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Application of Novel Extraction Technologies for Bioactives from Marine Algae

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Supporting Information

ABSTRACT: Marine algae are a rich source of bioactive compounds. This paper outlines the main bioactive compounds in marine algae and recent advances in novel technologies for extracting them. Novel extraction technologies reviewed include enzyme-assisted extraction, microwave-assisted extraction, ultrasound-assisted extraction, supercritical fluid extraction, and pressurized liquid extraction. These technologies are reviewed with respect to principles, benefits, and potential applications for marine algal bioactives. Advantages of novel technologies include higher yield, reduced treatment time, and lower cost compared to traditional solvent extraction techniques. Moreover, different combinations of novel techniques used for extraction and technologies suitable for thermolabile compounds are identified. The limitations of and challenges to employing these novel extraction technologies in industry are also highlighted.

KEYWORDS: *marine algae, extraction, bioactive, nutraceuticals, ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), pressurized liquid extraction (PLE)*

INTRODUCTION

The sea is habitat to half of the global biodiversity and is the largest remaining reservoir of bioactive compounds.[1] Marine algae are generally described as unicellular or multicellular, macroscopic, and benthic marine plants. Marine algae can be categorized into different pigmentation groups, namely, Rhodophyceae (red algae), Chlorophyceae (green algae), and Phaeophyceae (brown algae). The color of marine algae is attributed to pigments such as phycobilins for red, chlorophyll for green, and fucoxanthin for brown algae.[2]

The main pigments in brown algae are fucoxanthin, chlorophyll *a*, and chlorophyll *c*, which impart a greenish-brown color to the algae. Brown algae can grow up to lengths of 45 m or more. Globally there are 1500−2000 species of brown algae.[3] Some of the common genera of brown marine algae include *Ascophyllum*, *Sargassum*, and *Laminaria*. Red algae are a diverse group, consisting of over 700 genera and 6000 species. They are characterized by a red to violet thallus due to the presence of phycoerythrin. Red algae are widely utilized for the production of hydrocolloids. Green algae range from unicellular to multicellular groups of organisms with approximately 7000 species known worldwide.[4]

South Asian countries pioneered the utilization of marine algae for medicinal and food purposes. Traditionally the Western world has used marine algae for the production of phycocolloids.[5] It is estimated that around 18 million tonnes of marine algae and other aquatic plants are harvested annually with an estimated value of U.S. $5 billion.[6] Although global marine algae utilization is a multibillion dollar industry, it is mainly limited to farming of edible species or production of agar, carrageenan, and alginates.[7] The bioactive potential of marine algae is underexploited; however, many initiatives are currently being taken particularly in Europe to exploit marine algae for food ingredient applications. These initiatives are outlined in Table 1.

There is increasing awareness of foods as a source of functional health ingredients. Marine algae by virtue of their abundant availability in the marine ecosystem have potential to become excellent sources of bioactive compounds such as dietary fiber, omega-3 fatty acids, carotenoids, vitamins, and minerals. The bioactive potential of different marine algae has been extensively reviewed in the literature.[2,8−14]

Bioactive compounds are sensitive to extraction techniques based on heat or solvent use. In addition, these techniques are time-consuming and energy intensive.[15] It is necessary to identify and develop new efficient extraction processes to utilize the bioactives present in marine algae. To this end, marine algae researchers have been working toward the development of novel techniques that are more efficient in terms of yield, time, and cost and, in addition, are environmentally friendly. Extraction technologies such as enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), and pressurized liquid extraction (PLE) have been successfully used in food and pharmaceutical applications for extraction of bioactive compounds. This review describes novel extraction techniques that can be employed for the extraction of a range of bioactive compounds.

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Table 1. EU-Funded Research Initiatives on Algae

project name	objective	Web site
SWAFAX	to address a commercial opportunity to obtain bioactive compounds from seaweeds for application in food, health, and wellness products	http://www.seaweedforhealth.org/swafax/
HYFFI	to realize a commercial opportunity to produce low molecular weight polysaccharides from alginate and agar-bearing seaweeds for applications in food and health and wellness products	http://www.seaweedforhealth.org/node/1
MAREX	to explore marine resources for bioactive compounds	http://www.marex.fi/
NETALGAE	to create a European network of relevant stakeholders within the marine macroalgae sector	http://www.netalgae.eu/
NutraMara	to develop model foods enhanced with marine origin bioactives and capabilities to process marine-based materials for use by the functional ingredients sector	http://www.nutramara.ie/

