising since the IL can be recycled by distillation (Fig. 5) and separated from the Fx at the same time. The use of CO_2-based ILs, however, always leads to loss of the gas because it is not trapped in the condenser of the evaporator completely. Therefore, the concentration of the IL must be adjusted after each cycle. Nevertheless, the extraction efficiency of Fx is significantly higher (even up to 10-fold higher) compared to extraction by alcohols, ketones, esters or any other IL used. The authors discovered that extraction at lower temperature is desirable to prevent Fx degradation. Thus, the yield is significantly higher at 25 °C compared to 55 °C. Another example for carotenoid extraction was reported by Desai et al. [55], an extraction efficiency greater than 70 % of astaxanthin from *Haematococcus lacustris* using aqueous EMIM DBP as extractant at 45 °C for 90 min was achieved. In this case, however ethyl acetate was used for the liquid-liquid extraction to re-extract the pigments from the IL phase into the organic phase, which ultimately produces organic solvent waste. ILs have been utilized for carotenoid extraction from *Sargassum muticum* (Phaeophyceae) described by Vieira et al. [59]. Surface-active ILs are suitable for this process due to their hydrophobic side chains and ionic character, which enhance miscibility with water. However, the ILs used were halide-based, making them incompatible with industrial-scale stainless-steel reactors due to their corrosive nature. As a result, scaling up would require more costly glass reactors, posing a challenge for industrial applications. Similarly, Martins et al. [56] used halide-based ILs for the extraction of Chlorophyll and Fx from *Saccharina latissima* (Phaeophyceae). 4 imidazolium-based, 4 ammonium-based, and one phosphonium-based IL was tested at room temperature (rt). Sunflower oil was added as a co-solvent and the best extraction efficiency of the two analytes was achieved with the phosphonium based IL at rt extracted for 40 min. Despite the high pigment yield, the isolation is carried out by a back-extraction procedure with toluene, which makes the process unattractive for green industrialization.

3.2.10. DES extraction

Like ILs, depending on the reagents used to synthesize them, DES are also green solvents present in liquid state at ambient temperatures. Their hydrophobicity can be tuned by selecting the starting reagents and their mixing ratio (eutectic mixture of Lewis acids and bases). If the reagents are bio-based and biodegradable, the DES are labelled as natural based (NADES) [87]. As an example, Jayarani et al. [88] managed to extract 1.700 ± 0.006 mg Fx per g algae from *Sargassum wightii* (Phaeophyceae) with MeOH:Lactic acid mixture 1:2 (w/w) in 5 h at rt.

3.3. Greenness and recyclability of solvents used for Fx extraction

To design an efficient and economical process for pigment extraction from macroalgae, it is advisable to consider several metrics that describe not only the process units and their costs, but also the solvents used for extraction and pigment isolation (e.g. their origin, production costs, biodegradability and hazardousness, greenness, recyclability, reusability). Prat and co-workers [89] have creatively and thoroughly

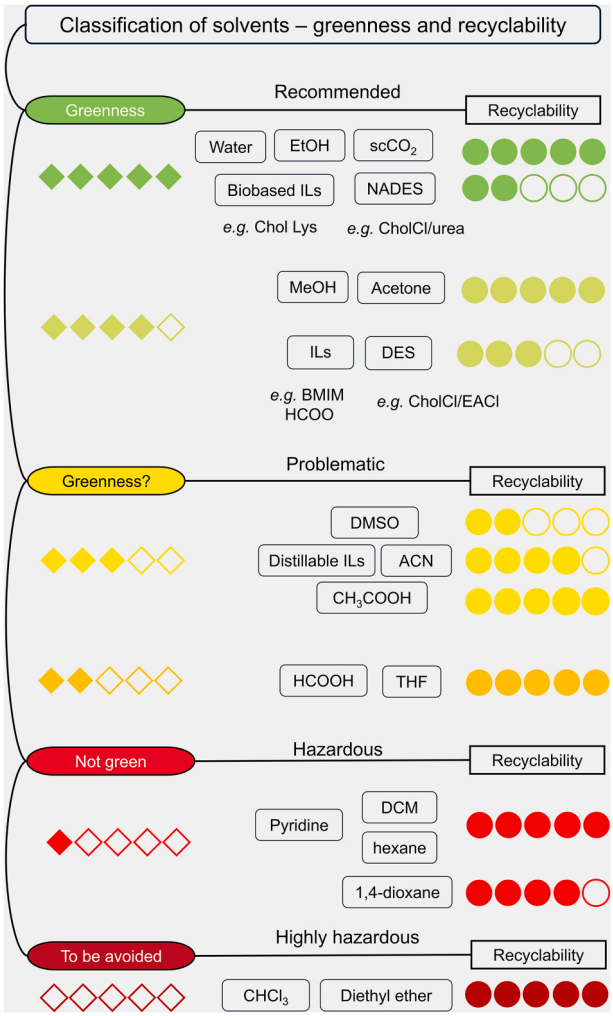

Fig. 6. Classification of conventional organic solvents, $scCO_2$ and various ILs and DES based on their green metrics and their recyclability (own work).

designed a selection guide for various conventional organic solvents and water used in chemical industries. Based on this research, green descriptors can be assigned to solvents based on their safety (e.g., flash point), health (e.g., exposure limits), and environmental features (e.g. n-octanol-water partition coefficient). The solvents are then ranked from recommended ones, problematic ones to hazardous and highly hazardous solvents, where it is recommended to design processes using the greenest alternatives or problematic solvents if the recommended solvents are not compatible with the given process. The replacement of hazardous and highly hazardous solvents is encouraged, and their use should be omitted. The selection of solvents for pigment extraction from macroalgae can be based on this methodology or on other literature discussing the greenness of non-conventional solvents such as supercritical fluids, ILs and DES. Despite the high complexity, these 3 substance classes can also be categorized with reference to the twelve principles of green chemistry [90]. Especially by addressing the issues of waste prevention (recyclability), use of less hazardous reagents (in the case of ILs and DES), energy efficiency (avoidance of costly production and recycling processes), use of renewable sources instead of fossil fuel-derived reagents, reduction of co-solvents and additional reagents (simplicity of the process) and (bio)degradability. $ScCO_2$ fulfils most of these criteria and can therefore be considered a recommended green solvent. Chen et al. [91] have discussed various ILs and DES used in different processes in detail and made recommendations for their improvement in terms of greenness. That is, to avoid the use of

Table 1
Summary of Analytical methods used for Fx assay determination in algae extracts.

Column	Column compartment temperature [°C]	Flow rate and mobile phase composition	Injection volume [μL]	Detection wavelength [nm]	Literature
C18 YMC-Triart (5[b]x4.6 mm, 5 μm)	n.d.[a]	1.0 mL/min MeOH/acetonitrile (ACN) 6:4 v/v	10	450	[11]
C18 Develosil® ODS UG-5 (250 × 4.6 mm, 5 μm) with 10 × 4.0 mm C18 guard column	28	1.0 mL/min MeOH/ACN 7:3 v/v	n.d.	450 (add. 210)	[33]
C18 J'sphere ODS-H80 (150 × 20 mm, 4 μm)	25	0.8 mL/min MeOH/water gradient	n.d.	440	[103]
C8 Zorbax (4.6 × 250 mm, 5 μm)	n.d.	1 mL/min MeOH/water gradient (15 % MeOH to 0 % in 15 min, 0 % MeOH till 28.5 min, change to 15 % MeOH till 29.5 min, 15 % MeOH till 30 min)	20	190–650	[104]
C18 Nova-Pak (150 × 3.9 mm, n. d.)	25	0.5 mL/min; A: 5 mM ammonium acetate (NH_4OAc) in water; B: 5 mM NH_4OAc in MeOH; C: Ethyl acetate; 5 %A+95 %B till 8min, 50 %B and 50 %C to 20min, 50 %A and 50 %B till 35min, 30 %A and 70 %B till end (40 min)	50	450–700	[63]
C18 Mightsil® (250 × 20 mm, 5 μm)	n.d.	MeOH/water 95:5, n.d. on flow rate	n.d.	450	[105]
C30 YMC (250 × 4.6 mm, 5 μm) with C18 Luna guard column (4 × 2.0 mm, 5 μm)	30	0.8 mL/min A: water; B: MeOH; C: t-butyl ether; Composition A/B/C: 0min: 10/90/0; 5 min, 4/81/15; 25 min, 4/81/15; 55 min, 4/31/65C till 65 min equilibrating	20	450	[106]
C18 ODS-2 (300 × 4 mm, 5 μm)	n.d.	0.6 mL/min MeOH/ACN 50:50 v/v	20	450	[107]
C18 Chromolith (3[b]x4.6 mm, 2 μm)	n.d.	1 mL/min; A: MeOH, B: water; Composition A/B: 0min 0/100; 2 min 100/0; 3 min 50/50; 4 min 25/75; 6 min 10/90; 8 min 5/95; till 15 min 0/100 end	20	445	[108]
C30 YMC (250 × 4.6 mm, 5 μm)	30	1 mL/min 100 % MeOH	10	450	[109]
C18 Develosil® ODS UG-5 (250 × 4.6 mm, 5 μm) with 10 × 4.0 mm C18 guard column	28	1.0 mL/min MeOH/ACN 7:3 v/v (same as [33])	n.d.	450	[101]
C18 ODS-3 (250 × 4.6 mm, 5 μm)	40	1.0 mL/min ACN/water 8:2 v/v	n.d.	445	[110]
C18 Zorbax SB (4.6 × 250 mm, 5 μm)	25	0.8 mL/min; A: ACN/water 80/20 v/v and B: methyl tert-butyl ether linear gradient	n.d.	450	[111]

[a] No data.
[b] Most certainly wrong column length.

unnecessarily unstable (thermally or chemically) and non-renewable reagents for ILs and DES, which are non-biodegradable, thereby avoiding toxic, flammable and explosive reagents (e.g. nitrate or perchlorate based ILs), energy-intensive downstream processes (expensive due to high viscosity and separation issues) and high recycling costs (non--distillable ILs and DES) due to the extensive use of purifying co-solvents, which should be avoided whenever possible. Based on these findings, a comprehensive categorization (greenness and recyclability) of the solvents used for pigment extraction from brown macroalgae was created (details in Fig. 6). Overgeneralization in the case of ILs and DES should be taken with caution. ILs can be completely bio-based and therefore highly recommended green solvents (e.g. Chol Lys), fossil fuel-derived and thus less green (e.g. 1-butyl-3-methylimidazolium acetate - BMIM OAc) or problematic (flammable and slightly toxic, but distillable protic ILs – e.g. pyridinium formate - Py HCOO). Similarly NADES may be highly recommended green solvents (e.g. choline chloride (CholCl): urea mixture) or less green (e.g. choline chloride mixed with non-bio-based ethyl ammonium chloride – CholCl/EACl).

