CHAPTER ELEVEN

Green and integrated processing approaches for the recovery of high-value compounds from brown seaweeds

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Abstract

The rapidly increasing interest in utilizing seaweeds as sustainable raw material from which multiple high-value bioactive compounds can be recovered, has necessitated the development of new processing methods to achieve this. Brown seaweeds contain a range of bioactive compounds that are of commercial interest, including hydrocolloid and non-hydrocolloid polysaccharides, antioxidant polyphenols and unique pigments. Traditional polysaccharide extraction from brown seaweeds has relied on methods that require harsh chemical processing and sometimes also large amounts of energy and

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organic solvents. Commercial operation requires high recovery of the targeted compounds on the one hand, but simultaneously also the preservation of biological activity of the products on the other, an objective that is difficult to achieve using conventional methods. In order to address this shortcoming and to move toward more environmentally friendly processing in general, several greener extraction methods are being developed which have lower environmental footprint and sometimes also greater process efficiency, including enzymatic, sub and supercritical fluid, ultrasonic and microwave extraction techniques. This chapter explores these extraction techniques within the context of particularly brown seaweeds and provides an overview of potential advantages compared to conventional technologies. The work further elucidates how certain physiological characteristics of brown seaweeds can impact the processing thereof and looks toward possible future developments within the field of brown seaweed processing.

1. Introduction

Seaweeds have been highlighted as a potentially sustainable biomass feedstock for production and recovery of energy and a unique range of products for feed, food, nutraceutical and pharmaceutical industries (Holdt & Kraan, 2011; Song, Duc Pham, Seon, & Chul Woo, 2015). Specifically brown seaweeds (Phaedophyta) harbor a range of high value compounds such as lamarinin, fucoidans, alginates and phlorotannins, which could potentially have widespread applications in the food, cosmetic and the pharmaceutical industry (Barbosa, Lopes, Andrade, & Valentão, 2019; Deniaud-Bouët, Hardouin, Potin, Kloareg, & Hervé, 2017; Pádua, Rocha, Gargiulo, & Ramos, 2015). The fact that seaweed cultivation does not compete with food crops for land use and fresh water resources is one of the biggest advantages, and it also does not require fertilizer application. Brown seaweed can reach high growth rates of up to ~13 kg dry weight $m^{-2}.y^{-1}$, which is significantly higher than some terrestrial crops like sugarcane at ~10 kg dry weight $m^{-2}.y^{-1}$ (Leu & Boussiba, 2014), which is a contributing factor to numerous efforts focused on commercializing brown seaweed cultivation.

Development of sustainable and high efficiency processing methods is a requirement to drive value-addition within the globally expanding seaweed industry (FAO, 2018). Conventional processing methods often employ harsh processing conditions, use organic solvents, or require extensive downstream processing for product separation and purification, which can lead to generation of hazardous wastes and create a significant carbon

footprint. Alternative and so-called 'greener' processing methods are being developed and research has shown that these can consistently yield better process efficacy, shorter processing times and result in enhanced bioactive properties of the products. These novel extraction methods range from enzyme assisted extraction (EAE), ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), pressurized liquid extraction (PLE), sub-critical water extraction (SWE) and super-critical fluid extraction (SFE).

This chapter reviews various emerging extraction techniques as being applied particularly to brown seaweeds and compares these to conventional methods. It further explores the coupling of various techniques in terms of increasing extraction efficiency and the benefits of considering the influence of different extraction processes on the functional characteristics of the product. The review was also extended to explore the physiological characteristics of brown seaweed over different seasons that could impact its processing and can lead to an integrated process development through employing a biorefinery approach.

2. Green extraction techniques for valorizing macroalgae

The extraction route for recovering products from seaweeds can be generalized to some extent, and is divided into pre-treatment of the raw material, followed by extraction in single or multiple stages, separation of valuable compounds from residues, and finally downstream recovery and concentration of the final products. Fig. 1 is a depiction of the general route that can be expected in a seaweed processing operation.

2.1 Macroalgae pre-treatments

In general, seaweed biomass is washed post-harvesting to remove impurities like sand and epiphytes and can be further dried in cases where immediate further processing is not feasible. Industrially, drying can be achieved through sun drying or using heated drying equipment (although this comes at a significant energy expenditure); freeze drying is reserved almost solely for laboratory investigations due to the high cost of this technique. A further commonly employed pre-treatment is size reduction of the material in order to enable more efficient mass transfer during compound extraction. Different milling/grinding techniques are used, and samples are classified into different sizes depending on the further processing requirements, which serves to enlarge the surface to volume ratio to improve extraction efficiency

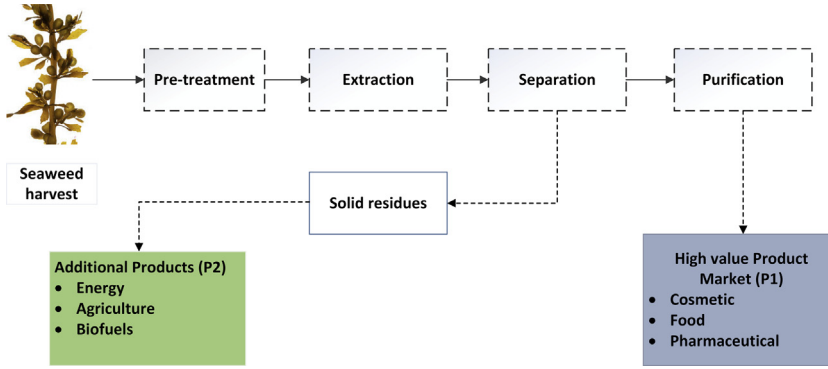

Fig. 1 A general schematic of the work-flow under a biorefinery approach showing different processing stages from harvesting to final recovery of products.

(Hahn, Lang, Ulber, & Muffler, 2012; Imbs, Ermakova, Malyarenko, Isakov, & Zvyagintseva, 2016). Proprietary cell burst and micronization techniques are increasingly being implemented for size reduction within the industry. While these routines are followed often, because of the nature of the algal biomass with water content ranging from 80% to 85%, drying can incur significant expenses upfront from an energy perspective. Developing extraction techniques which allows using size reduced wet samples would inevitably boost the workflow and can be either species-specific or end-product driven. However, storage is critical to account for seasonality and maintaining inputs through the macroalgal supply chain while maintaining bioactive functionalities of high value products within the raw materials at the same time. Drying and storage is currently a requirement as it increases the shelf life of the harvested fractions.

Based on the target end products, the samples are also pre-treated to avoid cross-contamination and removal of unwanted compounds from the sample. Mostly organic solvents are used for removing lipids (defatting), proteins and phenols (Hahn et al., 2012). Table 1 outlines different methods of pre-treatments used in various studies. Recently, alternative pre-treatment techniques have been favorably used to improve cell membrane permeability such as supercritical-CO_2 to defat algal biomass prior to extraction (Quitain, Kai, Sasaki, & Goto, 2013a), hydrothermal puffing, etc. Brown seaweed *Cystoseira trinodis* pre-treatment by fungal fermentation lead to reduced molecular weights of the fucoidan and alginate fraction with significantly improved antioxidant capacity (Hifney, Fawzy, Abdel-Gawad, & Gomaa, 2018).

Table 1 Different type of algal biomass pre-treatment methods employed prior to extraction of compounds.