BIOACTIVE COMPOUNDS IN MARINE ALGAE

Historically, compounds from marine algae have been used as gelling, thickening, and emulsifying agents in a range of food products. Traditionally, in the Western world, marine algae were not identified as a source of health-promoting compounds apart from being a good source of iodine.[16] Recent functional food ingredient research has shown that marine algae are a rich source of nutraceuticals with a variety of biological activities.[7] Although seaweeds are exposed to harsh environmental conditions such as light and high oxygen concentrations that lead to the formation of free radicals, they do not exhibit any significant photodynamic damage. Research studies have shown that marine algae generate bioactive compounds to protect against UV radiation, stress, and herbivores.[11] Variation in growing conditions such as water temperature, salt content, nutrients, and light can alter the content of these bioactive compounds in seaweed.

Polysaccharides. Polysaccharides are polymers of simple sugars that are monosaccharides. Algae have been cultivated in many regions of the world to produce phycocolloids such as alginates and carrageenan. Some of these polysaccharides impart functional value to food. Sulfated polysaccharides such as fucoidan, phorphyran, and furcellaran have been shown to demonstrate biological activities along with nonsulfated polysaccharide laminarin. Generally, these polysaccharides can be extracted with acid or water as solvent, and further precipitation is carried out using calcium chloride to separate alginates. Various methods of extraction of fucose-containing sulfated polysaccharides are outlined in the literature.[17,18]

Phenolic Compounds. Phenolic compounds are a group of compounds that contain hydroxyl (−OH) substituents on an aromatic hydrocarbon moiety. The presence of phenolic compounds in algae was first reported by Crato.[19] Polyphenols in algae are phenolic acids, tannins, flavonoids, catechins, and phlorotannins. Phlorotannins are polymers of phloroglucinol units (1,3,5-trihydroxybenzene). They are generally localized in highly refractive and colorless vesicles known as physodes in marine algae.[20] Molecular weights of phlorotannins vary from 126 Da to 650 kDa. The content of phlorotannins can vary from 1 to 14% in different marine alga species. Generally, brown algae contain higher amounts of phlorotannin as compared to red and green algae.[21] These phlorotannins have important biological activities such as antioxidant, antiproliferative, antibiotic, antidiabetic, anti-HIV, antiallergic, and anti-inflammatory properties.[11] Moreover, the total phenolic content and antioxidant activity of marine alga extracts is highly dependent upon the method of extraction. In general, 70% acetone is more efficient for polyphenol extraction than water.[22] Traditionally, phlorotannins have been extracted using ethanol or methanol as solvent. Further purification is carried out by high-pressure liquid chromatography (HPLC) or silica gel chromatography, and characterization is carried out using nuclear magnetic resonance (NMR) techniques.

Omega-3 Fatty Acids. Omega 3-fatty acids, especially eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), are of increasing importance due to their reported role in diminishing cardiovascular risks.[23] EPA and DHA are two important n-3 fatty acids that are mainly found in unicellular phytoplankton and marine algae. Both EPA and DHA accumulate in fish and other marine animals that consume algae and get passed on to other species through the food chain.[24] Omega-3 fatty acids have been found to have positive effects on the central nervous system for the development of brain, retinal, and neural tissues in fetuses and young children.[25] Traditionally these fatty acids have been extracted using chloroform solvent extraction, and subsequent fatty acid profiling is carried out by gas chromatography (GC) or thin layer chromatography.[26] Recently, extraction technologies such as supercritical fluid extraction and ultrasound have been employed to extract these fatty acids.[27,28]

Bioactive Peptides and Proteins. The protein content of seaweeds differs according to species. Generally, the protein fraction of brown seaweeds is lower (3−15% on a dry weight basis) compared to that of green or red seaweeds (10−47% on a dry weight basis).[29] Essential amino acids such as histidine, leucine, isoleucine, and valine are present in many seaweeds. The levels of isoleucine and threonine in *Palmaria palmata* are 3.5−3.7 and 3.6−4.1 g amino acid/100 g protein, which are similar to the levels found in legumes. Also, histidine is found in *Ulva pertusa* at levels of 4.0 g amino acid/100 g protein, which is similar to the level in egg white proteins.[30] Moreover, certain amino acids including taurine and mycosporine-like amino acids, which have potential biological activity as antioxidants, have been extensively studied.[31,32] To date, extraction and fractionation of macroalga proteins, peptides, and amino acids have mainly been performed on a laboratory scale.[33] In general, the main methods used for the extraction of macroalga protein fractions include aqueous and alkaline extraction in addition to polysaccharidase-aided extraction. Currently, techniques such as aqueous and polar partitioning and various chromatographic approaches (i.e., reverse phase high-performance liquid chromatography, ion-exchange, and affinity) are being utilized for the fractionation and purification of bioactive proteins, peptides, and amino acids. Furthermore, spectrophotometric methodology such as liquid chromatography−mass spectrophotometry (LC-MS), ultrafiltration, and gel permeation chromatography have been recently employed for the separation of protein-derived peptides into fractions having different molecular masses and structures.[34]