There are also some outliers among the discussed solvents, such as MeOH, which is indeed a problematic solvent due to its toxicity, however, it still is labelled as a recommended solvent due to allowed high exposure limits. Acetic acid, despite being environmentally benign, in some cases even bio-derived and safe solvent, it is nevertheless labelled as a problematic solvent due to being highly corrosive (H314), and furthermore, contrary to MeOH, the less toxic pyridine is classified as hazardous due to its very low permissible exposure limit [89]. We however, argue that pyridine based ILs can be classified as recommended or problematic solvents, due to their lower volatility, corrosivity and toxicity compared to pure pyridine or the acid from which they are synthesized [92]. Therefore, Py HCOO is classified as problematic, rather than hazardous in our master diagram. Other solvents with questionable greenness range from problematic ones, like acetonitrile (ACN), dimethyl sulfoxide (DMSO), formic acid (HCOOH), acetic acid (CH_3COOH) and tetrahydrofuran (THF), to hazardous ones like DCM, hexane, 1,4-dioxane, $CHCl_3$ and diethyl ether.

Besides the benefits of using green and recyclable solvents for pigment extraction, there are many challenges which prevent their implementation in larger scale processes. While $scCO_2$ has achieved commercial success in applications like decaffeination [93], IL and DES industrialization remains limited. One crucial aspect, which hinders successful industrialization of ILs, remains their production costs. Furthermore, both aprotic ILs and DES are significantly more viscous than conventional organic solvents, impeding effective mixing and substantially increasing extraction and downstream processing expenses. Additionally, problems associated with corrosion hinder the use of halide-based ILs in stainless steel extractors, which prevents their successful scale-up [94]. Changing to non-halide based ILs solves the corrosion issue, however, creates new challenges related to reactivity with extractable compounds. Belesov et al. [95] demonstrated that BMIM OAc can form covalent bonds with lignin monomer side chains during biomass fractionation. Similarly, van Erven and co-workers [96] observed covalent bonding between choline and lignin monomers during DES pulping of *Miscanthus* biomass. These reactions compromise the recyclability of the extraction media, presenting additional barriers to their sustainable implementation.

Nevertheless, exchanging existing extraction processes with sustainable alternatives, while implementing green and recyclable solvents, can positively contribute to the environmental impact reduction of outdated chemical processes, especially when hazardous petrochemicals and toxic waste accumulation can be replaced. A notable example of sustainable innovation comes from the leather industry, where protic ILs are used to demethylate lignin-based polyphenols, enabling chrome-free tanning and the production of eco-friendly, bio-based leather [97]. This approach avoids the use of toxic chromium (VI) while utilizing a bio-based tanning agent derived through green and eco-friendly ILs. As a result, the process not only circumvents harmful chemicals but also

minimizes waste generation, making it a truly sustainable alternative. Another compelling case study involves novel biorefinery processes, specifically the production of bioethanol and by-products from 2-hydroxymethylammonium acetate (MEAH OAc) pretreated sugarcane bagasse. While this process enhances mass efficiency through technical lignin recovery without compromising EtOH yield, it generates a substantial burden of wastewater and vinasse as well. Compared to conventional EtOH production, the MEAH OAc process generates 37 wt% more wastewater. Furthermore, the protic IL accumulates in the washing water. A detailed analysis however showed that the solvent biodegrades by over 95 % within five days, mitigating the environmental impact [98]. Therefore, a thorough literature analysis is essential, as evaluating the environmental impact of such processes is complex, and the use of green solvents in sustainable approaches may not always be the optimal solution. A similar approach is seen in the biorefinery concept, where the alga *Ulva rigida* (Chlorophyta), rich in carbohydrates, was pretreated using switchable, distillable, and low-viscosity ILs, replacing conventional organic solvents and sulfuric acid. A guanidinium-based IL dissolved over 66 wt% of the sugars, which were successfully recovered (>92 %) through precipitation and filtration. These sugars can be upcycled into valuable bio-based platform chemicals or biofuels like bioethanol [99]. However, the key challenge remains the efficient recycling of ILs to minimize solvent loss and optimize mass balance, as both factors are crucial in preventing waste accumulation and reducing environmental impact.

4. Analytical methods for Fx assay determination

In the Chemical Abstracts Service (CAS), the CAS Analytical Methods™ search engine tool listed 344 results for Fx labelled as the analyte (search was performed on the 3rd of June 2024). Most of the results listed use high performance liquid chromatography coupled to various detectors for the separation and quantification of Fx assay found in extracts. Reversed phase technique with a count of 56 predominates the choice of stationary phase. The detector listed with the highest count is the ultraviolet–visible (UV/Vis) spectrophotometer (count 58) followed by fluorescence spectrometer (count 14) and others.

A summary of high-performance liquid chromatography (HPLC) methods used (with all necessary hardware parameters listed) are shown in Table 1. The first column of Table 1 lists different HPLC columns used for analyte separation. The abbreviation YMC is the company's name which produces different chromatography columns and octadecylsilane (ODS) describes a type of stationary phase used.

As one can see the chromatography of Fx containing algal extracts is straightforward and uses standard reversed-phase columns, mostly with standard particle diameter (5 μm) and standard column lengths (either 150 mm or 250 mm). This makes the Fx assay determination fast and straightforward. However, one must consider that these brown macroalgal extracts are free of interfering analytes (no cross contamination from other carotenoids except for the baseline separated zeaxanthin are causing separation issues). And even the co-extracted chlorophyll (easily distinguishable) can be washed away by simple active charcoal washing step or silica gel column chromatography if necessary [14], therefore sample preparation and extract purifications are minimal. When analyzing mixtures of different carotenoids however (in a complex matrix), the analysis would become more technically challenging, and parameter optimization would be necessary to baseline separate different carotenoids or even use an alternative detector in parallel to the UV/Vis, like an electrospray ionization mass spectrometer to verify whether peaks representing Fx are not co-eluting with other carotenoids [100]. One additional feature from Fx assay determination from brown macroalgae is its purity. If one calculates the amount of Fx extracted per g fresh extract, the higher the concentration, the purer the Fx extract is. This is especially true when extracting Fx with polar solvents like MeOH or EtOH, because of chlorophyll and zeaxanthin coextraction [101]. To obtain high purity Fx (for instance at least reagent grade ≥95 wt%) the

Table 2
Amount of Fx found in commercially available algae, extraction method and extraction yield.

Source (species)	Extraction method	Extracted Concentration [μg/g dw algae]	Literature
Undaria pinnatifida	$scCO_2$ (4 mL/min) with EtOH as modifier (3.125 mL) at 414 bar, 50 °C, 240 min	282	[11]
Undaria pinnatifida	$scCO_2$ extraction with EtOH as co-solvent (2 mL/min flow rate, 3 % (v/v)), 200 bar, 50 °C, 50 min, mass flow rate not determined	$7.5 * 10^{-3}$	[113]
Undaria pinnatifida	MeOH 1:10 (w/v), 2x overnight	2300 ± 100	[114]
Undaria pinnatifida	$scCO_2$ extraction, 40 °C, 400 bar, 4 mL/min, 180 min, residue washed and combined with EtOH after extraction	546	[115]
Undaria pinnatifida	$scCO_2$ extraction, 60 °C, 400 bar, 3 mL/min, 270 min	59.5	[110]
	And same conditions + 0.5 mL/min EtOH as co-solvent	994.5	
Undaria pinnatifida	$scCO_2$ extraction with EtOH as co-solvent, 120 °C, 30 MPa, 30 min	737	[81]
Stems *of Undaria pinnatifida*	$scCO_2$ extraction with EtOH as co-solvent (5 mL in extraction chamber and 4.5 mL for trapping of crude extracts), 275.8 bar, 40 °C, 60 min	178 ± 3	[8]
Undaria pinnatifida – freshly harvested with seasonal variations	Extraction in MeOH at rt for 1 h and in dark, partitioning with n-hexane and $CHCl_3$	2080 ± 40	[36]
Undaria pinnatifida	Soxhlet extraction with EtOH	4580	[70]
	UAE with EtOH for 30 min	20910	
	MAE with EtOH for 3 min at 2 bar pressure	10010	
Undaria pinnatifida (commercial)	Maceration in MeOH for 2 days	323	[105]
Undaria pinnatifida	Conventional heat extraction at 45 °C, 70 % acetone in water for 61 min	5100	[63]
Undaria pinnatifida	UAE with water (960 W, 80 % amplitude, 20 kHz, 3 h)	3 % of total extract (no absolute value given)	[116]
Undaria pinnatifida fresh	1x extraction in 8 mL of MeOH/n-hexane/DCM 50/25/25 v/v/v and 2x extraction in 8 mL of acetone for 1 min	1119 + 122	[117]
Undaria pinnatifida dried	Extraction in MeOH over night	728 ± 57	[118]
Undaria pinnatifida powder		3090 ± 111	
Undaria pinnatifida	Extraction in DMSO/ water 4/1 v/v for 1 min	26.81 ± 0.79	[119]
Himanthalia elongata		2.79 ± 0.31	
Laminaria ochroleuca		14.21 ± 0.31	
Saccharina latissima	84 wt% of 350 mM aqueous phosphonium based chloride IL + 16 wt% sunflower oil extracted at rt for 30 min	1960 ± 80	[56]