Pre-treatment	Condition/source/coupled technique	Application	Reference
Dilute HCl	0.1 M HCl, 2 h, 60°C, solvent to algae ratio of 5 (mL g^{-1})	Used prior to fucoidan extraction by disrupting h−bonds between polysaccharides	(Anastyuk et al., 2017)
	0.1 M HCl, pH 2, 65°C, 3 h, 3 times, solvent to algae ratio of 10 (mL g^{-1})	For alginate extraction it helps remove unwanted impurities (polyphenols, proteins) while converting insoluble alginate salts to alginic acids for further extraction as sodium alginate	(Rostami, Tabarsa, You, & Rezaei, 2017)
Ethanol	85% ethanol, solvent to algae ratio of 10 (mL g^{-1}), stirring (2000 rpm), 24 h, room temperature	Use to selectively extract polyphenols, pigments and other low molecular weight compounds prior to subsequent extraction methods	(Alboofetileh et al., 2019)
Formaldehyde	2% (w/v) formaldehyde solution overnight, dried for 48 h, 40°C	Used to remove phenolics from the cell wall matrix and improve product purity	(Mohammed et al., 2018)
Enzymatic	a)5% solids content, alginate lyase (0.05% w/w), 37°C, 2 h, 125 rpm agitation	Helps in degradation of alginate in the cell wall matrix to improve extraction yield	(Billakanti, Catchpole, Fenton, Mitchell, & MacKenzie, 2013)
	b) Viscozyme intensified with microwave, 30 min, 50°C, pH 4.5	Coupling strategy was used to test pre-biotic potentials of different molecular weight fractions	(Charoensiddhi, Conlon, Vuaran, Franco, & Zhang, 2017)
Supercritical-CO_2	300 bar, 50°C, 4 h	Employed as defatting technique prior to polysaccharide extraction to enhance bioactivity of target polysaccharides	(Saravana et al., 2018)
Ultrasound assisted (UA)	750 W capacity and 20 kHz frequency for 60 min pre-treatment	UA pre-treatment was followed by alkali-based extraction of proteins	(Kadam, Álvarez, Tiwari, & O'Donnell, 2017)

2.2 Novel extraction techniques

The bottleneck in harnessing the potential of the seaweed compound repository lies in improving extraction efficiency to get viable yields, while also preserving the bioactive compounds functionalities. The macroalgal cell wall structure, with interlinked polysaccharide fractions, provides a very robust physical barrier which needs to be overcome in order to access various high value compounds that occur as part of the cell wall structure, or are found internally within the cells. The macroalgal matrix has a unique structural integrity and conventional extraction has relied on harsh treatments (high temperatures and high- and low pH, with or without the use of organic solvents) to overcome this barrier. However, these harsh extraction conditions and use of organic solvents raises safety issues for end use in food, pharma and cosmetics, in addition to environmental concerns. Downstream separation and purifications steps might further lead to production of waste that raise disposal concerns.

Novel and greener processes have started making headway recently, which have showed promise through improved yields and shorter extraction times, with lower environmental footprints and energy demands compared to conventional processes (Bowyer, Van Altena, & Scarlett, 2018; Flórez-Fernández, Torres, González-Muñoz, & Domínguez, 2019; Kadam, Tiwari, & O'Donnell, 2013; Nadar, Rao, & Rathod, 2018; Saravana et al., 2018). Although development of these novel processes require significant investment in terms of initial process development, piloting and scale up, long term economic prospects seem promising (Charoensiddhi et al., 2018).

2.2.1 Enzyme assisted extraction

Enzyme assisted extraction is gaining significant attention over conventional solvent based extraction techniques in terms of offering better extraction efficacy, specificity in targeting desired end products, reduced processing time and an environmentally friendly process devoid of the need for harsh solvents (Nadar et al., 2018; Wijesinghe & Jeon, 2012b). Macroalgae, specifically the brown seaweed species harbor a range of unique biomolecules such as phlorotannins, fucoxanthins, alginates and the sulfated polysaccharide fractions with illustrated bioactivities which are enmeshed within the structural makeup of the cell wall (Holdt & Kraan, 2011). Breaking down these structural barriers is pivotal to release the entrapped compounds and EAE is a promising technique in selectively breaking down the cellular

barriers under mild conditions (Charoensiddhi, Conlon, Franco, & Zhang, 2017).

EAE offers important advantages within integrated processing schemes. Extraction conditions enable the recovery of safe products suitable for use in nutraceutical, cosmetic and pharmaceutical industries. Furthermore, EAE products have been shown to possess enhanced bioactivities in various studies, which have been linked to the preservation of the structural integrity of target compounds (Casas, Conde, Domínguez, & Moure, 2018; Lee et al., 2012; Puspita et al., 2017; Rodrigues et al., 2015). EAE furthermore offers process scalability and good selectivity toward individual target compounds (Jaswir, Amid, Alam, Asiyanbi-H, & Ramli, 2013), and both individual enzymes or enzyme cocktails have proved valuable for targeting specific components of the macroalgae (Puspita et al., 2017).

Despite the promises of EAE, one of the main obstacles to this processing method in seaweeds is the fact that very few industrially available enzymes have been specifically tailored toward substrates found only in seaweed. Research on enzymatic seaweed processing to date has employed enzymes developed for use in terrestrial feedstocks due to lack of alternatives, and in some cases results have been variable. In the case of employing cellulases for release of fermentable sugars like glucose, results have been favorable and high glucose yields are achievable (Borines, De Leon, & Cuello, 2013; Noraini, Ong, Badrul, & Chong, 2014), although sometimes only after extended hydrolysis times. Other attempts to degrade seaweed polysaccharides using carbohydrases have been less successful, where a number of carbohydrases exhibited almost no ability to significantly solubilize seaweed biomass (Hardouin et al., 2016). The implication of this lack of industrial enzymes specifically tailored toward seaweed processing is that the terrestrial biomass-oriented enzymes available may exhibit slower enzyme kinetics and poorer substrate specificity, and that for some substrates found in seaweeds, no industrial enzymes are available to employ during processing. Thus, tapping into the potential of novel enzymes from marine sources more specific to the macroalgal chemistry can boost the EAE process significantly in terms of increased extraction efficiency, with likely improvement in the techno-economics of EAE-based seaweed processing operations.

Table 2 compiles the various enzymes and associated extracted product and its application from different brown seaweed species. EAE has been reported to deliver yields in the range of 20%–50% on a dry algal weight basis at enzyme dosing of 0.5%–5% (w/v), where target products included polyphenols, alginates, sulfated polysaccharides and proteins. Comparatively, the

Table 2 Applications of EAE for bioactive compounds from various brown macroalgae species.

Species	Enzyme	Conditions	Compounds	Application	Reference
Ecklonia radiate	Viscozyme® L, Celluclast® 1.5 L, Ultraflo® L, Alacalase® 2.4 L FG, Neutrase® 0.8 L and Flavourzyme® 1000 L	Enzymatic hydrolysis at 50°C for 24 h at pH 4.5−8 with buffers and water	Carbohydrates	Food industry	(Charoensiddhi et al., 2017)
Sargassum muticum (Yendo)	Neutrase, alcalase, ultraflo, amyloglucosidase (AMG), shearzyme, termamyl, viscozyme, and celluclast	3 h at 50°C followed by deactivation for 15 min at 90°C	Phenolic compounds	Antioxidant, antimicrobial, cytotoxicity activity, tyrosinase inhibition activity	(Puspita et al., 2017)
Nizamuddinia zanardinii	Alcalase, flavourzyme, celluclast, and viscozyme	5% (v/v), 50°C, 24 h at optimal pH of each enzyme	Sulfphated polysaccharides	Anticancer and immune-enhancing activities	(Alboofetileh, Rezaei, & Tabarsa, 2018)
Undaria pinnatifida	Alginate lyase	Enzyme pre-processing (0.05% w/w) followed by extraction using dimethyl ether (DME) with ethanol as a co-solvent. 37°C and pH 6.5	Fucoxanthin and lipids	Therapeutics	(Billakanti et al., 2013)

Ecklonia cava	Celluclast	24 h at 50°C followed by deactivation for 10 min at 100°C	Fucoidans	Anti-inflammatory activity	(Lee et al., 2012)
Sargassum muticum	Proteases (endo-peptidase, endo-protease) and carbohydrases (cellulase, xylanase, β-glucanase, arabanase)	50°C for 5 h	Crude hydrolysate	Anti-viral activity	(Hardouin et al., 2014)
Sargassum muticum	Celluclast, viscozyme, rapidase, amylase alcalase, protamex	3 h of extraction using 2%–5% (wt%) enzyme loads	Crude hydrolysate	Antioxidant, cytotoxicity activity	(Casas et al., 2018)
Sargassum muticum	Alcalase, flavourzyme, cellulase, viscozyme L	24 h at 50°C and deactivated by heating the sample at 90–100°C for 10 min.	Phenolics, sulfphated polysaccharides	Hydroxyl-radical scavenging activity, prebiotic activity	(Rodrigues et al., 2015)

fractions with different molecular weights from the native molecules, have often shown enhanced bioactivities, including antioxidant, anti-viral, anti-cancer and anti-inflammatory activities.

