



Advances in pre-treatment techniques and green extraction technologies for bioactives from seaweeds

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ABSTRACT

Background: A wide range of conventional and non-conventional technologies have been employed to extract a wide range of bioactive compounds from the complex matrices of seaweeds. Green extraction technologies are increasingly employed to improve extraction efficiencies.

Scope and approach: The objective of this review was to outline various approaches employed for the extraction of bioactives from seaweeds. This review covers various pretreatment methods generally employed prior to extraction, and their combinations with conventional and green extraction technologies. Novel technologies which can be employed with or without pretreatments to improve existing processes are also discussed.

Key findings: The role of pretreatments is of utmost importance and have significant impacts on the quality and quantity of target compounds. Combinations of different cell disruption technologies and extraction methods can enhance the extractability of compounds with higher purity and contribute towards improved process efficiency.

1. Introduction

Over the last decades, there has been an increased awareness of the impact of diet on health, which has led to various changes in diet and the development of functional foods, which are capable of providing health benefits beyond their nutritional value (Nowak, Livney, Niu, & Singh, 2019). The globalization of the food industry has seen a rise in demand for functional foods to meet the needs of the consumers (Adadi, Barakova, Muravyov, & Krivosheina, 2019). The revenue generated worldwide by the functional food market in 2019 was about 175 billion U.S. dollars and is projected to reach 275 billion U.S. dollars by 2025 (Shahbandeh, 2019).

Functional foods are defined as whole, fortified, or enriched with bioactives foods that provide health benefits beyond essential nutrition (e.g. vitamins, minerals), when consumed at sufficient levels as a part of a regular diet (Diplock et al., 1999). Bioactive compounds play a pivotal role in the development of functional foods. Bioactive compounds are essential and nonessential compounds (e.g., vitamins or polyphenols) that occur in nature which can be shown to affect human health (Biesalski et al., 2009). A range of bioactive compounds can be obtained

from both terrestrial and marine plants for a wide range of functional food applications (Chakraborty et al., 2018; Qin, 2018).

For example, deep-coloured vegetables including carrot, red beet-root, eggplant (Vinson, Hao, Su, & Zubik, 1998), mangrove trees (Dahibhate, Saddhe, & Kumar, 2019), tea (da Silva et al., 2017) and berry fruits (Szajdek & Borowska, 2008) are rich in bioactive compounds which display strong antioxidant capacity.

Among marine plants, seaweeds contain many bioactive compounds and functional carbohydrates including carrageenan, terpenoids, polyunsaturated fatty acids, sulphated polysaccharides and fucoidan (Smit, 2004).

These secondary metabolites display a wide range of bioactivities including antioxidant, antidiabetic, anticancer, anti-HIV, antiviral, anticoagulant, anti-inflammatory and cardiovascular protection. Bioactive compounds from seaweeds are considered to be natural and safe, and have potential application in nutritional supplements or therapeutic agents (Khalid, Abbas, Saeed, Bader-El-Ain, & Suleria, 2018).

A key challenge faced in obtaining bioactives from seaweed is the low recovery rates for these compounds, which is further limited by the

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rigidity of the seaweed matrix which retards the release of bioactive substances (Poojary et al., 2016). The composition of the cell-matrix also has a key effect on the disruption efficiency and yield of the functional compounds (Čikoš, Jokić, Šubarić, & Jerković, 2018). Selection of an appropriate pretreatment or cell disruption technique is dependent on the target bioactive compound and seaweed matrix. To overcome these challenges, a suitable pretreatment method before extraction or the application of novel extraction technologies can be employed to enhance the recovery of target compounds.

A biorefinery approach is required to achieve sustainable exploitation of seaweeds, and convert the seaweed biomass into a wide range of high value-added products which can be further exploited by the pharmaceutical and allied sectors (Serive, Kaas et al., 2012). Multiple bioactive compounds such as fucoxanthin, zeaxanthin, fucoidan, violaxanthin, laminarin, phlorotannins, lutein, glycoprotein etc can be obtained from seaweeds (Bikker et al., 2016).

Temperature sensitive bioactives such as carotenoids or polyphenols extracted from seaweeds must be carefully handled during downstream processing to ensure that the process does not have any negative effects on their functional properties.

This review considers the relevance of pretreatments and novel technologies to enhance the extraction of bioactives from seaweed, and outlines the range of unit operations involved in extraction processes including pre-treatment techniques.