Carotenoids. Carotenoids are isoprenoid molecules that are photosynthesized by plants, fungi, and algae. Green algae species contain β-carotene, lutein, violaxanthin, neoxanthin, and zeaxanthin, whereas red algae species contain mainly α- and β-

carotene, lutein, and zeaxanthin. β-Carotene, violaxanthin, and fucoxanthin are present in brown algae species.[35] Fucoxanthin is a characteristic orange xanthophyll present in edible brown seaweeds, such as *Undaria pinnatifida*, *Hijikia fusiformis*, *Laminaria japonica*, and *Sargassum fulvellum*.[36] It accounts for approximately 10% of the estimated total natural production of carotenoids.[37] Fucoxanthin has been found to have antioxidant, anticancer, antiobesity, antidiabetic, and antiphotoaging activity.[36] Various other major algal carotenoids such as astaxanthin, zeaxanthin, and β-carotenes are widely found in microalgae species.[38] Traditionally, for commercial purposes, algal carotenoids have been extracted by means of solvent extraction using hexane as a nonpolar solvent. However, recently, novel technologies have been developed for efficient extraction such as direct extraction using vegetable oils, supercritical fluid extraction, and pressurized liquid extraction.[39−41]

EXTRACTION TECHNOLOGIES FOR MARINE ALGAE BIOACTIVES

Pretreatment. Generally, marine algae are harvested from coastal areas or beaches. Marine algae are washed to remove any salt residues, impurities, or epiphytes. For extraction purposes, these algae are dried and milled to ensure uniform distributed mass as well as a higher surface-to-volume ratio.[18] Moreover, these pretreatments are vital in preventing the coextraction of other bioactive compounds from marine algae that have similar solubility properties. For example, in the case of fucoidan, pretreatment of algae with methanol/choloform/water at 4:2:1 (v/v/v) is found to be advantageous to prevent the coextraction of other algal compounds during the aqueous isolation of fucoidan.[42]

Traditional Methods of Extraction. Different mechanical and chemical processes such as solvent extraction and steam distillation are used for the extraction of compounds from plants.[43] Existing techniques used for extraction of bioactive compounds include Soxhlet, hydrodistillation, and maceration with alcohol.[44] The selection of the appropriate method varies according to the nature of the target compound to get maximum yield and highest purity. The mass transfer resistances due to involvement of more than one phase in the system often limit the use of conventional Soxhlet extraction techniques.[45] These methods may require a long time depending on the diffusion rates of solvents. Moreover, conventional extraction techniques are energy intensive.[15] Furthermore, conventional techniques are usually manual processes, and reproducibility is a challenge.[46] For sensitive bioactive components, for example, fucoxanthin, bioactivity is deteriorated by the heating process, leading to low extraction yields. These active molecules may be altered by the pH, temperature, and pressure conditions employed. In addition, the organic solvents required are harmful to the environment. For example *n*-hexane is ranked the highest of 189 hazardous air pollutants (HAPs) by the U.S. Environmental Protection Agency.[47] Thus, due to these limitations combined with the significant increase in the demand for marine algae bioactives, there is a need to develop appropriate, selective, cost-effective, and ecofriendly extraction technologies that are rapid, produce higher yields, and comply with relevant legislation.[48]

Novel Methods of Extraction. There are a number of novel extraction technologies, which have the potential to deliver many of the above listed requirements. They include EAE, MAE, UAE, SFE, and PLE techniques. These technologies will be discussed with reference to the principles and mechanisms of action, advantages and disadvantages over traditional methods, and potential suitability for extraction of marine algal bioactives.