2.2.2 Microwave assisted extraction

Microwave assisted extraction operates in the electromagnetic waves spectrum with frequencies ranging between 300 MHz and 300 GHz (Routray & Orsat, 2012). These waves are non-ionizing and the energy translates into heat which dissipates causing structural changes within the material matrix (Yuan & Macquarrie, 2015). The dielectric properties of the fluid within the matrix can be a deciding parameter for extraction efficiency. Absorption of the waves by the sample initiates two different phenomena, dipole rotation and ionic conduction which are influenced by temperature (Garcia-Vaquero, Rajauria, O'Doherty, & Sweeney, 2017). Ionic conduction happens due to the movement of the charged ions under the influence of the electrostatic field created by the microwaves and the resulting friction owing to these movements within the sample space creates heat. The other heat generating mechanism from the microwaves happens due to the tendency of the dipolar fractions in the sample to align according to the alternating electric field generated by the microwaves and collides among themselves and generates heat (Zhang, Yang, & Wang, 2011).

MAE has gained attention as a good alternative to solvent extraction techniques for extracting bioactive compounds from various natural matrices including macroalgae. MAE offers several advantages in terms of using less solvent, shorter processing times and increased extraction yields (Yuan et al., 2018). It enables deeper penetration of solvents into the biomass matrix which aid in better mass transfer and dissolution of solutes. Its industrial scale applicability has been recommended which does involve an initial capital expenditure, but more investigation is required in terms of operational logistics for large-scale operations (Leone, Tamborrino, Romaniello, Zagaria, & Sabella, 2014). In addition, due to the generation of heat during the process it could also hamper extraction of heat labile compounds and possibly contribute toward structural alteration of the compounds (Ren et al., 2017). A significant portion of polyphenols, up to 40% are cell-wall bound and thus allows better accessibility via MAE compared to solvent extraction. MAE increased polyphenols yields in the *Carpophyllum flexuosum* species by up to 70% compared to solvent extraction (Magnusson et al., 2017). Table 3 compiles the various MAE isolated products and its illustrated application from different brown seaweed species. Fucoidans and

Table 3 Applications of MAE for bioactive compounds from various brown macroalgae species.

Species	Conditions	Compounds	Application	Reference
Fucus vesiculosus	MAE at 120 psi, 1 min, solvent to algae ratio of 25 (mL g^{-1})	Fucoidans	Not characterized	(Rodriguez-Jasso, Mussatto, Pastrana, Aguilar, & Teixeira, 2011)
Ascophyllum nodosum	120°C for 15 min extraction, up to 300W	Fucoidans	Anti-oxidant activity	(Yuan & Macquarrie, 2015)
Undaria pinnatifida	Extraction time 1−3 min, microwave power 600 W, temperature 110−200°C	Fucoidans	Low-molecular-weight components (activity not evaluated)	(Quitain et al., 2013a)
Undaria pinnatifida and *Sargassum fusiforme*	Extraction time 10−30 min, microwave power 300−500 W, temperature 30−70°C,	Phytol Fucosterol	Not characterized	(Xiao, Yuan, & Li, 2013)
Saccharina japonica Aresch	Extraction time 5−25 min, microwave power 400−600 W, temperature 60°C	Phlorotannin	Inhibited HepG2 cancer cells	(He et al., 2013)
Ascophyllum nodosum, Laminaria japonica, Lessonia trabeculate and *Lessonia nigrecens*	Irradiation (2.45 GHz) for 15 min (5 min climbing and 10 min holding) at 110 °C. Agitation: 300 rpm	Phlorotannin	Antioxidant activities and inhibitory effects on α-amylase, α-glucosidase, pancreatic lipase and tyrosinase	(Yuan et al., 2018)

(*Continued*)

Table 3 Applications of MAE for bioactive compounds from various brown macroalgae species.—cont'd

Species	Conditions	Compounds	Application	Reference
Sargassum thunbergii	Extraction time 23 min, microwave power 547 W, extraction temperature 80°C, and solvent to algae ratio of 27 (mL g^{-1})	Polysaccharides	Antioxidant and hypoglycemic activities	(Ren et al., 2017)
Ten different species from Australia and New Zealand were studied	Biomass:solvent ratio 1:30, 160°C, 3 min, max power 850 W	Phenolic compounds	Antioxidant capacity	(Magnusson et al., 2017)
Sargassum vestitum	1200 W, frequency 2450 MHz extracted in 70% ethanol at solvent to algae ratio 50 (mL g^{-1})	Phenolic compounds	Antioxidant capacity	(Bowyer et al., 2018)

polyphenolic compounds from a number of different brown seaweed species have been isolated, and a range of bioactivities have been demonstrated, but MAE research can benefit from further functional characterization of the isolated products.

2.2.3 Ultrasound assisted extraction

Ultrasound assisted extraction has shown potential as a viable extraction technique for the bioactive compounds in the macroalgal cellular matrix (Kadam, Tiwari, Smyth, & O'Donnell, 2015). It works based on the propagation of high frequency sound waves (>20 kHz) through the medium in compression and rarefaction waves which leads to the formation of cavitation bubbles. Vapor from the liquid medium gets entrapped within the bubble which expands until the bubble collapses generating mechanical energy leading to the algal biomass matrix disruption through microturbulence (Vinatoru, Mason, & Calinescu, 2017). Liquid-solid suspensions (e.g. algal biomass) promotes the formation of asymmetrical bubbles which upon implosion forms micro-jets causing surface erosion, local thermal events and algal cell wall breakdown, thus enhancing extraction efficiency (Chemat et al., 2017; Shirsath, Sonawane, & Gogate, 2012). Additionally, it promotes mass transfer and micro-mixing within the matrix. A range of parameters influences the cavitation formation and its resulting physical impact such as frequency and intensity of the waves, viscosity and surface tension of the solvent along with operating temperature and pressure (Tiwari, 2015).

UAE is a favourable technique offering a range of benefits compared to conventional methods: it is an organic solvent-free process, has high extraction efficiency, it is a relatively fast extraction method, operates at mild temperatures, requires low maintenance of equipment, offers the possibility of direct scale-up to industrial scale, and it has been shown to be cost effective in a number of processing industries (Flórez-Fernández, López-García, González-Muñoz, Vilariño, & Domínguez, 2017; Garcia-Vaquero et al., 2017; Hmelkov, Zvyagintseva, Shevchenko, Rasin, & Ermakova, 2018; Peshkovsky, Peshkovsky, & Bystryak, 2013; Roselló-Soto et al., 2015). Dang et al. (2017) reported a 142.6% increase in total phenolics content using UAE for 1 h compared to conventional solvent extraction with ethanol for 12 h with higher antioxidant potential. Low temperature operation is favourable for extraction of thermo labile compounds, while the clean operational conditions that can be achieved with UAE open avenues in product applications within the food and pharma industries. However, UAE does

require significant process energy input, and initial equipment requires significant starting capital for operation at industrial scale.

Table 4 compiles the various UAE studies and associated extracted product and its application from different brown seaweed species. A range of fucoidans, polyphenols and laminarins has been isolated successfully at varying operating parameters, also with ethanol as a co-solvent primarily focusing on polyphenol extractions. An interesting fucoidan with previously unreported regular 1 → 3; 1 → 4-α-L-fucan structure, with the fucose residues sulfated at C2 and acetylated at C3, was isolated from a brown alga *Fucus evanescens* by ultrasound extraction, and was shown to possess anti-cancer activity (Hmelkov et al., 2018). Illustrated bioactivity of the isolated fractions achieved in a short time and at lower operating temperature with UAE compared to conventional processes, is one of its core advantages which needs to be translated in an industrial setting. Thus, while further research is warranted, UAE can be a promising methodology which can serve as a pre-treatment technique or can be used either as stand-alone extraction technique or in conjunction with other methods for developing an integrated extraction process.

2.2.4 Sub-critical water

Sub-critical water extraction is gaining traction owing to its zero toxic waste production, as water is employed as extraction solvent. SWE operates in the sub-critical region of water between 100 and 374 °C and pressures between 0.1 and 20 MPa to keep the water in liquid state, and allows manipulation of the properties of the water due to the temperature dependence of properties like polarity, viscosity, density and surface tension. Classical solvent extraction of non-polar compounds from macroalgae uses organic solvents such as hexane, methanol, chloroform, ethanol etc. Some of these solvents can be toxic and requires considerable downstream processing for both product and solvent recovery, and in order to adhere to the food and pharmaceutical industry regulations for safe end-product application. SWE in contrast offers a safe, green and high efficacy extraction technique for product use in therapeutics, food and cosmetic industry (Carr, Mammucari, & Foster, 2011).