2. Extraction of bioactive compounds

Naturally occurring bioactive compounds are synthesized in small amounts and are extracted along with other compounds during extraction, which makes their subsequent separation and purification time consuming and labour intensive (Lam, 2007). These compounds are generally embedded in the cellular matrices along with macromolecules (e.g. protein, fibre) and are difficult to extract. Extraction is a mass transfer process which is mainly dependent on the accessibility of target bioactive compounds to the solvent. Extraction involves diffusion of the solvent into the matrix, followed by the dissolution of bioactive compounds into the solvent, and separation of bioactive compounds from the solvent. Strategies adopted to enhance extraction yields with intact biological activities are well documented and include the use of classical and novel disruption techniques. Various cell disruption methods including mechanical, thermal and/or chemicals are used to enhance the mass transfer and thereby enhance the extraction yield (Romero-Díez et al., 2019).

Conventional extraction methods employed depend on the characteristics of the solvent used (viscosity, polarity, surface tension, dipole moment and dielectric constant), thermal treatment and mechanical agitation/mixing. These methods include Soxhlet, hydrodistillation, maceration (Azmir et al., 2013), infusion, digestion, decoction and percolation (Belwal et al., 2018) which may involve an alcohol-water mixture or non-polar solvent (Wang & Weller, 2006). The extraction method employed affects the qualitative (e.g. biological activities) and quantitative (e.g. yield) characteristics of bioactive compounds. Thus, it is critical to select the most appropriate solvent and extraction technique based on the target bioactive compound and proposed end application (Table 1).

It is desirable to use safe, affordable, and ecological extraction techniques to extract bioactive compounds sustainably and efficiently. This will not only enhance yields with minimal impact on the quality of end product but also comply with clean label requirements (Kadam, Tiwari, Smyth, & O'Donnell, 2015). It is also important that only food grade solvents are used if the target bioactive compounds are to be used for functional food applications. The use of green solvents obtained from renewable resources has been proposed to replace hazardous solvents (e.g. petroleum derived solvents). These solvents include water, subcritical and supercritical fluids, deep eutectic solvents and ionic liquids (Gomez et al., 2020, p. 116784).

Use of green solvents and novel extraction technologies has led to the development of the concept of green extraction, which is based on the discovery and design of extraction processes which will reduce energy consumption, allows the use of alternative solvents and renewable natural products, and ensure a safe and high quality extract/product (Chemat, Vian, & Cravotto, 2012).

Several novel extraction technologies, including microwave-assisted extraction (MAE), ultrasound assisted extraction (UAE), enzyme-assisted extraction (EAE), supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) have been employed for the extraction of a range of bioactive compounds in food as well as in the pharmaceutical applications (Kadam, Tiwari, et al., 2015).

These technologies facilitate the elimination or reduction of the use of toxic chemical solvents, enhance extraction efficiency as well as yield and quality of the extract obtained. They also reduce the extraction time and are less energy intensive. These novel extraction technologies can be classified as physical, chemical, biological and combinations of same (e.g. biochemical) as shown in Fig. 1. For example, physical extraction techniques include pretreatments such as milling, drying, puffing/extruding and mechanical pressing, followed by extraction processes such as heating, ultrasonication, microwave assisted extraction, sub- and supercritical fluid extraction and pressurized liquid extraction. Chemical extraction techniques include the use of organic and inorganic solvents, ionic liquids, etc while biological extraction involves the use of enzymes and microorganisms.

3. Seaweeds as a source of bioactive compounds

Seaweeds have been widely used as a functional food and medicinal herbs particularly in Asian countries (Liu, Heinrich, Myers, & Dworjanyn, 2012), however their potential importance has increased over the over recent decades due to global population growth and food security becoming an emerging issue (Rao & Mantri, 2006). The world production of seaweed has grown exponentially over the last 50 years (Loureiro, Gachon, & Rebours, 2015). Seaweeds are increasingly employed in the biomedicine and agri-food industries as they are a rich source of bioactive compounds including carotenoids, proteins, peptides, vitamins, minerals, oxylipins, phlorotannins, steroids, minerals, essential fatty acids, dietary fibres, polysaccharides and sulphated polysaccharides (Venkatesan et al., 2019). Dietary antioxidants help in reducing oxidative damage and chronic disease risks related to them, and also interferes with signal transduction regulation at various levels including inhibiting oncogenes, activating cancer cell death also known as apoptosis, decreasing inflammation, inhibiting angiogenesis and modulating hormone or growth factor activities (Russo, 2007).