(*continued on next page*)

Table 2 (*continued*)

Source (species)	Extraction method	Extracted Concentration [μg/g dw algae]	Literature
	and with a solid liquid ratio of 0.017 g/mL (w/v), supernatant obtained by centrifugation for 15 min		
15 different brown macroalgae samples	2x in MeOH, overnight, 1:10 w/v biomass loading	Ranging from 100 to 3700	[33]
Sargassum horneri	Maceration in absence of light with $CHCl_3$:MeOH	1350 - 4490 (Jun and Jan)	[34]
Seasonal variation	1:2 (v/v) for 1 h repeated 2x and once with $CHCl_3$:MeOH:H2O 1:2:0.8 (v/v/v) followed by evaporation and re-dissolution	630 - 4140 (Sep and Apr)	
*Stephanocystis hakodatensis (*formerly *Cystoseira. hakodatensis)*			
Seasonal variation			
Nizamuddinia zanardinii	Maceration in absence of light and in nitrogen atmosphere with $CHCl_3$: MeOH 1:2 (v/v) for 2 h repeated 3x and once with $CHCl_3$:MeOH:H2O 1:2:0.8 (v/v/v) followed by evaporation and re-dissolution	560–1650 (Feb and June)	[35]
Seasonal variation		2330–3560 (Apr and Dec)	
*Polycladia indica (*formerly *Cystoseira. indica)*			
Seasonal variation			
Saccharina japonica	$scCO_2$ extraction with sunflower oil as co-solvent (2 wt% in CO_2), 300 bar, 51 °C, 240 min	1421 ± 181	[120]
Saccharina japonica	$scCO_2$ extraction without co-solvent, 300 bar, 40 °C, 180 min	451 ± 27	[121]
Sargassum muticum	$scCO_2$ extraction at 50 °C, 300 bar, 25 g CO_2 per min for 60 min	15	[122]
	$scCO_2$ extraction with EtOH (10 wt%) at 50 °C, 200 bar, 25 g CO_2 per min, for 40 min	1350	
*Sargassum aquifolium (*formerly *Sargassum binderi)*	3x cold acetone/MeOH 7:3 v/v wash for 15 min; dry extract cleaned up by 3x partitioning between n-hexane and 90 % aqueous MeOH, aqueous phase diluted with diethyl ether and evaporated to dryness	1010 ± 100	[101]
*Sargassum ilicifolium (*formerly *Sargassum duplicatum)*		730 ± 390	
Saccharina japonica	1.0 g algae in 250 mL MeOH	90	[108]
Sargassum angustifolium	UAE with MeOH at 150 W and 100 % amplitude for 15 min with a biomass loading of 1:30 (w/v)	790 ± 10	[107]
Polycladia indica		810 ± 10	
Padina tetrastromatica	UAE of 1 g algae in 10 mL 80 % EtOH (v/v) at conditions: 50 Hz, 230 V, for 30 min at 50 °C	750	[123]
Undaria pinnatifida	MAE in EtOH at 50 °C for 10 min at a biomass loading of 1:10 (w/v) followed by high-speed counter current chromatography with hexane/ethyl acetate/ethanol/water (5:5:6:4 vol%)	727	[72]
Saccharina japonica		33	
Sargassum fusiforme		13	
Eisenia bicyclis	Pressurized liquid extraction, 110 °C, 90 % Ethanol loading	420	[124]

Table 2 (*continued*)

Source (species)	Extraction method	Extracted Concentration [μg/g dw algae]	Literature
Chaetoceros calcitrans	IL extraction with diallyl aqueous ammonium diallyl carbamate (90 % v/v) at 25 °C for 3 min	17510	[85]
Myagropsis myagroides	1 g of algae powder extracted with 100 mL of 80 % aqueous MeOH	9010	[103]
Dictyota coriacea	1 g of algae powder extracted with 100 mL of 80 % aqueous MeOH	6420	[103]
*Feldmannia mitchelliae (*formerly *Hincksia mitchelliae)*	UAE with ethyl acetate at rt, 3 × 10 min	5500	[125]
Fucus serratus	10 g algae powder in 100 mL hexane/acetone extracted at 50 °C for 24 h $scCO_2$ extraction, 2 g algae powder, 50 °C, 300 atm, 60 min and 1 mL/min CO_2 flowrate and 0.1 mL/min EtOH as a co-solvent $scCO_2$ extraction, 2 g algae powder, 50 °C, 300 atm, 105 min and 10 mL/min CO_2 flowrate	3570 2180 510	[104]
Sargassum siliquosum	1.0 g algae in 69 mL MeOH 37 °C for 15 h and 48 min	1410	[126]
Sargassum polycystum	1.0 g algae in 82 mL MeOH 37 °C for 17 h and 57 min	310	
Saccharina japonica	$scCO_2$ extraction with EtOH, 250 bar, 45 °C, 27 g CO_2 per min, for 2 h	410 ± 50	[127]
Sargassum horneri		770 ± 70	
Tisochrysis lutea	96 % EtOH, 30 °C, 1 h, 1:20 (w/v) loading	31100 ± 1500	[109]
Phaeodactylum tricornutum	100 % MeOH, 40 °C, 24 h, 1:10 (w/v) loading	13300 ± 300	
Sargassum wightii	$scCO_2$ extraction with 15 % EtOH, 400 bar, 45 °C, for 3 h	3000 ± 40	[88]
	NADES extraction MeOH:Lactic acid 1:2 (w/w)	1700 ± 6	
Laminaria ochroleuca	Sonication for 15 min in acetone/butylated hydroxytoluene 1:0.05 (v/v) followed by stirring at 600 rpm for 45 min (both steps repeated 3x)	163.7	[38]
Saccharina latissima		126.0	
Saccorhiza polyschides		236.0	
Fucus vesiculosus	Extraction with 62.2 % acetone at 30 °C and pH 5.7 for 36.5 min	751	[37]
Dunaliella tertiolecta (Chlorophyta)	MAE with acetone at 8.5 °C under argon atmosphere with an energy of 12.2 W for 3–10 min	4490 ± 80	[128]

isolation and purification downstream process becomes more and more complicated, involving multiple washing, evaporation and recrystallization steps [102]. At the end of the day, the necessary purity of the Fx extracts must meet the requirements for the end application.

5. Summary

The concentrations of extracted Fx in *Undaria pinnatifida* range from 0.008 to 20910 μg/g dry weight (dw) of algae. This wide range indicates

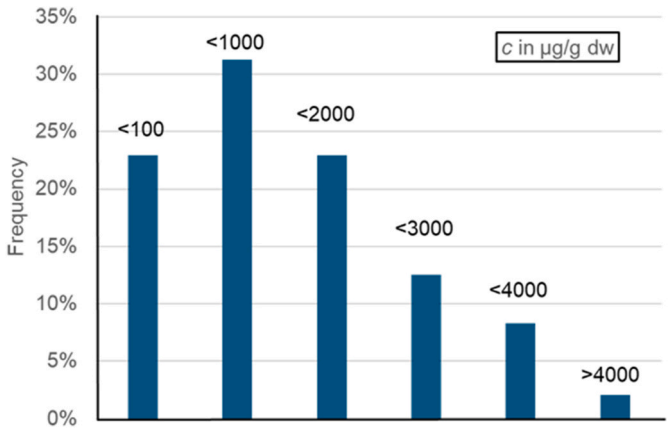

Fig. 7. Frequency [%] of results based on Fx concentrations reported in Table 2 (own work).

that the Fx yield is highly dependent on the extraction method used and on the fate and condition of the used algae. Not all samples contain the same total amount of Fx, even if it is a commercial Wakame sample from the same distributor, as its biosynthesis and accumulation in the algae varies depending on the season and physical growth parameters [104]. In addition, the extraction yield depends on the number of extraction cycles performed per batch. 3 extraction cycles result in a combined extract with higher Fx content than one cycle with the same method and solvent used. Therefore, it is very difficult to compare data and the true extraction efficiency of Fx is an unknown. The extraction yield of Fx extracted from various brown macroalgae and other species by using different extraction methods and solvents is summarized in Table 2.

When analyzing the data, a more realistic approach is to compare results by sorting them by concentration and calculate the frequencies of the occurring concentrations by count. The concentration ranges between 0.1 and 99, 100–999, 1000–1999, 2000–2999. 3000–3999 and above 4000 µg/g dw macroalgae have been sorted and visualized (Fig. 7). In addition, the median of all Fx concentration results lies at 994 µg/g dw for *Undaria pinnatifida*. A similar trend is observable for all other algal species incorporating Fx. For simplicity, the ranges in Fig. 7 have been labelled as $< c_i$, where c_i is the upper limit of a defined range. More than 75 % of all reported results lay in the concentration range between 0.1 and 1999 µg/g dw macroalgae.