A key operating principle of SWE is the ability to manipulate the properties of the extraction solvent and thereby to target specific classes of compounds. Water in ambient state is a polar solvent with a dielectric constant of 80 at 25°C which can be modulated in subcritical state and reduce to 40 at 200°C, thus enhancing solubilities of hydrophobic organic compounds (Reddy et al., 2014). The reduced dielectric constant of water at elevated

Table 4 Applications of UAE for bioactive compounds from various brown macroalgae species.

Species	Operational conditions	Compounds	Applications	Reference
Ascophyllum nodosum	20 kHz between ultrasound amplitude (22.8−114 μm) for 10 min	Total phenolics, fucose, and uronic acid	Bioactive properties	(Kadam et al., 2015)
Ascophyllum nodosum	20 kHz and 700 W, amplitude levels of 22.8 and 68.4 μm for 10 min	Proteins and amino acids	Bioactive properties	(Kadam et al., 2017)
Ascophyllum nodosum, *Fucus vesiculosus* and *Bifurcaria bifurcata*	Operated at room temperature for 30 min with water/ethanol (50:50, v:v) as The extraction solvent	Crude extract	Antioxidant potential	(Agregán et al., 2018)
Undaria pinnatifida	20 kHz/100−300 W/40−60°C for 30 min solvent to algae ratio of 30 (mL g^{-1}) with 410 rpm agitation at pH 11	Pigments: carotenoid and chlorophyll	Not evaluated in this study	(Zhu et al., 2017)
Sargassum muticum	24 kHz/400 W/20−60°C for 5−25 min	Post hydrolysis of crude fucoidan derived from hydrothermal treatment	Enhanced antiradical and cytotoxic properties	(Flórez-Fernández, González-Muñoz, & Domínguez, 2017)
Sargassum muticum	150 W and 40 kHz at room temperature (25°C) for 5−30 min	Crude fucoidan and phlorotannins	Antioxidant and anti-tumour activity	(Flórez-Fernández, López-García et al., 2017)

(*Continued*)

Table 4 Applications of UAE for bioactive compounds from various brown macroalgae species.—cont'd

Species	Operational conditions	Compounds	Applications	Reference
Sargassum muticum	400 W, 24 kHz ay 60% amplitude for 5 min and off periods of 25 min	Phenolics and carbohydrates	Antioxidant activity, cytotoxic activity	(Casas et al., 2018)
Sargassum muticum	400 W, 50/60 Hz for 60 min (sonicate for 10 min and pause for 2 min) at 50°C	Sulfated polysaccharides and phenolics	Antioxidant and prebiotic potential	(Rodrigues et al., 2015)
Sargassum muticum	Solvent to algae ratio of 20 (mL g^{-1}), 40 Hz/150W, 5−30 min and 25°C and amplitude of 40%−100%	Alginates	Cytotoxicity	(Flórez-Fernández, Torres, González et al., 2019)
Fucus evanescens	35 kHz/150 W at room temperature for 5−30 min	Fucoidans	Anticancer activity	(Hmelkov et al., 2018)
Hormosira banksii	50 Hz/150−250 W, 20 −60 min and 30−50°C. 70% ethanol (v/v) with a solvent to algae ratio of 50 (mL g^{-1})	Phenolic content	Antioxidant activity	(Dang et al., 2017)
Laminaria digitata	20 kHz/500 W, 10−30 min and 40−80°C and amplitude of 40−100%	Fucose, glucans	Antioxidant activity	(Garcia-Vaquero, Rajauria, Tiwari, Sweeney, & O'Doherty, 2018)

temperature in the sub-critical range is closer to organic solvents like ethanol (27) and methanol (33) (IAPWS, 1997). SWE entails considerably higher yields and shorter extraction times for a range of moderately non-polar compounds (Herrero, Cifuentes, & Ibañez, 2006). The SWE techniques follows a combination of reaction kinetics from desorption of the target compounds from the matrix, diffusion of the solvent into the matrix and finally dissolution of the compound into the solvent (Carr et al., 2011). In addition to the compound chemistry and biological matrix types, altered solvent properties, including surface tension, viscosity, density and polarity, plays a vital role in the overall SWE process efficiency. Surface tension of water significantly reduces with increasing temperature which entails enhanced wetting of the matrix and facilitates mass transfer. There is a 50% reduction in surface tension of water from 25 to 200°C (Plaza & Turner, 2015). Also, both density of water and viscosity reduces at elevated temperature which further enhances mass transfer and diffusion of analytes out of the matrix to the solvent phase. Density of water decreases from 0.997 g/mL at 25°C to 0.579 g/mL at 350°C (NIST, 2014). Cumulatively, all these parameter changes happen in parallel which contributes toward higher yields and lower extraction times with greater selectivity than most conventional techniques, all of which can be achieved in a clean and solvent-free extraction process. In addition to the use of water as a green solvent, the luxury of using wet algal biomass without the requirement of drying the biomass can cut down energy expenses up to 2–8 times (Reddy et al., 2014; Suganya, Varman, Masjuki, & Renganathan, 2016). A significant disadvantage of SWE is the higher equipment costs associated with the high pressure and high temperature operating regime, which necessitates high initial capital outlay, but also higher maintenance costs along with strict safety measures.

Table 5 compiles the various SWE studies and associated extracted product and its application from different brown seaweed species. A review of the recent investigations with SWE clearly puts it as a promising method for isolating a number of high-value products including fucoidans, alginates and polyphenols. The combination of temperature and pressure entails significantly higher overall yields compared to conventional as well as some novel methods with ~80% yields achieved in the 180–220°C/7–50 bar operating range (Flórez-Fernández, Torres, González et al., 2019; Saravana, Choi, Park, Woo, & Chun, 2016). The attributed bioactivity of the isolated products showed higher antioxidant activities along with antibacterial and anti-cancer activity. A number of studies also explored using catalysts like NaOH, acetic acid, formic acid, ionic liquids (ILs), and deep

Table 5 Applications of SWE for bioactive compounds from various brown macroalgae species.

Species	Operating conditions	Compounds	Applications	Reference
Saccharina japonica	Addition of 0.1% NaOH 100–180°C 100–300 rpm 20–80 bar solvent to algae ratio of 10–25 (mL g^{-1}), 10–20 min	Fucoidans, mannitol, amino acids	Antioxidant and anti-proliferation activity	(Saravana et al., 2018)
Saccharina japonica	1% acetic acid-assisted SWE 200–280°C 150 rpm 13–60 bar 28–42 min	Crude hydrolysate	Antibacterial	(Meillisa, Siahaan, Park, Woo, & Chun, 2013)
Saccharina japonica	1% formic acid-assisted SWE 180–260°C 140 rpm 15–65 bar	Monosaccharides and bioactive compounds	Antioxidant activity	(Meillisa, Woo, & Chun, 2015)
Saccharina japonica	180–420°C 150 rpm 13–520 bar for 5 min	Mannitol, amino acids	Fermentation industry	(Saravana et al., 2016)
Saccharina japonica	Ionic liquid-assisted SWE 100–250°C 200 rpm 50 bar 5 min	Phenolics	Antioxidant activity	(Vo Dinh, Saravana, Woo, & Chun, 2018)

Saccharina japonica	SWE with DES Optimized condition 150 °C, 19.85 bar, 70% water content and solvent to algae ratio of 38.8 (mL g^{-1})	Fucoidans and alginates	Antioxidant activity	(Saravana, Cho, Woo, & Chun, 2018)
Laminaria saccharina	Optimized condition, solvent to algae ratio of 10 (mL g^{-1}), 350°C 15 min	Biocrude	Bio-oil, biorefinery	(Anastasakis & Ross, 2011)
Nizamuddinia zanardinni	150 °C for 29 min	Fucoidans	Antibacterial and antiviral activity	(Alboofetileh, Ritta et al., 2019)
Laminaria ochroleuca	Non-isothermal autohydrolysis from temperature 120–220°C	Bioactive and gelling extracts	Antioxidant activity, viscoelastic properties, antitumor activity	(Flórez-Fernández, Torres, González-Muñoz et al., 2019)

eutectic solvents (DESs) to increase yields, some of which showed promising results; however, further investigation is needed with the use of some additives. Overall, SWE holds immense potential for scalability in industrial scale and research should be directed toward facilitating scaled-up operations.