Seaweeds are a good source of antioxidants (Nagai & Yukimoto, 2003). The main potential antioxidant compounds identified in seaweeds include pigments (astaxanthin, carotenoids, fucoxanthin) and polyphenols (phenolic acid, flavonoid, tannins, etc), which are known for their high antioxidative activities (Siriwardhana et al., 2004). Phenolic compounds are among the most abundant secondary metabolites and well-studied antioxidants, *in vivo* and *in vitro* in terrestrial plants and exhibit antioxidant activities by inducing antioxidant enzymes and by scavenging radicals (Kadam, Tiwari, & O'Donnell, 2013). They along with carotenoids, vitamins C and E, are referred to as antioxidants, and protect against oxidative stress and associated pathologies such as inflammation, cancer and coronary heart disease (Tapiero, Tew, Ba, & Mathe, 2002). Phlorotannins are another important bioactive compound found in seaweeds which are 10–100 times more stable and potent antioxidants than any other polyphenols (Namvar et al., 2012).

4. Extraction process

Recently use of new extraction technologies at various extraction stages has been reported. The stages at which these technologies are employed have a strong effect on extraction time, energy consumption,

Table 1

Extraction of seaweed target compounds using various mechanical cell disruption techniques.

Target Compound	Source (seaweed)	Extraction technology	Extraction solvent (optimised extraction condition)	Methodology	Result	Reference
Bead mill						
Protein	<i>Ulva sp.</i> and <i>Gracilaria sp.</i>	Bead mill	Buffer	Milling: 3 cycles of 60 s at 6500 rpm, breaks (120s) between cycles	High antioxidant activity shown by protein concentrates	Kazir et al. (2019)
High pressure Fucoidan	<i>N. decipiens</i>	High pressure homogenization and hydrothermal extraction process	Distilled water	1000g seaweed + water (1:15), subjected to high pressure homogenization at 40, 70 and 100 MPa, followed by extraction (70 °C for 30 min)	Fucoidan recovered by 70 and 100 MPa showed higher antioxidant activity than conventional method extracts	Li et al. (2017)
Hydrothermal liquefaction Mannitol and laminarin	<i>L. saccharina</i>	Hydrothermal liquefaction	Water	25 °C min ⁻¹ Biomass/water (5–20)%, 250–370 °C, Residence time 12–120min, Catalyst (0–100)% KOH	Max. bio crude (19.3%), obtained from 1:10 biomass-water ratio (350 °C), 15 min residence without catalyst. Sugars in aqueous phase included laminarin and mannitol	Anastasakis and Ross (2011)
Steam explosion Agar	<i>Gracilaria verrucosa</i>	Steam explosion	Water	90 °C, Multiple times	Extraction of agar was improved, and the agar showed low sulfate content and molecular weights	Talarico et al. (1990)
Agar	<i>Garcilaria dura</i>	Steam explosion	Water	Treatment with 0.1N HCl, neutralised with NaOH and washed with water to neutral pH. Steam explosion pretreatment: Algae soaked with 1M Na ₂ CO ₃ , steam explosion: 150 °C for 15sec. Extraction (95, 45 min, 0.05M phosphate buffer)	Agar extracted exhibited lower melting temperature, gel strength and apparent modulus of elasticity than native and alkali pretreated samples.	Murano et al. (1993)
Pulsed Electric Field Protein	<i>Ulva sp</i>	Pulsed Electric Field	Fresh biomass with water	PEF treatment at 247 kJ/kg, 50 kV (50 pulses), 70.3 mm electrode gap, 140 g fresh <i>Ulva</i>	7-fold increase in total protein extracted compared to osmotic shock samples	Robin, Kazir, et al. (2018)
Ultrasound Phenolics, uronic acid and fucose and	<i>A. nodosum</i>	Ultrasound	Concentration (0.03 M HCl)	740 W Ultrasonic probe Amplitude:114 µm, Extraction: 25 min, Acid: 0.03 M HCl	Efficient in extracting bioactive compounds	Kadam, Tiwari, et al. (2015)
Fucoidan	<i>Sargassum muticum</i>	Ultrasound	Water	Liquid: solid ratio 20:1, at 25 °C (RT), 5–30 min, 40 kHz, Intensity 1.5 A and 150 W	Fucose and sulfate content in extract increased during first 25 min of treatment, gave maximum antitumoral activity	Flórez-Fernández et al. (2017)
Carrageenan and alginates	<i>Sargassum binderi</i> and <i>Turbinaria ornate</i> <i>Kappaphycus alvarezii</i> and <i>Euchema denticulatum</i>	Ultrasound	Alginate: 2% NaOH Carrageenan: (water)	Alginate: 150 W ultrasound, algae/water ratio 10 g/l, 90 °C, pH 12, 30 min. Carrageenan: pH 7, 15 min	Extraction time decreased without affecting chemical structure and molar mass distribution	Youssef et al. (2017)
Phenolic and carbohydrates	<i>S. muticum</i>	EAE, UAE, Ultrasound-assisted enzymatic extraction (UAEE)	Enzymes in 0.1 M phosphate/0.1 M acetate buffer	EAE: Enzymes in buffer solution. 50 (v/w) (L/s) UAEE: 60% amplitude, (400 W, 24 kHz) Power discharges: 5 min and off periods of 25 min, on the buffer with or without enzyme	UAEE was better than EAE in extracting phenolics and increased antioxidant activity of extract. (UAE) more efficient in enhancing the total extraction yield and selective phenolic extraction than EAE.	Casas et al. (2019)