When analyzing the used solvents, interestingly the count of conventional organic solvents and the maceration extraction method used for Fx extraction is very high when compared to advanced extraction methods and nonconventional solvents used (supercritical fluids, DES and ILs). Nevertheless, the use of green solvents predominates (around 75 % of all solvents reported are recommended green solvents based on Fig. 6). Despite the high count of green solvents, ILs and DES, on the other hand, are highly underutilized, which leaves many opportunities for further research and optimization (Fig. 8). Especially considering that ILs have already been successfully used for the extraction of other xanthophylls. Astaxanthin, zeaxanthin and lutein have been extracted from different species deploying similar extraction methods as used for Fx, by applying ILs as the main extraction solvents in the processes [104]. These results are summarized in Table 3. Furthermore, there is a significant opportunity to explore the use of many more ILs for carotenoid extraction, as they have already demonstrated success in extracting other bioactive compounds from biomass samples. For instance, UAE of phenolic acids from various plant materials using 1-(4-sulfobutyl)-3-methylimidazolium hydrogen sulfate or BMIM bis (trifluoromethylsulfonyl)imide has been shown to double the yield of phenolics [112]. This suggests that ILs could similarly enhance the extraction of carotenoids, offering a promising direction for future research and optimization in this field.

6. Conclusion

The main aim of the research review was to summarize and evaluate environmentally friendly extraction methods and green solvents for the processing of biomass waste from natural sources, specifically from brown macroalgae waste. With proper processing of waste biomass, it is possible to obtain high quality and nutritious dietary supplements, containing bioactive, functional ingredients such as Fx, while at the same time adhering to the principles of green chemistry and recycling and thus contributing to the reduction of waste and soil pollution. The possibilities of processing large amounts of macroalgae waste biomass with ILs and $scCO_2$ are still underexplored and scarce. This represents great potential for the development and establishment of a sustainable, environmentally friendly and waste-free process based on this new

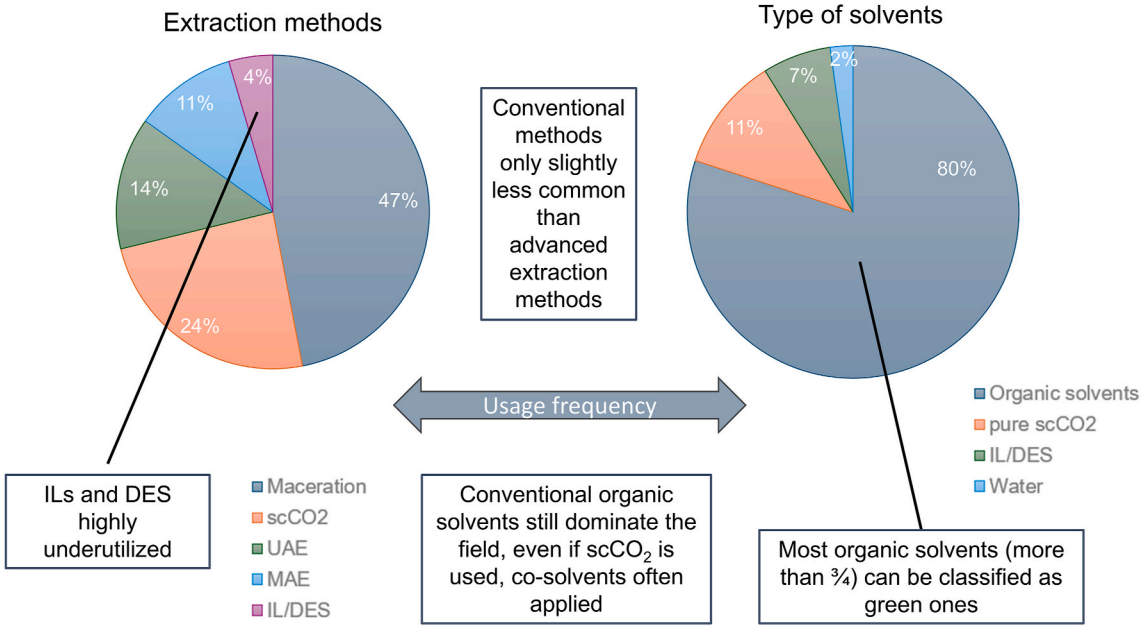

Fig. 8. Frequency of extraction methods and type of solvents used for Fx extraction from brown macroalgae.

Table 3
Amount of astaxanthin, lutein and zeaxanthin found in different types of species using ILs as extraction media.

Source (species)	Extraction method and solvent	Extracted Concentration [μg/g dw algae]	Literature
Haematococcus lacustris (Chlorophyta)	Astaxanthin: IL extraction (EMIM DBP, 45 °C, 90 min) + Ethyl acetate	~70	[55]
Chlorella (Chlorophyta) dried and fresh	Lutein: Aqueous 50 %(w/v) [BMIM] bromide, 60 min, 65 °C and re-extraction with diethyl ether Aqueous 10 %(w/v) [BMIM] bromide, 60 min, 65 °C and re-extraction with diethyl ether	4700 ± 10 5930 ± 140	[129]
*Thermostichus bigranulatus (*formerly *Synechococcus bigranulatus* (Cyanobacteria)	Zeaxanthin: Switchable IL extraction with N-ethylbutylamine and CO_2 for 30 min	20700	[130]

generation of green solvents, with shorter extraction times, less solvent consumption, milder extraction conditions and higher selectivity. In the future special emphasis must be paid to maximizing extraction and separation yields, as well as deep dive into the recyclability and reusability rates of these solvents to justify the scalability of the respective processes and minimizing their environmental impact at the same time. Even a combination of two or more green extraction methods and/or solvents is not excluded and could further reduce solvent consumption, extraction time and energy input.

CRediT authorship contribution statement

Marcell Gyurkač: Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation. **Taja Žitek Makoter:** Writing – review & editing, Visualization, Resources. **Miha Grilc:** Writing – review & editing, Supervision. **Blaž Likozar:** Supervision, Conceptualization. **Željko Knez:** Supervision, Project administration, Funding acquisition, Conceptualization. **Maša Knez Marevci:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Funding

Support from the Slovenian Research Agency through grant P2-0046 L2-3175 is gratefully acknowledged. The authors acknowledge the funding within the project "Upgrading national research infrastructures—RIUM", which was co-financed by the Republic of Slovenia, the Ministry of Higher Education, Science and Innovation, and the European Union from the European Regional Development Fund.

Declaration of competing interest

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

Acknowledgments

The authors would like to thank proionic GmbH for the shared know-how and advice on the discussed ionic liquids in this article. Moreover, this research did not receive any specific grant from funding agencies in commercial sector.

Data availability

No data was used for the research described in the article.