2.2.5 Super-critical fluid extraction

Super-critical fluid extraction operates above the critical points of temperature and pressure of the fluid while utilizing the modulated physico-chemical properties of the fluid to extract target compounds from a range of natural biomass matrices (Herrero et al., 2006). In the super-critical state, the density of the supercritical fluid is akin to a liquid, while the viscosity is in the intermediate range of liquid and gas. The relatively low viscosity and the associated increased diffusivity facilitates better mass transfer of the solutes from the matrix into the solvent (McHugh and Krukonis, 1994).

CO_2 is the most widely used fluid for Sc-FE and is increasingly being used for bioactive compounds extraction from plant materials, food by-products, macroalgae and microalgae (Esquivel-Hernández et al., 2017). Its critical points of temperature (30°C) and pressure (7.38 MPa) are favourable for extracting heat labile compounds. The use of CO_2 makes the process clean, non-toxic and environmentally safe which adheres to the safe regulatory requirements for solvents. Bioactive compounds extracted using Sc-FE has found application in the food and pharmaceutical industry (Herrero et al., 2006; Pérez-López et al., 2014). However, Sc-CO_2 is generally a non-polar solvent which is not ideal for extracting polar compounds. Addition of certain modifiers have been proven useful to considerably modulate the solvating capability of extracting polar natural compounds (Goto, Kanda, Wahyudiono & Machmudah, 2015). Table 6 compiles the various Sc-FE studies and associated extracted product and its application from different brown seaweed species. Sc-CO_2 extraction studies with brown seaweeds have been primarily focused on extracting fucoxanthin as a stand-alone method as well as with co-solvents. Significantly improved fucoxanthin yields have been reported with attributed bifunctionalities ranging from antioxidant activity, plant biostimulants and anti-hypersensitive activity. In addition, Sc-CO_2 have been used to extract both fatty acids and phenolic compounds with proposed application in the food and cosmetic industry. In a nutshell, further research is needed for its scale-up applicability given the environmentally benign and safe nature of the solvent.

Table 6 Applications of Sc-CO_2 for bioactive compounds from various brown macroalgae species.

Species	Conditions	Compounds	Application	Reference
Fucus vesiculosus	Raw material 9 wt% (air-dry raw material), temperature 60°C, pressure 304 bar, extraction time 60 min	Fatty acids, polyphenols	Bactericidal, fungicidal, and immunostimulating activities	(Bogolitsyn et al., 2017)
Saccharina japonica	45–55°C, pressures 200–300 bar, and co-solvent flow rates 0.50–2.00 (% of CO_2, w/w)	Fucoxanthin, phlorotannin	Antioxidant	(Saravana et al., 2017)
Sargassum hemiphyllum	40–50°C, 24.1–379 bar, 60 min	ω-3 fatty acids	Functional food supplements	(Cheung, Leung, & Ang, 1998)
Sargassum muticum	45°C, 290 bar with super-critical fluid flow of 10 kg h^{-1}	Glycolipids	Functional food	(Terme et al., 2017)
Undaria pinnatifida	40–70°C; 400 bar for 150 min	Fucoxanthin	Food ingredients	(Goto et al., 2015)
Undaria pinnatifida	40°C and 400 bar, extraction time of 180 min	Fucoxanthin	Food and pharmaceutical additive	(Quitain, Kai, Sasaki, & Goto, 2013b)
Ascophyllum nodosum	500 bar; 50°C; load mass 257 g; CO_2 passed through a bed of algal biomass 23.8 kg	Plant biostimulants	Agriculture and horticulture	(Michalak et al., 2016)

(*Continued*)

Table 6 Applications of Sc-CO_2 for bioactive compounds from various brown macroalgae species.—cont'd

Species	Conditions	Compounds	Application	Reference
Sargassum muticum	100 bar, 50°C	Fatty acids, phenolics and fucoxanthin	Anti-browning activity, inhibition of lipogenesis and cytotoxicity effects	(Conde, Moure, & Domínguez, 2015)
Saccharina japonica and *Sargassum horneri*	45°C and pressure of 250 bar. The flow rate of CO_2 27 g min^{-1} and ethanol as co-solvent	Fucoxanthin in oil	Antihypertensive and antioxidant activity	(Sivagnanam et al., 2015)

2.2.6 Other novel green solvents

Developing green solvents has got a great impetus in the quest for replacing the toxic organic solvents employed in various industrial processes. Deep eutectic solvents (DESs) are mixtures formed by combinations of Brønsted Lewis bases and acids with different types of anionic and cationic groups imparting specific tunable properties (Saravana et al., 2018). These mixtures have lower melting points compared to any of its individual components, partly owing to intermolecular hydrogen bonds (Dai, van Spronsen, Witkamp, Verpoorte, & Choi, 2013). They are biodegradable, easy to handle and have decreased toxicity (Ruesgas-Ramón, Figueroa-Espinoza, & Durand, 2017). Ionic liquids (ILs) are another category of multimolecular solvents comprising of an organic cation with a coordinating inorganic or organic anion (Han & Row, 2010). In addition to their tunable properties, ILs have the advantage of possessing low vapor pressure, are non-flammable and are stable at higher temperature (Ruesgas-Ramón et al., 2017). However, despite these advantages the significant economic challenge of high solvent costs remains an obstacle for process commercialization.

3. Coupling of techniques for enhanced extractions of bioactive compounds

Recent research is driven toward development of eco-friendly and cost-effective extraction techniques which leads to high yields and shorter processing times, while recovering bioactive compounds with preserved or enhanced functionalities. Each extraction technique has its own advantages and disadvantages in terms of operational logistics, capital costs and downstream processing requirements of the desired end-products (Table 7). Also, the comparison of different methods clearly illustrates that there is a variation across methods in terms of process efficacy as well as compound selectivity. A good extraction technique is a function of multiple variables ranging from the species type, target compounds and the desired application of the products. Products for food and cosmetic industry needs adherence to normally strict regulations, while pharmaceutical applications need to follow extremely stringent standards. Greener extraction techniques hold a distinct advantage in comparison to conventional solid-liquid extractions which often employ toxic organic solvents. Coupling of extraction methodologies offers the advantages of circumventing some pitfalls of any single method which can lead to better yields and product recovery and lead to the development of more efficient and profitable processes.

Table 7 Comparison of the advantages and disadvantages of some novel green extraction methodologies currently in focus.

Extraction technique	Advantages	Disadvantages	Reference
Enzyme-assisted extraction	Green technology, mild operating conditions, water can be used, enhanced bioactive properties of extracts	Extended hydrolysis time, enzyme specificity and activity, expensive leading to high cost for industrial scale-up	(Nadar et al., 2018; Rhein-Knudsen, Ale, & Meyer, 2015; Wijesinghe & Jeon, 2012b)
Microwave-assisted extraction	Shorter processing time, no harsh co-solvents involved, environment friendly, low cost, both open and closed vessel operation is possible at ambient temperature	Structural modification to the compound structure may alter/ or limit bioactive properties, recovery of non-polar compound- High extraction temperature	(Joana Gil-Chávez et al., 2013; Ren et al., 2017; Yuan et al., 2018; Yuan & Macquarrie, 2015)
Ultrasound-assisted extraction	Non-thermal extraction technique, low solvent requirement, high extraction yields, greater level of automation, Affordable and reproducible method	Economic feasibility for scale up, Non-linearity of the process, scale-up operation needs to be validated, high energy inputs	(Chemat et al., 2017; Kadam et al., 2015; Roselló-Soto et al., 2015; Tiwari, 2015; Vinatoru et al., 2017)
Sub-critical water extraction	Green and sustainable process, tuneable properties of water under sub-critical conditions, higher yields, enhanced mass transfer and lower extraction time, relatively simple equipment set-up	High pressure and temperature operation, high initial capital costs, May not be suitable of thermolabile compounds	(Meillisa et al., 2013; Plaza, Amigo-Benavent, del Castillo, Ibáñez, & Herrero, 2010; Saravana, Cho, et al., 2018; Saravana, Tilasun, et al., 2018)

Super-critical fluid extraction	Non-toxic and solvent free, mild extraction parameters, highly selective	High power consumption, high operating pressure, expensive set-up, laborious sample processing	(Bogolitsyn et al., 2017; Conde et al., 2015; Michalak, Chojnacka, & Saeid, 2017)
Deep eutectic solvents	Biodegradable, easy to handle and have lower environmental footprints then organic solvents, non-volatile, non-flammable, wide range of selectivity for compounds,	High densities and viscosities of DES, recovery of target compounds can be difficult due to low vapor pressure, re-cyclability and industrial applicability yet to be evaluated	(Dai et al., 2013; Ruesgas-Ramón et al., 2017; Saravana et al., 2018)
Ionic liquids	Low vapor pressure, non-flammable and stability at higher temperature	Expensive, water stability, high toxicity, issues in purifying, poor biodegradability	(Han & Row, 2010; Ruesgas-Ramón et al., 2017)