Enzyme-Assisted Extraction. Marine algae cell walls and cuticles are made up of chemically complex and heterogeneous biomolecules, that is, sulfated and branched polysaccharides, which are associated with proteins and various bound ions including calcium and potassium.[49,50] It is necessary to break down these complex molecules to extract marine algal bioactives. Applications of cell wall degrading enzymes such as carbohydrases and proteases to marine algae at optimal temperature and pH conditions have been found to break down the cell wall and release the desired bioactive. Some of the commonly used enzymes are Viscozyme, Cellucast, Termamyl, Ultraflo, carragenanase, agarase, xylanase, Kojizyme, Neutrase, Alcalase, and Umamizyme.[51−53]

EAE is ecofriendly and nontoxic as it alleviates the use of solvents in the process. It is a high bioactive yielding technology by which unnecessary components from cell walls are removed and desired bioactives are released. It also removes the barriers of water solubility and insolubility for bioactive compounds. It is a relatively low-cost technology that uses common food grade enzymes such as cellulase, α-amylase, and pepsin.[54] This technology offers the benefits of high catalytic efficiency and preserves original efficacy of the compounds to a high degree.[55] Food grade enzymes can be used to separate different algal biomass for industrial purposes. Specific catalytic property and mode of action of enzymes on substrates should be considered for selection of enzymes.[15] Protocols for EAE emphasize the importance of maintaining optimum treatment time and temperature conditions for enzymes to maximize extraction yield.[54]

Many studies have been carried out to determine the antioxidant activity of seaweed extracts. Enzyme-assisted extracts have higher antioxidant activity compared to extracts obtained using conventional extraction methods.[52] Different EAE approaches employed for marine algae are summarized in Tables 2 and 3. Billakanti et al.[56] reported on improving the yields of total lipids and fucoxanthin from *Undaria pinnatifida* using an EAE process followed by dimethyl ether extraction. Alginase lyase enzymes were employed to degrade cell wall polysaccharides to oligosaccharides prior to the extraction of fucoxanthin from the residual biomass using near-critical dimethyl ether with and without ethanol as a cosolvent. The temperature and pH were optimized at 37 °C and 6.2, respectively. A significant improvement in the extraction yield with EAE was observed. Lipid yields were 15−20% higher, whereas the yield of fucoxanthin increased by 50%. EAE is well suited to the extraction of phlorotannins and other phenolic compounds from seaweeds, as it assists in breaking the complex

Table 2. Applications of EAE for Bioactive Compounds from Marine Algae

marine algae	bioactive compound	enzymes used for extraction	ref
Undaria pinnatifida	fucoxanthin	alginase lyase enzymes, temperature of 37 °C, and pH of 6.2	56
Sargassum horneri	antioxidant rich extracts	carbohydrases and proteases	52
brown seaweed species	antioxidant rich extracts	carbohydrases and proteases	53

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Table 3. Enzymes, pH, and Temperature Employed for EAE of Bioactive Compounds from Marine Algae

enzyme	temperature (°C)	pH	enzyme composition
Viscozyme	50	4.5	arabanase, cellulase, β-glucanase, hemicellulase, and xylanase
Cellucast	50	4.5	group of enzymes catalyzing the breakdown of cellulose into glucose, cellobiose, and higher glucose polymer
Termamyl	60	6.0	heat-stable α-amylase
Ultraflo	60	7.0	heat-stable multiactive β-glucanase
Neutrase	50	6.0	endoprotease
Flavourzyme	50	7.0	endoprotease and exopeptidase activities
Alcalase	50	8.0	α-endoprotease

bonding between phenolics and proteins in seaweed. EAE technology is a feasible alternative to traditional methods of extraction for algal bioactives. The technology can also be applied for large-scale operations.

Microwave-Assisted Extraction. Microwaves are nonionizing electromagnetic radiation with a frequency from 300 MHz to 300 GHz. The use of microwaves for extraction of various compounds was first reported in 1986.[57] MAE transfers energy to the solution, which is heated by twin mechanisms of dipole rotation and ionic conduction. The radiation frequency corresponds to the rotational motion of molecules; in condensed matter, energy absorption immediately causes energy redistribution between molecules and homogeneous heating of the medium.[58] MAE causes disruptions of hydrogen bonds and migration of dissolved ions, resulting in increased penetration of solvent into the matrix, which facilitates the extraction of target compounds. Due to significant pressure developed inside the matrix, there is an increase in the porosity of the biological matrix resulting in higher penetration of solvent into the matrix.[59] There are two main types of MAE systems, namely, closed vessel and open vessel. Closed vessels are used for extraction of target compounds at higher temperature and pressure conditions, whereas open vessel systems are used for extractions carried out at atmospheric pressure conditions.[60]

MAE is increasingly viewed as a viable option for the extraction of bioactives from plants and herbs due to distinct advantages over traditional solvent extraction techniques.[61] Advantages include improved extraction rate, lower use of solvents, and improved extraction yield. MAE is also a more economically feasible option compared to SFE. However, MAE does require an additional separation process to remove solid residues compared to SFE.[44] In addition, MAE is not suitable for use with heat-sensitive bioactives.