References

[1] L. Pereira, Macroalgae, Encyclopedia 1 (2021) 177–188, https://doi.org/10.3390/encyclopedia1010017.
[2] H.-M.D. Wang, C.-C. Chen, P. Huynh, J.-S. Chang, Exploring the potential of using algae in cosmetics, Bioresour. Technol. 184 (2015) 355–362, https://doi.org/10.1016/j.biortech.2014.12.001.
[3] E.-S. Biris-Dorhoi, D. Michiu, C.R. Pop, A.M. Rotar, M. Tofana, O.L. Pop, S. A. Socaci, A.C. Farcas, Macroalgae-A sustainable source of chemical compounds with biological activities, Nutrients 12 (2020), https://doi.org/10.3390/nu12103085.
[4] S. Pardilhó, J. Cotas, L. Pereira, M.B. Oliveira, J.M. Dias, Marine macroalgae in a circular economy context: a comprehensive analysis focused on residual biomass, Biotechnol. Adv. 60 (2022), https://doi.org/10.1016/j.biotechadv.2022.107987.
[5] A.G. Pereira, P. Otero, J. Echave, A. Carreira-Casais, F. Chamorro, N. Collazo, A. Jaboui, C. Lourenco-Lopes, J. Simal-Gandara, M.A. Prieto, Xanthophylls from the sea: algae as source of bioactive carotenoids, Mar. Drugs 19 (2021), https://doi.org/10.3390/md19040188.
[6] F. and A.O. of the U. Nations, The state of world fisheries and aquaculture 2024 – blue transformation in action, Food Agric. Organ.United Nat. (2024), https://doi.org/10.4060/cd0683en. Rome.
[7] Seaweed Insights, Global production of undaria. https://seaweedinsights.com/global-production-undaria/, 2024. (Accessed 15 January 2025).
[8] S. Yin, M. Shibata, T. Hagiwara, Extraction of bioactive compounds from stems of undaria pinnatifida, Food Sci. Technol. Res. 25 (2019) 765–773, https://doi.org/10.3136/fstr.25.765.
[9] E. Susanto, A.S. Fahmi, M. Abe, M. Hosokawa, K. Miyashita, Lipids, fatty acids, and fucoxanthin content from temperate and tropical Brown seaweeds, Aquat Procedia 7 (2016) 66–75, https://doi.org/10.1016/j.aqpro.2016.07.009.
[10] A. Cheng, W.Y. Lim, P.E. Lim, A. Yang Amri, S.W. Poong, S.L. Song, Z. Ilham, Marine autotroph-herbivore synergies: unravelling the roles of macroalgae in marine ecosystem dynamics, Biology 11 (2022), https://doi.org/10.3390/biology11081209.
[11] S.P. Yin, L.Q. Niu, M. Shibata, Y.F. Liu, T. Hagiwara, Optimization of fucoxanthin extraction obtained from natural by-products from Undaria pinnatifida stem using supercritical CO2 extraction method, Front. Nutr. 9 (2022), https://doi.org/10.3389/fnut.2022.981176.
[12] J.H. Yun, S.D. Archer, N.N. Price, Valorization of waste materials from seaweed industry: an industry survey based biorefinery approach, Rev. Aquacult. (2022) 1–8, https://doi.org/10.1111/raq.12748.
[13] J. Cai, Global status of seaweed production, trade and utilization. World Aquaculture Performance Indicators (WAPI) - Seaweed Innovation Forum Belize, 2021, p. 18.
[14] K. Kanazawa, Y. Ozaki, T. Hashimoto, S.K. Das, S. Matsushita, M. Hirano, T. Okada, A. Komoto, N. Mori, M. Nakatsuka, Commercial-scale preparation of biofunctional fucoxanthin from waste parts of Brown sea algae *Laminalia japonica*, Food Sci. Technol. Res. 14 (2008) 573, https://doi.org/10.3136/fstr.14.573.
[15] F.B. Pimentel, R.C. Alves, P.A. Harnedy, R.J. FitzGerald, M. Oliveira, Macroalgal-derived protein hydrolysates and bioactive peptides: enzymatic release and potential health enhancing properties, Trends Food Sci. Technol. 93 (2019) 106–124, https://doi.org/10.1016/j.tifs.2019.09.006.
[16] D.R. Nobles, S.R. Manning, Extraction and characterization of lipids from macroalgae, in: V. Balan (Ed.), Microbial Lipid Production. Methods in Molecular Biology, 2019, pp. 131–140, https://doi.org/10.1007/978-1-4939-9484-7_7.
[17] E.J. Yun, H.T. Kim, K.M. Cho, S. Yu, S. Kim, I.G. Choi, K.H. Kim, Pretreatment and saccharification of red macroalgae to produce fermentable sugars, Bioresour. Technol. 199 (2016) 311–318, https://doi.org/10.1016/j.biortech.2015.08.001.
[18] B. Machado, S.M. Costa, I. Costa, R. Fangueiro, D.P. Ferreira, The potential of algae as a source of cellulose and its derivatives for biomedical applications, Cellulose 31 (2024) 3353–3376, https://doi.org/10.1007/s10570-024-05816-w.
[19] C. Author, M. Kawaroe, R. Wahyudi, D. Fauzia Lestari, Comparison of biogas productionfrom macroalgae eucheuma cottonii in anaerobic degradation under different salinity conditions, World Appl. Sci. J. 35 (2017) 344–351, https://doi.org/10.5829/idosi.wasj.2017.344.351.
[20] S.V. Vassilev, C.G. Vassileva, Composition, properties and challenges of algae biomass for biofuel application: an overview, Fuel 181 (2016) 1–33, https://doi.org/10.1016/j.fuel.2016.04.106.
[21] M. Sneha, S. Sowmya, M. Premalatha, K. Mathivanan, K. Muthukumar, T. Mathimani, Multifarious extraction methodologies for ameliorating lipid recovery from algae, Environ. Res. 218 (2023), https://doi.org/10.1016/j.envres.2022.114978.
[22] H. Jang, J. Lee, Y.K. Park, J.Y. Lee, Exploring the health benefits and concerns of brown seaweed consumption: a comprehensive review of bioactive compounds in brown seaweed and its potential therapeutic effects, J. Agric. Food Res. 17 (2024), https://doi.org/10.1016/j.jafr.2024.101215.
[23] S.A.O. Santos, R. Félix, A.C.S. Pais, S.M. Rocha, A.J.D. Silvestre, The quest for phenolic compounds from macroalgae: a review of extraction and identification methodologies, Biomolecules 9 (2019), https://doi.org/10.3390/biom9120847.