Deep eutectic solvent combined with subcritical water extraction was used to improve the extraction of seaweed polysaccharides (fucoidans and alginates) from *Saccharina japonica* (Saravana et al., 2018). A combined EAE-pressurized liquid extraction process was studied by Sánchez-Camargo et al. (2016) to extract phlorotannins from the seaweed *Sargassum muticum* for antioxidant potential, but suggested further research with more substrate-specific enzymes is required to selectively recover the target compounds. Ionic liquid assisted SWE has been shown to enhance the extraction of phenolic compounds from the brown macroalgae *Saccharina japonica* with good antioxidant activity (Vo Dinh et al., 2018). Casas et al. (2018) illustrated EAE coupled with ultrasound extraction to be effective in selectively enhancing the phenolic extraction with good antioxidant activity in addition to improved yields in *S. muticum*. The extracts also had additional bioactive properties in terms of cytotoxic activity against murine melanoma B16F10 cells and human liposarcoma SW872 cells. Recently an EDTA based extraction of fucoidans from *Laminaria japonica* resulted in high yield with antioxidant and anti-cancer activity (Zhao, Xu, & Xu, 2018). Supercritical CO_2 (Sc-CO_2) defatted *S. japonica* samples were coupled with SWE to extract fucoidans with good antioxidant and anti-proliferative potential suggesting potential industrial applications (Saravana et al., 2018).

There is an intricate link between the extracted product, structural characteristics and its bioactivity to the extraction techniques used (Okolie, Akanbi, Mason, Udenigwe, & Aryee, 2018; Zhu et al., 2016). Linking extraction techniques to the products and its characteristics and decoupling various facets can help gain significant insight in integrating processes toward maximizing the valorized macroalgal biomass potential. Alginates extracted from the species *Colpomenia peregrina* through enzymatic hydrolysis showed lowest protein (<0.7%) and polyphenol (<5 mg gallic acid/g sample) contents in comparison to extractions using water and acid (Rostami et al., 2017). Enzyme assisted methodologies have been suggested to be less effective for polyphenol recovery but much more effective for proteins, uronic acids and sulfate groups (Hardouin et al., 2014; Puspita et al., 2017). EAE, while being effective, also ranks as one of the safest extraction methods for food grade products end-uses (Nadar et al., 2018; Rodrigues et al., 2015). Polyphenol recovery from *S. muticum* through EAE in combination with ultrasound significantly improved in selectively recovering polyphenols than enzyme alone extractions (Casas et al., 2018). Choice of enzyme is particularly critical owing to its specificities to target compounds (Jaswir et al., 2013). In comparison to a range of carbohydrase, and protease and

hot water extractions from *Nizamuddinia zanardinii*, Alcalase was the most effective in extracting fucoidans with higher molecular weight and sulfur content with enhanced anticancer and immune-enhancing activities (Alboofetileh et al., 2018). Quitain et al. (2013a) illustrated the advantages of using microwave aided extraction in comparison to conventional heating to generate low-molecular-weight fucoidan components of about 5–30 kDa, from wakame de-oiled using Sc-CO_2. Charoensiddhi et al. (2017) coupled ethanol pre-treatment of *Ecklonia radiata* to extract polyphenols and outlined a sequential process by combining enzyme aided (Viscozyme) with microwave-intensified extraction to generate different molecular weight polysaccharide with good prebiotic potential. Other techniques like SWE often results in structural modifications and leads to neoformation of compounds with potent antioxidant capacity. Further research is warranted pertaining to characterizing these compounds and assessing their application as nutraceuticals and functional food components. Thus, with further research novel techniques can cater toward a sustainable process development.

4. Potential high-value seaweed products

There is a broad range of potentially valuable compounds found in brown seaweeds, including polyphenols and other metabolites, polysaccharides, proteins, lipids, minerals and a host of other minor constituents. Various of these compounds are valued for their nutritional value or potential positive impacts on health via favourable biological activities. Table 8 provides a summary of the bioactivities characterized in various brown seaweeds and to which types of compounds these activities were linked. Seaweeds are often the subject of bioprospecting investigations in further efforts to discover novel compounds. Two of the classes of compounds which have potentially high-value applications and which are unique to seaweeds, are the different polysaccharides and polyphenols found in these organisms.

4.1 Polysaccharides

Fucoidan, laminarin and alginate constitutes the three major valuable polysaccharide fractions in the brown seaweeds, and are increasingly finding applications in the food, cosmetic and the pharmaceutical industries, mainly owing to their functional and bioactive characteristics.

Table 8 Compilation of some routinely investigated brown seaweed species and their illustrated bioactivities.

Brown seaweed species	Compounds	Application	Reference
Fucus vesiculosus	Fucoidans, fatty acids, polyphenols	Anticancer and anti-angiogenic potential, bactericidal and immunostimulating activities	(Bogolitsyn et al., 2017; Oliveira et al., 2019)
Laminaria digitata	Fucose, glucans	Antioxidant activities	(Garcia-Vaquero et al., 2018)
Laminaria ochroleuca	Fucoidans, phlorotannins, proteins, alginates	Antioxidant, antitumor	(Flórez-Fernández, Torres, González-Muñoz et al., 2019)
Sargassum muticum	Phlorotannins, phenolics, sulphated polysaccharides, alginates, glycolipids, fatty acids	Antioxidant, hydroxyl-radical scavenging activity, prebiotic activity	(Conde et al., 2015; Flórez-Fernández et al., 2019b; Pérez-Larrán et al., 2019; Rodrigues et al., 2015; Sánchez-Camargo et al., 2016; Terme et al., 2017)
Halidrys siliquosa	Phlorotannins	Sunscreen, antioxidant, and bactericide	(Le Lann et al., 2016)
Laminaria japonica	Fucoidans, phlorotannin	Antioxidant and antitumor activity	(Yuan et al., 2018; Zhao et al., 2018)
Himanthalia elongata (Linnaeus)	Protein	Food industry	(Garcia-Vaquero, Lopez-Alonso, & Hayes, 2017)
Sargassum turbinarioides	Alginates	Food and non- food application	(Fenoradosoa et al., 2010)
Ecklonia radiata	Polysaccharides and phlorotannins	Dietary supplements with pre-biotic potential	(Charoensiddhi et al., 2017)

Himanthalia elongata	Phenolic compounds	Antioxidants	(Rajauria, Foley, & Abu-Ghannam, 2016)
Nizamuddinia zanardinii	Fucoidans	Antibacterial and antiviral activity	(Alboofetileh et al., 2019)
Durvillaea potatorum	Fucoidans, alginates and laminarin	Different molecular weight distribution for various uses	(Abraham, Su, Puri, Raston, & Zhang, 2019)
Fucus evanescens	Fucoidans	Anticancer activity	(Hmelkov et al., 2018)
Silvetia compressa and *Ecklonia arborea*	Dietary fiber, total polyphenol	Food and nutraceutical industries	(Tapia-Salazar et al., 2019)
Padina pavonica, *Dictyopteris membranaceae* and *Cystoseira compressa*	Alginates	Gastroprotective activity	(Ammar et al., 2018)
Colpomenia peregrina	Alginates	Immunomodulatory and antioxidant properties	(Rostami et al., 2017)
Coccophora langsdorfii	Fucoidans	Anticancer activity	(Imbs et al., 2016)
Sargassum hemiphyllum	Fucoidans	Neuroprotective effects	(Huang, Kuo, & Chen, 2017)
Cystoseira trinodis	Fucoidans, alginates	Antioxidants	(Hifney et al., 2018)
Cystoseira abies-marina	Phlorotannin	Biological properties	(Sánchez-Camargo et al., 2016)

Laminarins are unique internal storage polysaccharides usually found within the cell vacuoles comprising of linear β-1,3 D-glucose units along with β-1,6-linkages which influences the water solubility of the compounds (Rioux, Turgeon, & Beaulieu, 2010). They are low molecular weight fractions of ~ 5 kDa and can account for up to 35% DW of the biomass, depending on the season, species and growth conditions (Kadam, Tiwari, & O'Donnell, 2015). Structural variations are dependent on the degree of polymerization (25) and the ratio of (1,3)- and (1,6)-glycosidic bonds. Laminarins have demonstrated good bioactive properties such as antibacterial, antioxidative and anticoagulant potential (Zhang & Row, 2015). Complex laminarin structures are stabilized by inter-chained hydrogen bonds thus rendering them resistant to hydrolysis in the upper gastrointestinal tract and are therefore classified as dietary fibers (O'Sullivan et al., 2010). The basic structural units of laminarin are shown in Fig. 2.