Recently, MAE has been employed to extract fucoidans, carotenoids, and minerals from marine macroalgae and microalgae. Different MAE approaches applied for marine algae are summarized in Table 4. Pasquet et al.[62] studied the performance of MAE against cold/hot soaking and UAE for carotenoid extraction from *Dunaliella tertiolecta* and *Cylindrotheca closterium*. They also studied the potential of vacuum microwave-assisted extraction (VMAE) for *Dunaliella tertiolecta*. VMAE was performed at 22 °C, which enabled a rapid and efficient extraction but at lower extraction yields compared to MAE, which the authors reported as the preferred extraction process for pigments from marine microalgae, as it combined rapidity, efficiency, and protection against thermal denaturation.

MAE has also been applied for extraction of sulfated polysaccharides (fucoidan) from brown seaweed *Fucus vesiculosus*.[63] Different conditions of pressure (200−800 kPa), extraction time (1−31 min), and alga/water ratio (1:25 to 5:25 g/mL) were evaluated during this process. The algal degradation (%), total sugar yield (%), and SO_3 content (%) were also determined for each experimental condition. During this study, extraction yield was significantly affected by all variables investigated. MAE at 800 kPa, for 1 min of treatment time, using 1 g of alga per 25 mL of water was the optimum condition for fucoidan recovery.

In a separate study researchers combined MAE and UAE technologies for extraction of oils rich in DHA from microalgae.[48] They concluded that both technologies used either alone or combined could improve extraction rate and yield and reduce costs compared to conventional extraction processes. Also, these technologies can be successfully applied to two-step extraction and transesterification for the production of biofuels.

Ultrasound-Assisted Extraction. Ultrasound waves are high-frequency sound waves above human hearing capacity, that is, above 20 kHz. Unlike electromagnetic waves, they are mechanical waves, which pass through solid, gas, and liquid media. These waves propagate by rarefactions and compression. These expansions cause negative pressure in the liquid. If the pressure exceeds the tensile strength of the liquid, then formation of vapor bubbles occurs. These vapor bubbles undergo implosive collapse in strong ultrasound fields, which is known as cavitation.[64] The implosion of cavitation bubbles generates macroturbulence, high-velocity interparticle collisions, and perturbation in microporous particles of the biomass.[43] Cavitation near liquid−solid interfaces directs a fast-moving stream of liquid through the cavity at the surface. Impingement by these microjets results in surface peeling, erosion, and particle breakdown, facilitating release of bioactives from the biological matrix. This effect increases the efficiency of extraction by increasing mass transfer by eddy and internal diffusion mechanisms.[65] There are two main types of ultrasound equipment that can be employed for extraction purposes, namely, an ultrasonic water bath and an ultrasonic

Table 4. Applications of MAE for Bioactive Compounds from Marine Algae

marine algae	bioactive compound	conditions	ref
Dunaliella tertiolecta	carotenoids	temperature of 56 °C and atmospheric pressure conditions	62
Fucus vesiculosus	fucoidan	pressure of 200−800 kPa, extraction time 1−31 min,, and alga/water ratio of 1/25 to 5/25g mL^{-1}	63
Porphyra (nori), *Palmaria* (dulse), *Undaria pinnatifida* (wakame), *Himanthalia elongata* (sea spaghetti), *Laminaria ochroleuca* (kombu), *Ulva rigida* (sea lettuce)	iodine	temperature of 200 °C, power of 1000 W, holding time of 0−5 min	92

probe system fitted with horn transducers.[48] Figure 1 shows an UAE system employing an ultrasonic probe.

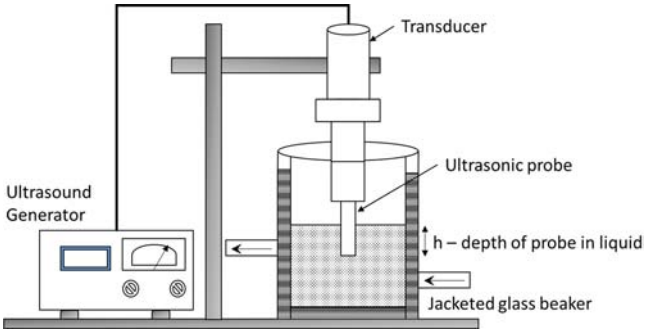

Figure 1. Schematic diagram of ultrasound-assisted extraction (UAE).