[24] N.A.S. Din, A.S. Mohd Alayudin, N.S. Sofian-Seng, H.A. Rahman, N.S. Mohd Razali, S.J. Lim, W.A. Wan Mustapha, Brown algae as functional food source of fucoxanthin: a review, Foods 11 (2022), https://doi.org/10.3390/foods11152235.
[25] N. D'Orazio, E. Gemello, M.A. Gammone, M. de Girolamo, C. Ficoneri, G. Riccioni, Fucoxantin: a treasure from the sea, Mar. Drugs 10 (2012) 604–616, https://doi.org/10.3390/md10030604.
[26] E. Christaki, E. Bonos, I. Giannenasa, P. Florou-Paneria, Functional properties of carotenoids originating from algae, J. Sci. Food Agric. 93 (2013) 5–11, https://doi.org/10.1002/jsfa.5902.
[27] Y. Li, Y. Zheng, Y. Zhang, Y. Yang, P. Wang, B. Imre, A.C.Y. Wong, Y.S.Y. Hsieh, D. Wang, Brown algae carbohydrates: structures, pharmaceutical properties, and research challenges, Mar. Drugs 19 (2021), https://doi.org/10.3390/md19110620.
[28] J. Olsson, G.B. Toth, E. Albers, Biochemical composition of red, green and brown seaweeds on the Swedish west coast, J. Appl. Phycol. 32 (2020) 3305–3317, https://doi.org/10.1007/s10811-020-02145-w.
[29] M. Øverland, L.T. Mydland, A. Skrede, Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals, J. Sci. Food Agric. 99 (2019) 13–24, https://doi.org/10.1002/jsfa.9143.
[30] A. Dobrinčić, S. Balbino, Z. Zorić, S. Pedisić, D.B. Kovačević, I.E. Garofulić, V. Dragović-Uzelac, Advanced technologies for the extraction of marine brown algal polysaccharides, Mar. Drugs 18 (2020), https://doi.org/10.3390/md18030168.
[31] D. Noviendri, D. Fithriani, R.F. Hasrini, Fucoxanthin, A xanthophyll from macro- and microalgae: extraction techniques, bioactivities and their potential application in nutra- and cosmeceutical industries. Nternational Conference on Agribusiness and Rural Development (IConARD 2020), 2021, p. 232, https://doi.org/10.1051/e3sconf/202123203010.
[32] K. Miyashita, S. Nishikawa, F. Beppu, T. Tsukui, M. Abe, M. Hosokawa, The allenic carotenoid fucoxanthin, a novel marine nutraceutical from brown seaweeds, J. Sci. Food Agric. 91 (2011) 1166–1174, https://doi.org/10.1002/jsfa.4353.
[33] M. Terasaki, A. Hirose, B. Narayan, Y. Baba, C. Kawagoe, H. Yasui, N. Saga, M. Hosokawa, K. Miyashita, Evaluation of recoverable functional lipid components of several brown seaweeds (Phaeophyta) from Japan with special reference to fucoxanthin and fucosterol contents, J. Phycol. 45 (2009) 974–980, https://doi.org/10.1111/j.1529-8817.2009.00706.x.
[34] M. Nomura, H. Kamogawa, E. Susanto, C. Kawagoe, H. Yasui, N. Saga, M. Hosokawa, K. Miyashita, Seasonal variations of total lipids, fatty acid composition, and fucoxanthin contents of Sargassum horneri (Turner) and Cystoseira hakodatensis (Yendo) from the northern seashore of Japan, J. Appl. Phycol. 25 (2013) 1159–1169, https://doi.org/10.1007/s10811-012-9934-x.
[35] G.A. Fariman, S.J. Shastan, M.M. Zahedi, Seasonal variation of total lipid, fatty acids, fucoxanthin content, and antioxidant properties of two tropical brown algae (Nizamuddinia zanardinii and Cystoseira indica) from Iran, J. Appl. Phycol. 28 (2016) 1323–1331, https://doi.org/10.1007/s10811-015-0645-y.
[36] A. Fung, N. Hamid, J. Lu, Fucoxanthin content and antioxidant properties of Undaria pinnatifida, Food Chem. 136 (2013) 1055–1062, https://doi.org/10.1016/j.foodchem.2012.09.024.
[37] E. Shannon, N. Abu-Ghannam, Optimisation of fucoxanthin extraction from Irish seaweeds by response surface methodology, J. Appl. Phycol. 29 (2017) 1027–1036, https://doi.org/10.1007/s10811-016-0983-4.
[38] F. Fernandes, M. Barbosa, A.P. Oliveira, I.C. Azevedo, I. Sousa-Pinto, P. Valentão, P.B. Andrade, The pigments of kelps (Ochrophyta) as part of the flexible response to highly variable marine environments, J. Appl. Phycol. 28 (2016) 3689–3696, https://doi.org/10.1007/s10811-016-0883-7.
[39] Food and Agricultural Organization of the United Nations, Global seaweeds and microalgae production, 1950 – 2019, 172, https://www.fao.org/fishery/en/publications/280709, 2021.
[40] R. Todd Lorenz, Gerald R. Cysewsk, Commercial potential for Haematococcus microalgae as a natural source of astaxanthin, Trends Biotechnol. 18 (4) (2000) 160–167.
[41] C. Galasso, C. Corinaldesi, C. Sansone, Carotenoids from marine organisms: biological functions and industrial applications, Antioxidants 6 (2017), https://doi.org/10.3390/antiox6040096.
[42] M. Mrowicka, J. Mrowicki, E. Kucharska, I. Majsterek, Lutein and zeaxanthin and their roles in age-related macular degeneration—neurodegenerative disease, Nutrients 14 (2022), https://doi.org/10.3390/nu14040827.
[43] R.R. Ambati, P.S. Moi, S. Ravi, R.G. Aswathanarayana, Astaxanthin: sources, extraction, stability, biological activities and its commercial applications - a review, Mar. Drugs 12 (2014) 128–152, https://doi.org/10.3390/md12010128.
[44] A. Zarekarizi, L. Hoffmann, D. Burritt, Approaches for the sustainable production of fucoxanthin, a xanthophyll with potential health benefits, J. Appl. Phycol. 31 (2019) 281–299, https://doi.org/10.1007/s10811-018-1558-3.
[45] European Commssion, Communication from the commission to the European parliament, the European council, the European economic and social committee and the committee of the regions, in: https://ec.europa.eu/info/law/law-making-process/evaluating-and-improving-existing-laws/refit, 2019.
[46] C. Kornpointner, A. Sainz Martinez, M. Schnürch, H. Halbwirth, K. Bica-Schröder, Combined ionic liquid and supercritical carbon dioxide based dynamic extraction of six cannabinoids from: cannabis sativa L, Green Chem. 23 (2021) 10079–10089, https://doi.org/10.1039/d1gc03516a.
[47] A.A. Salmeán, D. Duffieux, J. Harholt, F. Qin, G. Michel, M. Czjzek, W.G. T. Willats, C. Hervé, Insoluble (1 → 3), (1 → 4)-β-Dglucan is a component of cell walls in brown algae (Phaeophyceae) and is masked by alginates in tissues, Sci. Rep. 7 (2017), https://doi.org/10.1038/s41598-017-03081-5.
[48] M.M. Poojary, F.J. Barba, B. Aliakbarian, F. Donsì, G. Pataro, D.A. Dias, P. Juliano, Innovative alternative technologies to extract carotenoids from microalgae and seaweeds, Mar. Drugs 14 (2016) 1–34, https://doi.org/10.3390/md14110214.
[49] D.D. Patel, J.M. Lee, Applications of ionic liquids, Chem. Rec. 12 (2012) 329–355, https://doi.org/10.1002/tcr.201100036.
[50] R.S. Kalb, E.N. Stepurko, V.N. Emel'yanenko, S.P. Verevkin, Carbonate based ionic liquid synthesis (CBILS®): thermodynamic analysis, Phys. Chem. Chem. Phys. 18 (2016) 31904–31913, https://doi.org/10.1039/c6cp06594e.
[51] R.S. Kalb, M. Damm, S.P. Verevkin, Carbonate Based Ionic Liquid Synthesis (CBILS®): development of the continuous flow method for preparation of ultra-pure ionic liquids, React. Chem. Eng. 2 (2017) 432–436, https://doi.org/10.1039/c7re00028f.
[52] C. Froschauer, M. Hummel, M. Iakovlev, A. Roselli, H. Schottenberger, H. Sixta, Separation of hemicellulose and cellulose from wood pulp by means of ionic liquid/cosolvent systems, Biomacromolecules 14 (2013) 1741–1750, https://doi.org/10.1021/bm400106h.
[53] R. Parthasarathi, A.M. Socha, J. Shi, S. Zhang, V. Stavila, K.L. Sale, B.A. Simmons, S. Singh, Understanding Pretreatment Efficacy of Four Cholinium and Imidazolium Ionic Liquids by Chemistry and Computation 3 4 Ning Sun, n.d.
[54] R.K. Desai, Ionic Liquid Pre-treatment of Microalgae and Extraction of Biomolecules, [internal PhD, Wu, Wageningen University, 2016, https://doi.org/10.18174/393439.
[55] R.K. Desai, M. Streefland, R.H. Wijffels, M.H.M. Eppink, Novel astaxanthin extraction from Haematococcus pluvialis using cell permeabilising ionic liquids, Green Chem. 18 (2016) 1261–1267, https://doi.org/10.1039/c5gc01301a.
[56] B.M.C. Vaz, M. Martins, L.M. de S. Mesquita, M.C. Neves, A.P.M. Fernandes, D.C. G.A. Pinto, M.G.P.M.S. Neves, J.A.P. Coutinho, S.P.M. Ventura, Using aqueous solutions of ionic liquids as chlorophyll eluents in solid-phase extraction processes, Chem. Eng. J. 428 (2022), https://doi.org/10.1016/j.cej.2021.131073.
[57] D. Han, T. Zhu, K.H. Row, Ultrasonic extraction of phenolic compounds from Laminaria japonica aresch using ionic liquid as extraction solvent, Bull. Kor. Chem. Soc. 32 (2011) 2212–2216, https://doi.org/10.5012/bkcs.2011.32.7.2212.
[58] M. Martins, L.M. de Souza Mesquita, B.M.C. Vaz, A.C.R. Dias V, M.A. Torres-Acosta, B. Queguineur, J.A.P. Coutinho, S.P.M. Ventura, Extraction and fractionation of pigments from Saccharina latissima (linnaeus, 2006) using an ionic liquid plus oil plus water system, ACS Sustain. Chem. Eng. 9 (2021) 6599–6612, https://doi.org/10.1021/acssuschemeng.0c09110.
[59] F.A. Vieira, R.J.R. Guilherme, M.C. Neves, A. Rego, M.H. Abreu, J.A.P. Coutinho, S.P.M. Ventura, Recovery of carotenoids from brown seaweeds using aqueous solutions of surface-active ionic liquids and anionic surfactants, Sep. Purif. Technol. 196 (2018) 300–308, https://doi.org/10.1016/j.seppur.2017.05.006.
[60] V.C.A. Orr, N.V. Plechkova, K.R. Seddon, L. Rehmann, Disruption and wet extraction of the microalgae Chlorella vulgaris using room-temperature ionic liquids, ACS Sustainable Chem. Eng. 4 (2016) 591–600, https://doi.org/10.1021/acssuschemeng.5b00967.
[61] C. Lourenço-Lopes, P. Garcia-Oliveira, M. Carpena, M. Fraga-Corral, C. Jimenez-Lopez, A.G. Pereira, M.A. Prieto, J. Simal-Gandara, Scientific approaches on extraction, purification and stability for the commercialization of fucoxanthin recovered from brown algae, Foods 9 (2020), https://doi.org/10.3390/foods9081113.
[62] A.K. Gupta, K. Seth, K. Maheshwari, P.K. Baroliya, M. Meena, A. Kumar, V. Vinayak, Harish, Biosynthesis and extraction of high-value carotenoid from algae, Frontiers in Bioscience - Landmark 26 (2021) 171–190, https://doi.org/10.52586/4932.
[63] C. Lourenço-Lopes, M. Fraga-Corral, A. Soria-Lopez, B. Nuñes-Estevez, M. Barral-Martinez, A. Silva, N. Li, C. Liu, J. Simal-Gandara, M.A. Prieto, Fucoxanthin's optimization from undaria pinnatifida using conventional heat extraction, bioactivity assays and in silico studies, Antioxidants 11 (2022), https://doi.org/10.3390/antiox11071296.
[64] S. Núñez, M.S. Valero, A.M. Mustafa, G. Caprioli, F. Maggi, C. Gómez-Rincón, V. López, Ultrasound-assisted extraction traditional Soxhlet apparatus for the obtention of polyphenols, carotenoids and tocopherols from L. flowers, J Sci Food Agric n/a (2024), https://doi.org/10.1002/jsfa.14105.
[65] A. Carreira-Casais, P. Otero, P. Garcia-Perez, P. Garcia-Oliveira, A.G. Pereira, M. Carpena, A. Soria-Lopez, J. Simal-Gandara, M.A. Prieto, Benefits and drawbacks of ultrasound-assisted extraction for the recovery of bioactive compounds from marine algae, Int. J. Environ. Res. Publ. Health 18 (2021), https://doi.org/10.3390/ijerph18179153.
[66] C.K.R. Pocha, W.Y. Chia, K.W. Chew, H.S.H. Munawaroh, P.L. Show, Current advances in recovery and biorefinery of fucoxanthin from Phaeodactylum tricornutum, Algal Res. 65 (2022), https://doi.org/10.1016/j.algal.2022.102735.
[67] A.-M. Cikoš, K. Aladić, D. Velić, S. Tomas, P. Lončarić, I. Jerković, Evaluation of ultrasound-assisted extraction of fucoxanthin and total pigments from three Croatian macroalgal species, Chem. Pap. (2022), https://doi.org/10.1007/s11696-022-02524-2.
[68] M.D. Macías-Sánchez, C. Mantell, M. Rodríguez, E. Martínez de la Ossa, L. M. Lubián, O. Montero, Comparison of supercritical fluid and ultrasound-assisted extraction of carotenoids and chlorophyll a from Dunaliella salina, Talanta 77 (2009) 948–952, https://doi.org/10.1016/j.talanta.2008.07.032.