Fucoidans are sulfated polysaccharides comprising of (1,2) or (1,3) -linked α-L-fucopyranose residues along with other monomers such as galactose, mannose, xylose, glucose and glucuronic acid (Ale, Mikkelsen, & Meyer, 2011). These hetropolysaccharides are mostly found in the cell walls and the intercellular spaces (Mizuno et al., 2009). They are generally large macromolecules, with molecular weight ranges from 100 to 1600 kDa and are mostly soluble in water and dilute acids (Zhang & Row, 2015). The functional and bioactivity of these polysaccharides are influenced by their molecular weight, composition of the monosaccharide units, sulfate content and the position of the sulfate ester group and the extraction technique used for isolation of the compound (Hahn et al., 2012). Seasonal variations in terms of quantity and structure of fucoidans for brown macroalgae species have also been found (Fletcher, Biller, Ross, & Adams, 2017). Fucoidans have generated significant interest in recent times due to their broad spectrum of applications in cosmetic, food and

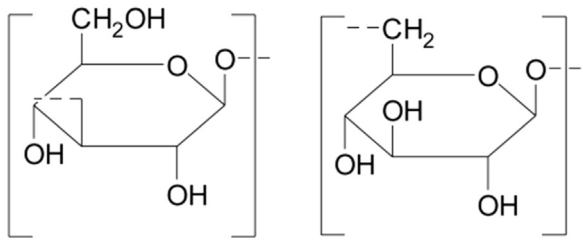

Fig. 2 Basic chemical units of laminarin, made up of β-(1,3) and β-(1,6) linked glucose (O'Sullivan et al., 2010) (Used under open access license CC BY 3.0).

the pharmaceutical industry (Fitton, Stringer, & Karpiniec, 2015). It has shown good bioactivities such as antiviral, anti-inflammatory (Lee et al., 2012), immunomodulatory, antithrombotic, anticoagulant (Wijesinghe & Jeon, 2012a), antioxidative (Hifney, Fawzy, Abdel-Gawad, & Gomaa, 2016; Yuan & Macquarrie, 2015; Zhao et al., 2018), antitumor (Wu et al., 2016), antibacterial, and anticancer activity (Ale, Maruyama, Tamauchi, Mikkelsen, & Meyer, 2011). The structure of fucoidan is shown in Fig. 3.

Alginates are linear unbranched polymers comprising of D-mannuronic (M) and L-guluronic (G) acid with β(1,4)-linkages. The monomeric unit arrangement sequence varies from homopolymeric blocks (MM and GG) to heteropolymeric blocks (MG or GM). Their molecular weight ranges from 500 to 1000 kDa. Alginate forms gels in presence of divalent cations (e.g. Ba^{2+}, Ca^{2+}, Mg^{2+}), and can stabilize emulsions which is a critical property finding widespread application in the food, textile and the cosmetic industry (Borazjani, Tabarsa, You, & Rezaei, 2017; Flórez-Fernández et al., 2019a; O'Sullivan et al., 2010). The gel properties in terms of strength and viscosity of the alginate solution is influenced by the concentration and the length of the polymer chains, and the ratio of G:M monomers (Lee & Mooney, 2012). Higher guluronic acid concentrations imparts greater strength to the gels while mannuronic acid and heterogenous blocks (MG or GM) forms weak but more flexible gels (Costa et al., 2018). Alginates also finds application in the biomedical field in wound dressing, cell immobilization and is a viable tool for drug delivery due to favourable properties like biocompatibility, biodegradability and non-toxicity (Lee & Mooney, 2012). The different building blocks of alginate are shown in Fig. 4.

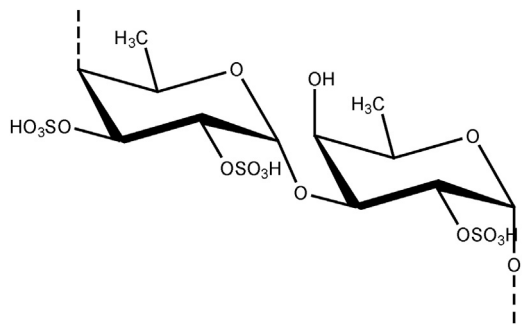

Fig. 3 Chemical structure of fucoidan (Thomas & Kim, 2013) (Used under open access license CC BY 3.0).

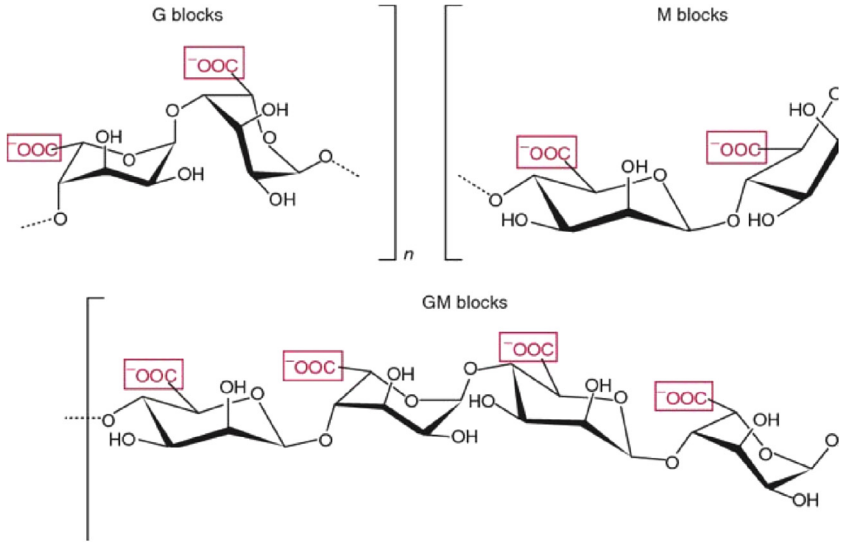

Fig. 4 Brown seaweed alginates showing units of (A) poly-D-glucoronic acid (G blocks), (B) poly-D-mannuronic acid (M blocks), and (C) alternate D-glucoronic and D-mannuronic acid (GM block) (Vera, Castro, Gonzalez, & Moenne, 2011) (Image reproduced under open access license CC BY 3.0).

4.2 Polyphenols

Polyphenols in macroalgae comprise of phenolic acids, flavonoids, tannins and phlorotannins. Brown seaweed polyphenols are known as phlorotannins which are formed by polymerization of phloroglucinol units and are unique to these species. Phlorotannins can be classified based on their monomer linkage of phloroglucinol units which are known as fucols, phlorethols, fucophlorethols and eckols (Isaza Martínez & Torres Castañeda, 2013). These hydrophilic compounds have molecular weights ranging between 126 Da and 650 kDa and its content can vary from 1 to 14% between species (Kadam et al., 2013). They reside in special vesicles (physodes), form an intricate linkage with alginates in the cell wall structure and impart protection against microbes and other environmental stressors (Pádua et al., 2015). Barbosa et al. (2019) have comprehensively reviewed the anti-inflammatory and anti-allergic potential of phlorotannins isolated from brown seaweeds. Polyphenols content in brown algae varies as a function of both taxonomy and geography (Magnusson et al., 2017) and can exhibit different bioactive and functional properties (Agregán et al., 2018; Li, Wijesekara, Li, & Kim, 2011; Puspita et al., 2017). The structure of some phlorotannins are shown in Fig. 5.