UAE is a simple, cost-effective, and efficient alternative to traditional extraction techniques. Benefits of using ultrasound in solid–liquid extraction include an increase of extraction yield and faster kinetics. Ultrasound facilitates the extraction of heat-sensitive compounds with minimal damage. Equipment costs are lower than other novel extraction techniques. It may also be used with a wide variety of solvents including the aqueous extraction of bioactives, that is, for water-soluble components.[66] However, wave attenuation in dispersed phase systems is a particular challenge in UAE technologies due to the decrease in sound wave amplitude with distance.

UAE extraction technology for bioactives has been employed from laboratory-scale level to large-scale industry operations, that is, full-scale commercialized extraction applications.[66] Combined effects with other novel technologies such as MAE and SFE have also been studied.[67] Reported applications of UAE applied to marine algae are summarized in Table 5. Klejdus et al.[68] developed a hyphenated technique using UAE-SFE followed by fast chromatography with tandem mass chromatography for extraction of isoflavones from algae. In their study UAE was employed as a pretreatment using an ultrasonic bath or ultrasound probe instrument. For longer treatment times the probe instrument was shown to be more efficient. In another study, UAE was combined with MAE to extract vegetable oil rich in DHA from marine microalgae. The disruption of the tough algal cell wall with ultrasound improved the extraction yield to 25.9% compared to 4.8% using Soxhlet.[69] Following ultrasound-assisted acid leaching, trace elements in Atlantic marine algae were investigated using inductive coupled plasma optical emission spectroscopy (ICP-OES). It was found that ultrasound at 17 kHz, temperature of 65 °C, and pretreatment time of 10 min were the optimum extraction conditions.[70]

Supercritical Fluid Extraction. A supercritical fluid is a fluid whose temperature and pressure are above its critical limit. In this state, the density of the fluid is similar to that of a liquid, its viscosity is similar to that of a gas, and its diffusivity is intermediate between those of a liquid and a gas. Thus, due to low viscosity and high diffusivity, supercritical fluids possess better transport properties than liquid. An important characteristic of SFE is that fluid density can be altered by changing the temperature and pressure of the fluid. This means that the dissolving power of a fluid can be altered by temperature and pressure changes, as it is dependent upon the density of fluid.[71,72] A typical schematic diagram of an SFE system is presented in Figure 2.

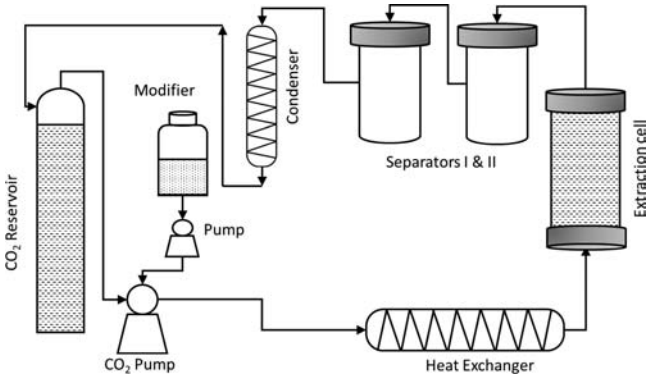

Figure 2. Schematic diagram of supercritical fluid extraction (SFE).

Hannay and Hogarth[73] first reported the use of supercritical fluid technology for extraction purposes in 1879. Due to recent advances in SFE technology, increased applications in research and industry have been reported.[71,74] SFE is a suitable technology for extraction of nutraceuticals. Bioactive compounds can also be extracted without any loss of volatility. SFE offers a fast extraction rate and high yield and is an ecofriendly technology with minimal or no use of organic solvents. Drawbacks of SFE including high investment cost and low polarity of supercritical CO_2 limit widespread use of SFE. SFE has been widely employed in the food, pharmaceutical, pesticide, and fuel industries. Reported applications of SFE to marine algae are summarized in Table 6. Extraction of flavors and bioactive compounds is the main reported food application.[75] Subra and Boissinot[76] postulated that SFE can obtain algal extracts with different compositions and yields by changing the applied pressure.

Astaxanthin is an important bioactive carotenoid. However, sources of astaxanthin are limited, and few extraction studies have been reported to date. Fujii[77] developed a novel method with higher yields for astaxanthin extraction from vegetative microalgae *Haematococcus pluvialis* cysts. This method involved a combination of SFE and acid extraction. The novelty of the method was that acids such as H_2SO_4 and HCl were used for separation of chlorophyll from astaxanthin rather than ethanol. Macías-Sánchez et al.[78] found that a pressure of 40 MPa and a temperature of 60 °C were the optimum conditions to extract lutein and β-carotene from *Scenedesmus almeriensis* using SFE.