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Table 1 (continued)

Target Compound	Source (seaweed)	Extraction technology	Extraction solvent (optimised extraction condition)	Methodology	Result	Reference
Bead mill						
Polyphenols, phlorotannins and antioxidants	<i>Fucus serratus</i> , <i>Fucus vesiculosus</i> , <i>Fucus spiralis</i> , <i>H. elongata</i> , <i>Halidrys siliquosa</i> , <i>Laminaria digitata</i> , <i>L. saccharina</i> , <i>Laminaria hyperborea</i> , <i>A. nodosum</i> , <i>Alaria esculenta</i> and <i>Pelvetia canaliculata</i>	UAE and conventional extraction method	30, 50 and 70% ethanol	Optimisation using <i>F. vesiculosus</i> , ultrasound conditions 35 and 130 kHz, 30, 50 and 70% ethanol, for 10 and 30 min. Optimised conditions used for all 11 seaweeds and compared with solvent extraction method.	Optimised conditions (35 kHz, 30 min and 50% ethanol). Significant improvement in extraction yield (1.5-fold–2.2-fold) in all seaweeds compared to conventional extraction	
Fucose sulphated polysaccharides, total soluble carbohydrate and antioxidants	<i>A. nodosum</i>	UAE, MAE or UMAE	Maceration with 0.1 M HCl for 10 min	UAE (500 W, 20 kHz), MAE (2450 MHz) or UMAE (US; 500W, 20 kHz and MW 2450 MHz) for 2 and 5 min	Maximum yields of compounds achieved using UMAE	(Garcia-Vaquero et al., 2020)
Fucose and glucan	<i>L. digitata</i> , <i>L. hyperborea</i> and <i>A. nodosum</i>	Ultrasound assisted extraction	0.1 M HCl (1:10, w/v) for time (10 min)	Power 500 W, 20 kHz, 76 °C, 10 min, 100% amplitude	UAE was found to enhance the yield of polysaccharides and its antioxidant activities. UAE was more efficient than conventional extraction in terms of higher TPC and antioxidant activities.	Garcia-Vaquero, Rajauria, Tiwari, Sweeney, and O'Doherty (2018)
Phenolics and antioxidant activity	<i>Hormosira banksii</i>	UAE	70% ethanol, solvent:sample 50 (ml/g)	50 Hz, 220 V and 250 W. Optimum conditions: 30 °C, 60% power for 60 min, 150 W.	UAE was more efficient than conventional extraction in terms of higher TPC and antioxidant activities.	Dang et al. (2017)
Microwave Fucoidan	<i>A. nodosum</i>	Pre extraction with ethanol followed by Microwave assisted extraction	0.1 M HCl	Microwave heating (120 °C), 15 min	Highest yield with optimum conditions. MAE was found to be faster and more efficient. MW 90 °C showed similar composition, DPPH scavenging as conventional. But has higher reducing power than conventional. Molecular weight and sulfate content of fucoidan increased with decreasing extraction time.	(Yuan & Macquarrie, 2015c)
Fucoidan	<i>F. vesiculosus</i>	MAE	Distilled water	MAE in digestion oven model (MDS-2000) 120 psi, 1 min and 1/25 g/mL (alga/water)	MAE short extraction time and use of non-corrosive solvents, resulting in reduced costs	Rodriguez-Jasso, Mussatto, Pastrana, Aguilar, and Teixeira (2011)
Phlorotannin and antioxidant	<i>Ecklonia radiata</i>	Microwave assisted enzymatic	Buffer solution	Microwave-assisted Viscozyme extraction for 5–30 min	Extraction time (5–30 min), most effective process. High phlorotannins contents and antioxidant activities	Charoensiddhi et al. (2015)
Fucoidan	<i>Nizamuddiniazanardinii</i>	Viscozyme, alcalase, cellulase, flavourzyme, ultrasound, microwaves, subcritical water, alcalase-ultrasound (EUAE), and simultaneous ultrasound-microwave (UMA) and conventional hot water extraction.	Water	Subcritical water (1500 W (150 °C), SWE, 10 min runs (2)	Highest fucoidan yield by SWE, lowest yield by UAE. Antibacterial assays: fucoidans extracted by microwave & subcritical water inhibited <i>E. coli</i> . Growth. Fucoidans extracted from enzyme-US, US-microwave and subcritical water showed inhibition against <i>P. aeruginosa</i> (2 mg/mL)	Alboofetileh, Rezaei, Tabarsa, Rittà, et al. (2019)
Sulphated polysaccharides	<i>Ulva prolifera</i>	Microwave assisted hydrothermal extraction	Aqueous solution with different HCl concentrations	2.45 GHz, 500 W, 120 °C, 0.01 M HCl for yield	Molecular weight and chemical composition were influenced. Polysaccharides extracted (90 °C, 0.05 M HCl) had best water-holding and oil-	Yuan et al. (2018)