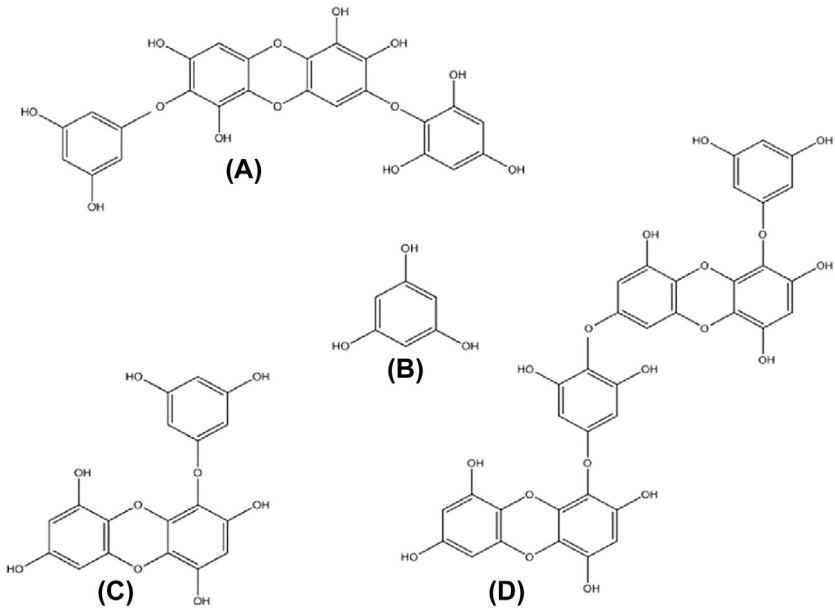

Fig. 5 Chemical structures of some phlorotannins: (A) diphlorethohydroxycarmalol; (B) phloroglucinol; (C) eckol; (D) dieckol (Thomas & Kim, 2013). *(Image reproduced under open access licence CC BY 3.0).*

5. Impact of physiological characteristics and seasonality on processing

The processing of seaweeds cannot be developed independently from the organisms physiology and seasonal variation in composition. Careful consideration of these factors during processing operations could contribute to improved scheduling of harvesting, and more optimal resource utilization. Seaweed growth and productivity are intricately tied to environmental and seasonally dependent factors like solar irradiance, nutrient availability, temperature, salinity and water movement. Seasonal variability thus plays a key role in seaweed physiological characteristics, as most of these factors change with seasons. Chemical composition of seaweeds has also been linked to the seasonal variability which culminates through a range of abiotic factors and nutrient synthesis (Marinho-Soriano, Fonseca, Carneiro, & Moreira, 2006).

Seasonal variations are furthermore different for the various compound classes found in brown seaweeds. Investigations of seasonality of fucoidan in three brown macroalgae species revealed significant difference in the

quantity and structure of fucoidans across species and seasons (Fletcher et al., 2017), with the highest fucoidan content being reported for the autumn months. Metabolomic and bioactivity profile study of *Fucus vesiculosus* as a function of seasonal variation revealed significant changes in compound concentrations, where phlorotannin content was highest in the summer months, while lipids and carotenoids increased over the winter months (Heavisides et al., 2018). Nutritional composition of the *Sargassum oligocystem* species was reported to be most favourable during the summer season, while highest anti-proliferative activity was found from samples from the monsoon months (Praiboon, Palakas, Noiraksa, & Miyashita, 2018). Significant seasonal variation in the total phenolics content and antioxidant capacity for the two brown macroalgae *Macrocystis pyrifera* and *Lessonia spicata* was found, with the higher concentration of phenolics reported during winter (Beratto-Ramos, Castillo-Felices, Troncoso-Leon, Agurto-Muñoz, & Agurto-Muñoz, 2019).

6. Seaweed biorefinery

Substantial improvements in resource utilization, environmental impact, and in some instances, processing profitability can be achieved through employing the biorefinery approach. The biorefinery concept involves integration of various sequential processing steps which enables maximum utilization of the seaweed biomass, by producing multiple products and minimizing residues. The focus is to extract maximum value through biomass fractionation and to produce a range of products that find application in different industries, including relatively higher value markets like cosmetics, food and pharmaceuticals, and in bulk industries like energy and platform chemicals (Milledge, Nielsen, & Bailey, 2016). A key aspect within a biorefinery approach is to preserve the structural integrity and thus bioactive properties of the higher-value components derived from seaweeds, which requires selection of appropriate extraction and purification steps.

Green processing methods that minimize or eliminate waste production, decrease energy requirements and avoid the use of carbon-intensive organic solvents may lead to substantial reduction of environmental impacts, and may also offer improved overall process efficiency (Balboa, Moure, & Domínguez, 2015). There are substantial advantages of using seaweed biomass for biorefineries compared to terrestrial biomass, including no

competition for agricultural land use and zero agricultural inputs like fresh water and fertilizer. Development and optimization of green seaweed processing methods and implementing these within a traditionally carbon-intensive processing sector can substantially improve sustainability (Ibañez & Cifuentes, 2013). However, the commercial success of seaweed based biorefineries will be dependent on the financial viability, which in turn will be determined by the specific biomass processed, processing efficiency and the market worth of the product spectrum followed by the associated logistics of running a large scale industrial facility (Jung, Lim, Kim, & Park, 2013; Suganya et al., 2016; Trivedi, Gupta, Reddy, & Jha, 2013).

Despite these potential advantages, empirical evidence on the economics associated with seaweed biorefineries is extremely limited, as is the important associated components such as quantitative environmental footprint data, sustainability and scale-up feasibility of newer and so-called green processing techniques. Balboa et al. (2015) valorized *S. muticum* under a biorefinery concept to recover six different products and favored the integration of greener techniques. A cascading biorefinery process targeting ulvans along with seaweed salts and proteins were defined by Glasson, Sims, Carnachan, de Nys, and Magnusson (2017) investigating eight biorefinery processes. Flórez-Fernández, Torres, González-Muñoz et al. (2019) proposed a biorefinery approach by using autohydrolysis of *S. muticum* biomass enabling simultaneous extraction of alginate, fucoidan and phlorotannin fractions. Charoensiddhi et al. (2018) did a techno-economic case study for a simulated industrial scale production of functional food from *Ecklonia radiata* and demonstrated economic feasibility with a best-case scenario showing a payback time and net present value (NPV) of 1.1 years and US$ 89.43 m respectively.

7. Conclusion and future perspectives

Seaweed biomass as a renewable feedstock has gained significant attention in the quest to move away from a fossil fuel-based economy. Macroalgal biomass can play an important role in providing food and energy products in future. Efforts toward commercial cultivation of brown seaweeds are making substantial progress, and brown seaweed biomass can be particularly suited to build processing operations around due to high yields and the range of unique compounds that it contains, with widespread application in the food, cosmetic, pharmaceutical and agricultural industries.

The economic success of future seaweed processing enterprises is likely to depend on how successfully such enterprises can move away from environmentally unsustainable processing operations. For this reason, the development of so-called green processing methods that are less energy and resource intensive, produce less waste and avoid utilization of high carbon footprint organic solvents are particularly desirable. Substantial progress has been made toward adapting technologies developed for terrestrial biomass processing for seaweed processing, and individual technologies are being optimized mostly at the proof-of concept and laboratory demonstration stages. The concept of integrated seaweed biorefineries needs to be demonstrated beyond just modeling investigations in order to decrease technical risk for investors, and to demonstrate the technical viability of integrated approaches.

A major shortcoming in especially enzyme-assisted seaweed processing is the lack of commercially available enzymes that have been tailored to seaweed biomass. While it is acknowledged that enzymatic processing is relatively mild and environmentally friendly, currently available commercial enzyme preparations are invariably tailored to terrestrial biomass and therefore the seaweed cellular matrix thus presents a unique challenge to efficient processing. In brown seaweeds, the gelling properties of alginate can lead to mass transfer limitations during extraction, as well as difficulties in downstream processing. There is therefore a distinct need for enzymes which are more specific to substrates found in seaweed, which could improve extraction efficiency during seaweed processing and thus lead to improved economic performance of such operations.

Substantial future research is required to further sustainability and economic performance of processing of the various brown seaweeds around the world. The integration and coupling of different extraction and downstream processing methods need to be optimized, and the impact of processing steps needs to describe impacts on bioactivity-structure relationship of target compounds. Furthermore, taking into account physiological characteristics and seasonality in the composition of the raw material is important to ensure appropriate scheduling of harvesting and processing operations. Pertaining to the biorefinery concept, there is a scarcity of data regarding the downstream processing stages post-extraction and their associated efficiencies, which increases the uncertainty around technical feasibility and techno-economic analysis of current seaweed biorefinery research.

Overall, brown seaweed biomass has been highlighted as being a potentially sustainable biomass feedstock for the future. If processing technologies

can be optimized and implemented successfully, the processing of brown seaweed can contribute significantly toward the development of a strong bio-based economy.

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