Table 5. Applications of UAE for Bioactive Compounds from Marine Algae

marine algae	bioactive compound	conditions	ref
Sargassum muticum, *Sargassum vulgare*, *Hypnea spinella*, *Porphyra* sp., *Undaria pinnatifida*, *Chondrus crispus*, and *Halopytis incurvus*	isoflavones	sonication treatment time 30 min	68
Porphyra, *Palmaria* (red marine algae), *Undaria pinnatifida*, *Himanthalia elongata*, and *Laminaria ochroleuca* (brown marine algae)	minerals	frequency of 17 kHz, temperature of 65 °C	93

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Table 6. Applications of SFE for Bioactive Compounds from Marine Algae

marine algae	bioactive compound	conditions	ref
Haematococcus pluvialis	astaxanthin	ethanol and acids were used as solvents for extraction	77
Scenedesmus almeriensis	carotenoids	pressure of 40 MPa and temperature of 60 °C	78
Dunaliella salina	chlorophyll	methanol as solvent	79
Hypneacharoides sp.	polyunsaturated fatty acids	temperature ranges from 40 to 50 °C and pressure from 24.1 to 37.9 MPa	27
Botryococcus braunii, Chlorella vulgaris, Dunaliella salina	β-carotene	pressure of 30 MPa and temperature of 40 °C	81
Sargassum muticum	polyphenols	extractions were performed using CO_2 modified with 12% ethanol at 15.2 MPa pressure and 60 °C during 90 min	91

Moreover, Macías-Sánchez et al.[79] and Klejdus et al.[68] reported that SFE was more selective for recovery of bioactives than UAE as the solubility of a fluid can be changed as required by altering temperature and pressure conditions. Cheung[27] studied the effect of extraction conditions for obtaining fatty acids from *Hypnea charoides* algae using supercritical CO_2. Temperature values from 40 to 50 °C and pressure values from 24.1 and 37.9 MPa were investigated. Lipid recovery, as well as the ratio of unsaturated fatty acids, were found to increase with extraction pressure and temperature. Algal extracts from SFE demonstrate antimicrobial activity due to the presence of an indolic derivative.[80] This indolic derivative was detected in the SFE extract, together with polyunsaturated fatty acids and compounds related to carotene metabolism, including β-ionone and neophytadiene. The highest antimicrobial activity was obtained using conditions of 31.4 MPa and 9.8 °C. Mendes et al.[81] used SFE to extract β-carotene at 30 MPa pressure and 40 °C temperature from *Dunaliella salina*. They reported that increasing pressure resulted in an increase in extraction yield.

Pressurized Liquid Extraction. The use of PLE for bioactives was first reported in 1996.[82] PLE is also referred to as accelerated solvent extraction (ASE), high-pressure solvent extraction (HPSE), pressurized fluid extraction (PFE), and enhanced solvent extraction (ESE).[83] The temperature and pressure conditions employed in PLE are in the ranges of 50−200 °C and 3.5−20 MPa, respectively. This pressure causes the solvents to rise above their boiling point temperature. The increased temperature accelerates the extraction by increasing solubility and mass transfer rate. Moreover, the increased temperature reduces the viscosity and surface tension of solvents, helping to spread them evenly over the biological matrix and improve the extraction rate. In some cases, pressurized hot water is used as a solvent for extraction instead of an organic solvent. This process is known as pressurized hot water extraction (PHWE) or subcritical water extraction (SWE).[84] A typical schematic diagram of a PLE system is shown in Figure 3. The equipment for PLE involves an extraction cell where the sample is introduced. The cell is filled with solvent, which is heated. High temperature and pressure are maintained to facilitate faster extraction. A pressure relief valve is installed to protect against overpressurization of the cell. All residual solvent is purged with nitrogen.[60] Extraction solvent, temperature, pressure, static time, and number of cycles are reported to influence extraction yield and rate.[83]

PLE significantly reduces the quantity of solvents used. Moreover, PHWE completely removes the requirement to use solvents. PLE is a much faster technique than other solvent extraction techniques.[85] Also in comparison with SFE, a wider range of solvents can be used for PLE extraction. However, PLE is not suitable for thermolabile compounds sensitive to high temperature and pressure conditions, and it is not as selective as SFE.[48]