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Table 1 (continued)

Target Compound	Source (seaweed)	Extraction technology	Extraction solvent (optimised extraction condition)	Methodology	Result	Reference
Bead mill						
Phytosterols and phytol	<i>Undaria pinnatifida</i> and <i>Sargassum fusiforme</i>	Microwave assisted extraction	Saponification using ethanolic solution of KOH	1.5 mol/l ethanolic KOH, 2g homogenised sample	holding capacity. 0.05 M HCl, 150 °C: best foaming properties 0.1M HCl, 150 °C: highest antioxidant activity Microwave was found to be an efficient extraction method. MW and high speed counter current chromatography combination was efficient in separation and purification of compounds. MAHE resulted in reduction of treatment time, without extracting agents. By altering the extraction temperature, the viscosity and molecular weight of polysaccharides can be controlled.	(X.-H. Xiao, Yuan, & Li, 2013)
Sulphated polysaccharides	<i>Ulva spp.</i> and <i>Monostroma latissimum</i>	Microwave assisted hydrothermal extraction (MAHE)	Distilled water	1/20 sample to solvent ratio, Microwave: 2.45 GHz, Thermal history based on 4 min come up time, extraction time 10 min, temp 100–180 °C.		Tsubaki, Oono, Hiraoka, Onda, and Mitani (2016)
Subcritical water Fucoidan	<i>N. zanardinii</i>	Subcritical water	Subcritical water	29 min extraction, 150 °C, and 21 g/mL (material to water)	Higher yield of fucoidan than conventional method. Fucoidan showed appropriate antioxidant, immunomodulatory and anticancer activity	Alboofetileh, Rezaei, Tabarsa, You, et al. (2019)
Polysaccharides (alginate and fucoidan)	<i>S. japonica</i>	SWE + DES	DES- water solution	150 °C, 36.81 mL/g L/s ratio 70% water content, 19.85 bar.	High alginate and fucoidan yield	Saravana, Cho, Woo, and Chun (2018)
Phenolics	<i>S. japonica</i>	Ionic liquid-assisted subcritical water (IL + SWE)	0.25 M [C4Clim] [BF4] solution in distilled water	0.25 M solvent, 175 °C, 50 bar, extraction time 5 min	Antioxidant activity was enhanced in SWE + IL, being correlated to phenolics. SWE + IL showed enhancement in extraction Quantity and quality of phenolics in Subcritical water extraction + Ionic liquid and Subcritical water extraction higher than Solid liquid extraction	Dinh et al. (2018)
Carrageenan	<i>K. alvarezii</i>	Ionic liquid assisted subcritical water extraction	1% ionized liquid or distilled water	Pressure 5 MPa, temperature (60–180 °C), 1% 1- butyl-3methylimidazolium acetate, 1/80 g/ml	High yield, Gel strength and viscosity minimal, emulsification index higher than SWE and conventional. Antioxidant activity of sample by SWE + IL was low due to low sulfate content	Gereniu et al. (2018)
Polysaccharides (alginate and fucoidan)	<i>S. japonica</i>	SWE + DES	DES- water solution	150 °C, 19.85 bar, 70% water content, 36.81 mL/g L/s ratio	High alginate and fucoidan yield	Saravana et al. (2018)
Pressurized liquid extraction Fucoidan	<i>S. japonica</i>	Pressurized liquid extraction	Water or sodium hydroxide or ethanol	140 °C temperature and 50 bar pressure, 0.1% sodium hydroxide	Increased crude fucoidan yield. Extracts showed antioxidant activity, radical scavenging activity and good emulsion stabilizing properties	Saravana et al. (2016)
Proteins	<i>Porphyra umbilicalis</i> , <i>Ulva lactuca</i> and <i>Saccharina latissima</i>	a) Sonication b) pH-shift protein extraction c) accelerated solvent extraction (ASE) to extract lipids and	a) Water b) Water c) 70% food grade acetone in water	a) 1-h sonication, followed by stirring and protein precipitation by ammonium sulfate b) sample to water 1:6 (w/v), homogenization,	pH-shift method showed highest protein concentration.	Harrysson et al. (2018)