[69] F. Chemat, M.A. Vian, G. Cravotto, Green extraction of natural products: concept and principles, Int. J. Mol. Sci. 13 (2012) 8615–8627, https://doi.org/10.3390/ijms13078615.
[70] C. Lourenço-Lopes, A. Carreira-Casais, M. Carperna, M. Barral-Martinez, F. Chamorro, C. Jiménez-López, L. Cassani, J. Simal-Gandara, M.A. Prieto, Emerging technologies to extract fucoxanthin from undaria pinnatifida: microwave vs. Ultrasound assisted extractions, Mar. Drugs 21 (2023), https://doi.org/10.3390/md21050282.
[71] J. Nie, D. Chen, J. Ye, Y. Lu, Z. Dai, Optimization and kinetic modeling of ultrasonic-assisted extraction of fucoxanthin from edible brown algae Sargassum fusiforme using green solvents, Ultrason. Sonochem. 77 (2021), https://doi.org/10.1016/j.ultsonch.2021.105671.
[72] X. Xiao, X. Si, Z. Yuan, X. Xu, G. Li, Isolation of fucoxanthin from edible brown algae by microwave-assisted extraction coupled with high-speed countercurrent chromatography, J. Separ. Sci. 35 (2012) 2313–2317, https://doi.org/10.1002/jssc.201200231.
[73] E. Brglez Mojzer, M. Knez Hrnčič, M. Škerget, Ž. Knez, U. Bren, Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects, Molecules 21 (2016), https://doi.org/10.3390/molecules21070901.
[74] I. Fatima, M. Munir, R. Qureshi, U. Hanif, N. Gulzar, A.A. Sheikh, Advanced methods of algal pigments extraction: a review, Crit. Rev. Food Sci. Nutr. (2023), https://doi.org/10.1080/10408398.2023.2216782.
[75] J.M. Billakanti, O.J. Catchpole, T.A. Fenton, K.A. Mitchell, A.D. MacKenzie, Enzyme-assisted extraction of fucoxanthin and lipids containing polyunsaturated fatty acids from Undaria pinnatifida using dimethyl ether and ethanol, Process Biochem. 48 (2013) 1999–2008, https://doi.org/10.1016/j.procbio.2013.09.015.
[76] MERCK Sigma-Aldrich, Alginate lyase - product number: a1603 - CAS number: 9024-15-1. https://www.sigmaaldrich.com/SI/en/product/sigma/a1603, 2025. (Accessed 20 January 2025).
[77] A. Gan, S. Baroutian, Current status and trends in extraction of bioactives from brown macroalgae using supercritical CO2 and subcritical water, J. Chem. Technol. Biotechnol. (n.d.). https://doi.org/10.1002/jctb.7063.
[78] Ž. Knez, D. Cör, M. Knez Hrnčič, Solubility of solids in sub- and supercritical fluids: a review 2010-2017, J. Chem. Eng. Data 63 (2018) 860–884, https://doi.org/10.1021/acs.jced.7b00778.
[79] I. Michalak, A. Dmytryk, P.P. Wieczorek, E. Rój, B. Łęska, B. Górka, B. Messyasz, J. Lipok, M. Mikulewicz, R. Wilk, G. Schroeder, K. Chojnacka, Supercritical algal extracts: a source of biologically active compounds from nature, J. Chem. 2015 (2015), https://doi.org/10.1155/2015/597140.
[80] T. Arumugham, R. K, S.W. Hasan, P.L. Show, J. Rinklebe, F. Banat, Supercritical carbon dioxide extraction of plant phytochemicals for biological and environmental applications – a review, Chemosphere 271 (2021) 129525, https://doi.org/10.1016/j.chemosphere.2020.129525.
[81] M. Honda, K. Murakami, S. Takasu, M. Goto, Extraction of fucoxanthin isomers from the edible Brown seaweed undaria pinnatifida using supercritical CO2: effects of extraction conditions on isomerization and recovery of fucoxanthin, J. Oleo Sci. 71 (2022) 1097–1106, https://doi.org/10.5650/jos.ess22077.
[82] S.O. Essien, B. Young, S. Baroutian, Recent advances in subcritical water and supercritical carbon dioxide extraction of bioactive compounds from plant materials, Trends Food Sci. Technol. 97 (2020) 156–169, https://doi.org/10.1016/j.tifs.2020.01.014.
[83] T.Q. To, K. Procter, B.A. Simmons, S. Subashchandrabose, R. Atkin, Low cost ionic liquid-water mixtures for effective extraction of carbohydrate and lipid from algae, Faraday Discuss 206 (2018) 93–112, https://doi.org/10.1039/c7fd00158d.
[84] E. Suarez Garcia, C.F. Miranda, M.T. Cesario, R.H. Wijffels, C. van den Berg, M.H. M. Eppink, Ionic liquid-assisted selective extraction and partitioning of biomolecules from macroalgae, ACS Sustain. Chem. Eng. 11 (2023) 1752–1762, https://doi.org/10.1021/acssuschemeng.2c05823.
[85] K.S. Khoo, C.W. Ooi, K.W. Chew, S.C. Foo, P.L. Show, Bioprocessing of Chaetoceros calcitrans for the recovery of fucoxanthin using CO2-based alkyl carbamate ionic liquids, Bioresour. Technol. 322 (2021), https://doi.org/10.1016/j.biortech.2020.124520.
[86] R. Vijayaraghavan, D.R. Macfarlane, CO2-based alkyl carbamate ionic liquids as distillable extraction solvents, in: ACS Sustain Chem Eng, American Chemical Society, 2014, pp. 1724–1728, https://doi.org/10.1021/sc5002066.
[87] V. Fischer, Properties and Applications of Deep Eutectic Solvents and Low-Melting Mixtures, 2015.
[88] R. Jayarani, N.S. Chatterjee, R.G. K Lekshmi, P. Kumar Dara, R. Anandan, S. Mathew, Fucoxanthin Content and Antioxidant Activity in Supercritical CO 2 , Enzymatic and Natural Hydrophobic Deep Eutectic Solvent Extracts of Sargassum Wightii Seaweed, 2021.
[89] D. Prat, A. Wells, J. Hayler, H. Sneddon, C.R. McElroy, S. Abou-Shehada, P. J. Dunn, CHEM21 selection guide of classical- and less classical-solvents, Green Chem. 18 (2015) 288–296, https://doi.org/10.1039/c5gc01008j.
[90] P. Anastas, N. Eghbali, Green chemistry: principles and practice, Chem. Soc. Rev. 39 (2010) 301–312, https://doi.org/10.1039/b918763b.
[91] Y. Chen, T. Mu, Revisiting greenness of ionic liquids and deep eutectic solvents, Green Chem. Eng. 2 (2021) 174–186, https://doi.org/10.1016/j.gce.2021.01.004.
[92] S. Sowmiah, V. Srinivasadesikan, M.-C. Tseng, Y.-H. Chu, On the chemical stabilities of ionic liquids, Molecules 14 (2009) 3780–3813, https://doi.org/10.3390/molecules14093780.
[93] P. Munshi, S. Bhaduri, Supercritical CO2 : a twenty-first century solvent for the chemical industry, Curr. Sci. 97 (2009) 63.
[94] R.S. Kalb, Toward industrialization of ionic liquids, in: M.B. Shiflett (Ed.), Commercial Applications of Ionic Liquids, Springer International Publishing, Cham, 2020, pp. 261–282, https://doi.org/10.1007/978-3-030-35245-5_11.
[95] A.V. Belesov, D.M. Mazur, A.V. Faleva, I.S. Varsegov, I.I. Pikovskoi, N. V. Ulyanovskii, D.S. Kosyakov, 1-Butyl-3-methylimidazolium-Based ionic liquid in biomass fractionation—green solvent or active reagent toward lignin compounds? Int. J. Mol. Sci. 25 (2024) https://doi.org/10.3390/ijms252312623.
[96] G. van Erven, V.J.P. Boerkamp, J.W. van Groenestijn, R.J.A. Gosselink, Choline and lactic acid covalently incorporate into the lignin structure during deep eutectic solvent pulping, Green Chem. 26 (2024) 7101–7112, https://doi.org/10.1039/d4gc00909f.
[97] Q. Luo, C. Li, W. Zhao, W. Ding, Y. Liu, W. Xiao, H. Liu, X. Pang, J. Sun, Lignin demethylated by protic ionic liquid as a novel and sustainable chrome-free tanning agent for eco-leather production, ACS Sustain. Chem. Eng. 12 (2024) 9682–9694, https://doi.org/10.1021/acssuschemeng.4c01036.
[98] J.F. Leal Silva, P.Y.S. Nakasu, A.C. da Costa, R. Maciel Filho, S.C. Rabelo, Techno-economic analysis of the production of 2G ethanol and technical lignin via a protic ionic liquid pretreatment of sugarcane bagasse, Ind. Crops Prod. 189 (2022), https://doi.org/10.1016/j.indcrop.2022.115788.
[99] R. Pezoa-Conte, A. Leyton, I. Anugwom, S. von Schoultz, J. Paranko, P. Mäki-Arvela, S. Willför, M. Muszyński, J. Nowicki, M.E. Lienqueo, J.P. Mikkola, Deconstruction of the green alga Ulva rigida in ionic liquids: closing the mass balance, Algal Res. 12 (2015) 262–273, https://doi.org/10.1016/j.algal.2015.09.011.
[100] A. Piovan, R. Seraglia, B. Bresin, R. Caniato, R. Filippini, Fucoxanthin from undaria pinnatifida: photostability and coextractive effects, Molecules 18 (2013) 6298–6310, https://doi.org/10.3390/molecules18066298.
[101] I. Jaswir, D. Noviendri, H.M. Salleh, K. Miyashita, Fucoxanthin extractions of Brown seaweeds and analysis of their lipid fraction in methanol, Food Sci. Technol. Res. 18 (2012) 251–257, https://doi.org/10.3136/fstr.18.251.
[102] R.K. Sangeetha, N. Bhaskar, V. Baskaran, Comparative effects of β-carotene and fucoxanthin on retinol deficiency induced oxidative stress in rats, Mol. Cell. Biochem. 331 (2009) 59–67, https://doi.org/10.1007/s11010-009-0145-y.
[103] S.J. Heo, W.J. Yoon, K.N. Kim, G.N. Ahn, S.M. Kang, D.H. Kang, A. affan, C. Oh, W.K. Jung, Y.J. Jeon, Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide-stimulated RAW 264.7 macrophages, Food Chem. Toxicol. 48 (2010) 2045–2051, https://doi.org/10.1016/j.fct.2010.05.003.
[104] N. Heffernan, T.J. Smyth, R.J. FitzGerald, A. Vila-Soler, J. Mendiola, E. Ibáñez, N. P. Brunton, Comparison of extraction methods for selected carotenoids from macroalgae and the assessment of their seasonal/spatial variation, Innov. Food Sci. Emerg. Technol. 37 (2016) 221–228, https://doi.org/10.1016/j.ifset.2016.06.004.
[105] K. Mori, T. Ooi, M. Hiraoka, N. Oka, H. Hamada, M. Tamura, T. Kusumi, Fucoxanthin and its metabolites in edible Brown algae cultivated in deep seawater, Mar. Drugs 2 (2004) 63–72. www.mdpi.net/marinedrugs/.
[106] B.C. Liau, C.T. Shen, F.P. Liang, S.E. Hong, S.L. Hsu, T.T. Jong, C.M.J. Chang, Supercritical fluids extraction and anti-solvent purification of carotenoids from microalgae and associated bioactivity, J. Supercrit. Fluids 55 (2010) 169–175, https://doi.org/10.1016/j.supflu.2010.07.002.
[107] N. Oliyaei, M. Moosavi-Nasab, Ultrasound-assisted extraction of fucoxanthin from Sargassum angustifolium and Cystoseira indica brown algae, J. Food Process. Preserv. 45 (2021), https://doi.org/10.1111/jfpp.15929.
[108] S.C. Foo, F.M. Yusoff, M. Ismail, M. Basri, S.K. Yau, N.M.H. Khong, K.W. Chan, M. Ebrahimi, Antioxidant capacities of fucoxanthin-producing algae as influenced by their carotenoid and phenolic contents, J. Biotechnol. 241 (2017) 175–183, https://doi.org/10.1016/j.jbiotec.2016.11.026.
[109] A. Delbrut, P. Albina, T. Lapierre, R. Pradelles, E. Dubreucq, Fucoxanthin and polyunsaturated fatty acids Co-extraction by a green process, Molecules 23 (2018), https://doi.org/10.3390/molecules23040874.
[110] H. Kanda, Y. Kamo, S. Machmudah, Wahyudiono, M. Goto, Extraction of fucoxanthin from raw macroalgae excluding drying and cell wall disruption by liquefied dimethyl ether, Mar. Drugs 12 (2014) 2383–2396, https://doi.org/10.3390/md12052383.
[111] J. Zhu, X. Sun, X. Chen, S. Wang, D. Wang, Chemical cleavage of fucoxanthin from Undaria pinnatifida and formation of apo-fucoxanthinones and apo-fucoxanthinals identified using LC-DAD-APCI-MS/MS, Food Chem. 211 (2016) 365–373, https://doi.org/10.1016/j.foodchem.2016.05.064.
[112] T. Lefebvre, E. Destandau, E. Lesellier, Selective extraction of bioactive compounds from plants using recent extraction techniques: a review, J. Chromatogr. A 1635 (2021), https://doi.org/10.1016/j.chroma.2020.461770.
[113] M.-K. Roh, MdS. Uddin, B.-S. Chun, Extraction of fucoxanthin and polyphenol from Undaria pinnatifida using supercritical carbon dioxide with co-solvent, Biotechnol. Bioproc. Eng. 13 (2008) 724–729, https://doi.org/10.1007/s12257-008-0104-6.
[114] M. Terasaki, B. Narayan, H. Kamogawa, M. Nomura, N.M. Stephen, C. Kawagoe, M. Hosokawa, K. Miyashita, Carotenoid profile of edible Japanese seaweeds: an improved HPLC method for separation of major carotenoids, J. Chem. Technol. Biotechnol. 21 (2012) 468–479, https://doi.org/10.1080/10498850.2011.610025.
[115] A.T. Quitain, T. Kai, M. Sasaki, M. Goto, Supercritical carbon dioxide extraction of fucoxanthin from undaria pinnatifida, J. Agric. Food Chem. 61 (2013) 5792–5797, https://doi.org/10.1021/jf400740p.
[116] S.J. Eom, Y.E. Kim, J.E. Kim, J. Park, Y.H. Kim, K.M. Song, N.H. Lee, Production of Undaria pinnatifida sporophyll extract using pilot-scale ultrasound-assisted