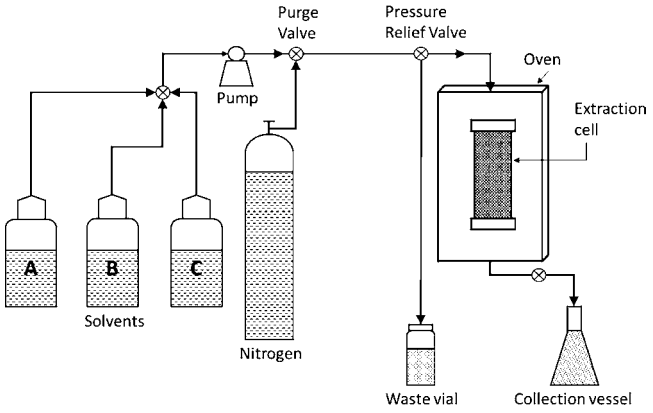

Figure 3. Schematic diagram of pressurized liquid extraction (PLE).

There is significant potential to employ PLE for extracting bioactives from marine algae and microalgae. A limited number of studies have investigated the application of PLE to brown macroalgae and microalgae. Reported applications of PLE applied to marine algae are summarized in Table 7. It was reported that the optimized extraction temperature and ethanol concentration for fucoxanthin from brown marine algae *Eisenia bicyclis* was 110 °C and 90%, respectively.[86] The use of PLE and solid-phase extraction (SPE) to extract phenolic compounds from fresh water algae and marine algae has been reported.[87] It was found that the best extraction was obtained at a pressure of 13 MPa and a temperature 130 °C. Using optimal extraction conditions, the average recovery for studied phenols was 96%. PLE was also studied by Koo et al.[88] for the extraction of zeaxanthin from *C. ellipsoidea*. Among the solvents investigated,

Table 7. Applications of PLE for Bioactive Compounds from Marine Algae

marine algae	bioactive compound	conditions	ref
Eisenia bicyclis	fucoxanthin	temperature 110 °C and 90% ethanol concentration	86
Chlorella ellipsoidea	zeaxanthin	temperature and time for extraction were 115.4 °C and 23.3 min, respectively	88
Dunaliella salina	bioactive phenols	temperatures of 40, 100, and 160 °C and times of 5, 17.5, and 30 min	39
Himanthalia elongata	bioactive phenols	temperatures of 50, 100, 150, and 200 °C for 20 min	89
Undaria pinnatifida	antioxidants	water as solvent	90
Sargassum muticum	polyphenols	pressure of 10.3 MPa at 120 °C temperature for 6 min	91

ethanol was the most efficient. Temperature had the strongest influence on zeaxanthin yield. They reported that the optimum extraction temperature and treatment time for zeaxanthin were 115.4 °C and 23.3 min, respectively. Similar studies were carried out to investigate the effect of process parameters on PLE extraction of bioactives from *Dunaliella salina*, and *Himanthalia elongata*.[39,89] Application of PHWE/SWE for the extraction of antioxidants from *Undaria pinnatifida* resulted in the generation of neo-antioxidants during SWE processing according to Herrero et al.[90] PLE using ethanol (70% v/v) enabled a good extraction of phenolic compounds (10.18 ± 0.25% dry weight) from *Sargassum muticum*.[91]

CONCLUSIONS

The significant potential of marine algae as a functional food ingredient is increasingly being recognized. Marine algae are a rich source of dietary fiber, sulfated polysaccharides, omega-3 fatty acids, amino acids, bioactive peptides, vitamins, minerals, and carotenoids. Moreover, these algae are abundantly present in nature. To better exploit this potential, there is a need to develop new and enhanced novel extraction technologies. Conventional extraction techniques are time-consuming and are not ecofriendly due to the use of organic solvents. Yields obtained with traditional solvent extraction techniques are also limited compared to the novel extraction technologies outlined in this paper, which have the potential to significantly improve extraction efficiency. For industrial applications, EAE offers benefits such as reduction in extraction time, minimization of solvent use, and increased yield. However, the maximum benefits of EAE can be achieved only if limitations of high cost, unavailability of substrate-specific enzymes, and difficulty in maintaining suitable bioreactor conditions are overcome. High-temperature operation of MAE and PLE is a major obstacle to its application to thermolabile compounds. UAE has been used for extraction of bioactive compounds from natural sources at industrial level and, similarly, it can be used for marine algae as well. Although SFE is an expensive process, it can be used successfully to extract high-value compounds from marine algae for dietary supplements. Future research priorities in this area should be concentrated on overcoming the challenges of employing these novel technologies on an industrial scale so that the significant benefits to be obtained by improved extraction of bioactives from algae are exploited by industry.

ASSOCIATED CONTENT

Supporting Information

Chemical structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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