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Table 1 (continued)

Target Compound	Source (seaweed)	Extraction technology	Extraction solvent (optimised extraction condition)	Methodology	Result	Reference
Bead mill						
		phlorotannin and carbohydrates before protein		milling, pH adjustment to 12, centrifugation.		
				c) for lipids, phlorotannin and carbohydrates: 1000 psi and 0 °C. Extraction for 1 cycle of 7 min for proteins: 50% methanol-water, 1500 psi, 37 °C, 2 cycles of 5 min		
Antioxidant	<i>A. nodosum</i> , <i>F. vesiculosus</i> , <i>F. serratus</i>	Accelerated Solvent extraction, using different solvents	80% ethanol/ 20% H ₂ O	100 T (°C)/6.9 P (MPa). Static mode of extraction. Sample dispersion: Silica (sample: silica ratio1:3 (w/w)) and diatomaceous earth	Ascophyllum extracts (80% aqueous ethanol), gave highest antioxidant potential, based on ability to protect against oxidant-induced DNA damage	O'Sullivan et al. (2013)
Polyphenol	<i>F. serratus</i> , <i>G. gracilis</i> , <i>C. fragile</i> , <i>L. digitata</i> ,	Solid- liquid extraction, PLE	Cold water	Cold water, shaker 24 h, filtered twice	SLE with Cold water extracts showed max TPC from <i>F. serratus</i> . The antioxidant activity and TPC for Solid liquid extraction were greater than Pressurized Liquid Extraction using same solvents. SLE was better in yield obtained, low capital cost and ease. <i>F. serratus</i> showed best yields.	Heffernan, Smyth, FitzGerald, Soler-Vila, and Brunton (2014)
Fucoidan	<i>S. muticum</i>	Hot, compressed water (hydrothermal processing)	Water	170 °C, 30:1 (w/w, dry basis) liquid/solid	Hot water processing-subcritical conditions: effective, gave simultaneous extraction, depolymerization of fucoidans. Fucoidan and sugar content decreased with the temperature	(E. Balboa, Rivas, Moure, Domínguez, & Parajó, 2013)
Fucoidan	<i>Sargassum glaucescens</i>	Compressional puffing hydrothermal extraction	Hydrothermal extraction: Double distilled water (w/v 1:10)	Puffed samples, after removal of protein, pigments and lipids were given Hydrothermal extraction: Double distilled water (w/v 1:10), 80 °C for 1 h	Compressional puffing disrupted cellular structure and enhances extraction with hot water. It was simple and the samples showed antioxidant activity. Fucoidan yield found to be more than conventional method	Huang et al. (2016)
Isoflavones	<i>S. vulgare</i> , <i>Porphyra sp.</i> , <i>Undaria pinnatifida</i> , <i>Sargassum muticum</i> , <i>Chondrus crispus</i> , <i>Hypnea spinella</i> and <i>Halopytis incurvus</i> ,	Sonication pretreatment followed by supercritical CO ₂ fluid extraction.	SFE modifier (MeOH: H ₂ O 1:9, v/v)	US pretreatment for 30 min. SFE: 35 MPa, 40 °C for 60 min	Sonication pretreatment led to higher recovery.	Klejdus, Lojková, Plaza, Šnoblóvá, and Šterbová (2010)
Enzymatic extraction Phlorotannin	<i>S. muticum</i>	Enzymatic pretreatment Pressurized liquids	Alcalase and viscozyme enzyme Water and ethanol sonicated for 10 min	- Alcalase: 50 °C, 7.0 pH, 0.1 M phosphate buffer - Viscozyme enzyme 50 °C, 4.5 pH, 0.1 M sodium acetate-acetic acid buffer, for 2 or 4 h. PLE: static extraction time: 20 min, 1500 psi; 120 °C; extraction solvent (75:25 ethanol: water) (v/v).	PLE alone gave highest yields. Viscozyme, 2 h with pressurized liquids, gave higher antioxidant rich extracts compared to PLE alone. Optimum conditions were 160 °C, Pressurized solvent: 95% ethanol	del Pilar Sánchez-Camargo et al. (2016)
Fucoanthin	<i>U. pinnatifida</i>	Enzyme pretreated followed by Diethyl ether and ethanol as co solvent	Water	Fresh (wet) seaweed Enzyme pretreatment	Extraction yield increased with enzyme pre-processing. Enzyme pretreatment followed by removal of	Billakanti et al. (2013)