extraction: extract characteristics and antioxidant and anti-inflammatory activities, Algal Res. 51 (2020), https://doi.org/10.1016/j.algal.2020.102039.
[117] K. Amorim, M.A. Lage-Yusty, J. Lopez-Hernandez, Changes in bioactive compounds content and antioxidant activity of seaweed after cooking processing, CyTA - J. Food 10 (2012) 321–324, https://doi.org/10.1080/19476337.2012.658871.
[118] R. Sugimura, M. Suda, A. Sho, T. Takahashi, T. Sashima, M. Abe, M. Hosokawa, K. Miyashita, Stability of fucoxanthin in dried undaria pinnatifida (wakame) and baked products (scones) containing wakame powder, Food Sci. Technol. Res. 18 (2012) 687–693, https://doi.org/10.3136/fstr.18.687.
[119] C. Osorio, S. Machado, J. Peixoto, S. Bessada, F.B. Pimentel, R.C. Alves, M. Oliveira, Pigments content (chlorophylls, fucoxanthin and phycobiliproteins) of different commercial dried algae, Separations 7 (2020), https://doi.org/10.3390/separations7020033.
[120] P.S. Saravana, A.T. Getachew, Y.J. Cho, J.H. Choi, Y.B. Park, H.C. Woo, B.S. Chun, Influence of co-solvents on fucoxanthin and phlorotannin recovery from brown seaweed using supercritical CO2, J. Supercrit. Fluids 120 (2017) 295–303, https://doi.org/10.1016/j.supflu.2016.05.037.
[121] A.T. Getachew, P.S. Saravana, Y.J. Cho, H.C. Woo, B.S. Chun, Concurrent extraction of oil from roasted coffee (Coffea arabica) and fucoxanthin from brown seaweed (Saccharina japonica) using supercritical carbon dioxide, J. CO2 Util. 25 (2018) 137–146, https://doi.org/10.1016/j.jcou.2018.03.018.
[122] E. Conde, A. Moure, H. Domínguez, Supercritical CO2 extraction of fatty acids, phenolics and fucoxanthin from freeze-dried Sargassum muticum, J. Appl. Phycol. 27 (2015) 957–964, https://doi.org/10.1007/s10811-014-0389-0.
[123] V. Raguraman, S.A. L, D. MubarakAli, G. Narendrakumar, R. Thirugnanasambandam, R. Kirubagaran, N. Thajuddin, Unraveling rapid extraction of fucoxanthin from Padina tetrastromatica: purification, characterization and biomedical application, Process Biochem. 73 (2018) 211–219, https://doi.org/10.1016/j.procbio.2018.08.006.
[124] Y.F. Shang, S.M. Kim, W.J. Lee, B.H. Um, Pressurized liquid method for fucoxanthin extraction from Eisenia bicyclis (Kjellman) Setchell, J. Biosci. Bioeng. 111 (2011) 237–241, https://doi.org/10.1016/j.jbiosc.2010.10.008.
[125] C.R. Chen, D.M. Lin, C.M.J. Chang, H.N. Chou, J.J. Wu, Supercritical carbon dioxide anti-solvent crystallization of fucoxanthin chromatographically purified from Hincksia mitchellae P.C. Silva, J. Supercrit. Fluids 119 (2017) 1–8, https://doi.org/10.1016/j.supflu.2016.08.013.
[126] H. Nagappan, P.P. Pee, S.H.Y. Kee, J.T. Ow, S.W. Yan, L.Y. Chew, K.W. Kong, Malaysian brown seaweeds Sargassum siliquosum and Sargassum polycystum: low density lipoprotein (LDL) oxidation, angiotensin converting enzyme (ACE), α-amylase, and α-glucosidase inhibition activities, Food Res. Int. 99 (2017) 950–958, https://doi.org/10.1016/j.foodres.2017.01.023.
[127] S.P. Sivagnanam, S. Yin, J.H. Choi, Y.B. Park, H.C. Woo, B.S. Chun, Biological properties of fucoxanthin in oil recovered from two brown seaweeds using supercritical CO2 extraction, Mar. Drugs 13 (2015) 3422–3442, https://doi.org/10.3390/md13063422.
[128] V. Pasquet, J.R. Chérouvrier, F. Farhat, V. Thiéry, J.M. Piot, J.B. Bérard, R. Kaas, B. Serive, T. Patrice, J.P. Cadoret, L. Picot, Study on the microalgal pigments extraction process: performance of microwave assisted extraction, Process Biochem. 46 (2011) 59–67, https://doi.org/10.1016/j.procbio.2010.07.009.
[129] Y. Zhu, X. Li, Y. Wang, L. Ren, Q. Zhao, Lutein extraction by imidazolium-based ionic liquid-water mixture from dried and fresh Chlorella sp, Algal Res. 60 (2021), https://doi.org/10.1016/j.algal.2021.102528.
[130] L. D'Elia, P. Imbimbo, D. Liberti, F. Bolinesi, A. Pollio, O. Mangoni, W. Brilman, G. Olivieri, D.M. Monti, Switchable solvent selective extraction of hydrophobic antioxidants from synechococcus bigranulatus, ACS Sustain. Chem. Eng. 9 (2021) 13798–13806, https://doi.org/10.1021/acssuschemeng.1c04400.