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Table 1 (continued)

Target Compound	Source (seaweed)	Extraction technology	Extraction solvent (optimised extraction condition)	Methodology	Result	Reference
Bead mill					water-soluble compounds from hydrolysed seaweed by centrifugation prior to DME doubled the throughput. Lipids rich in w-3 and w-6 polyunsaturated fatty acid were generated. The DME + ethanol co solvent extraction resulted in high yields.	
Fermentable sugars	<i>Enteromorpha</i> sp.	Enzymatic degradation	Various acid	Nitric acid, dilute sulphuric acid, steam flashing, pretreatment followed by enzymatic degradation	Enzymatic hydrolysis was found to be efficient	Nahak, Nahak, Pradhan, and Sahu (2011)
Supercritical carbon dioxide extraction Fucoxanthin, phenolic compounds	<i>S. horneri</i> and <i>S. japonica</i>	SC-CO ₂ with EtOH as Co-Solvent	Ethanol as co solvent	45 °C, 250 bar, CO ₂ flow rate: 27 g/min, extraction: 2 h. 96% Ethanol, as a co-solvent, 1 mL/min flow rate	SC-CO ₂ extraction was efficient in extracting high yields (oil, FAs, and fucoxanthin content, phenolic compounds) Oil from SC-CO ₂ , exhibited strong antioxidants, antimicrobial, phenolics, and antihypertensive activities. Oil obtained from <i>Sargassum horneri</i> via SC-CO ₂ , gave high fucoxanthin yields and better biological activities compared to <i>S. japonica</i> .	Sivagnanam et al. (2015)
Fucoidan	<i>Saccharina japonica</i> and <i>Sargassum oligocystum</i>	Co solvents using supercritical CO ₂	Ethanol as co-solvent	Pressure = 550 bar, Temperature = 60 °C, 5% ethanol as co-solvent	Supercritical CO ₂ with 5% ethanol gave an improved yield of fucoidan	Men'shova et al. (2013)
Fucoxanthin and phlorotannin, carotenoids	<i>S. japonica</i>	Co solvents using supercritical CO ₂	Sunflower oils	Fucoxanthin and carotenoids: 50.62 °C, 300 bar, 2% Sunflower oil Phlorotannin: 2% water, 48.94 °C and 300 bar and	Vegetable oil and water addition as co solvent, enhanced efficiency of SC CO ₂ . Sunflower oil was found be most effective in extracting carotenoids and fucoxanthin, while water improved yield of phlorotannin. Oil obtained via SC CO ₂ and sunflower oil showed high antioxidant activity and stability and fatty acids. Oil rich in bioactives was obtained	Saravana et al. (2017)
Fucoxanthin, alginate, phlorotannin and fucoidan	<i>S. muticum</i>	SFE		45 °C and pressure was set at 10 and 35 MPa, flow rate of 25 g CO ₂ min ⁻¹	Enhanced purity of extracts and fucoxanthin yield	(E. M. Balboa et al., 2015)

yield and bioactivity/functionality of the target compound. The use of extraction technologies as a pretreatment of seaweed biomass or as the main extraction technique alone or in combination with conventional or other novel technology with and without green solvents is shown in Fig. 2.

4.1. Pretreatment techniques

Pretreatment of biomass is one of the most common but least investigated unit operation and is often considered as an extraction technique. Pre-treatments have a crucial role in the extraction of

compounds and bioconversion processes (Michalak & Chojnacka, 2014). Pretreatments of biomass have been reported to enhance the availability of target compounds in extraction of bioactives (Billakanti, Catchpole, Fenton, Mitchell, & MacKenzie, 2013), microbial hydrolysis for biogas production (Thompson, Young, & Baroutian, 2019) and the production of fermentable sugars (Yun et al., 2016). Several conventional pretreatment techniques including physical, chemical and biological, and application of emerging technologies to disrupt the cell matrix and to facilitate mass transfer are outlined below.

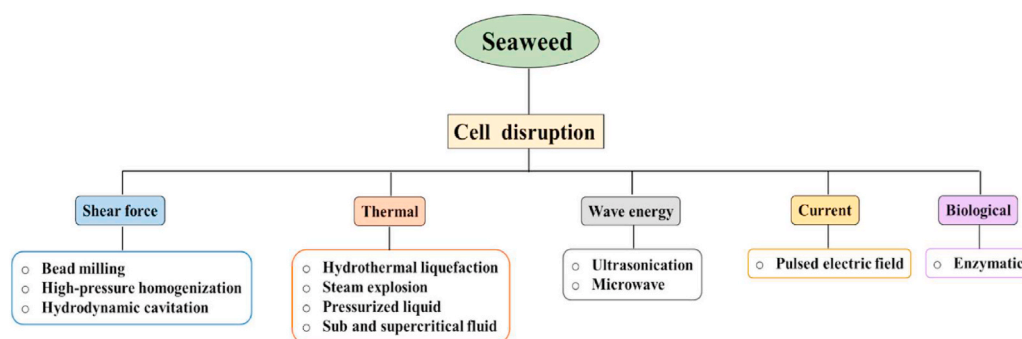


Fig. 1. Classification of cell disruption methods employed in seaweed applications.

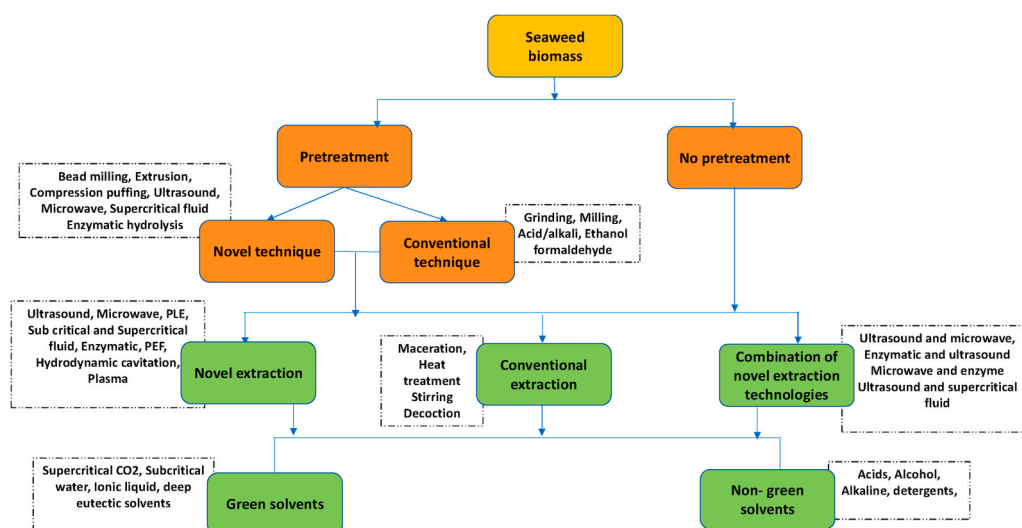


Fig. 2. Overview of extraction processes for extraction of seaweed bioactive compounds.

4.1.1. Conventional pretreatment techniques

Conventional physical pretreatment methods including hot air drying and milling are generally employed to modify the permeability of the cell membranes and accelerate mass transfer in seaweed. Drying not only helps in the storage and transportation of the seaweeds but also impacts the extractability of bioactive compounds and their quality. The most commonly employed drying methods include solar drying, hot air drying, and freeze drying. However drying requires significant amounts of energy and may cause losses of certain valuable compounds and nutritional attributes (Chemat, Rombaut, Meullemiestre, et al., 2017). Chan, Cheung, & Ang (1997) reported that the various methods of drying including solar drying, oven drying and freeze drying greatly affected the nutritional composition (amino acids, vitamin C, minerals and fatty acids) of *Sargassum hemiphyllum*. Another study reported that different drying temperatures had an impact on the phytochemicals present in *Himanthalia elongata* (Gupta, Cox, & Abu-Ghannam, 2011). Many similar studies highlight the effects of the drying methods employed and temperature profile on the composition of seaweeds.

Chemical pretreatments using acids, salts and surfactants have been employed for disruption of seaweed cell walls followed by solvent assisted extraction. For example most fucoidan extraction processes involve a pretreatment using ethanol to remove pigments, proteins, mannitol and some salts (Yuan & Macquarrie, 2015b). Studies have also been reported for extraction of polysaccharides (García-Vaquero, Rajauria, O'doherty, & Sweeney, 2017) using alkaline pretreatment (Sasuga, Yamanashi, Nakayama, Ono, & Mikami, 2017), mild acid treatment (Sudhakar, Merlyn, Arunkumar, & Perumal, 2016) and formalin (Cajko, Novak, & Likozar, 2019).

Biological techniques including fermentation and the use of enzymes are widely used as a pretreatment for extraction. For example, fungi produce a range of extracellular enzymes that can breakdown seaweed polysaccharides into mono and oligosaccharides. A study on fungal fermentation of *Palisada perforata* (Rhodophyceae) and *Sargassum* seaweed species by Gomaa, Hifney, Fawzy, Issa, and Abdel-Gawad (2015) reported that along with the fungal growth on the macroalgae, certain enzymes such as fucodanase and alginate lyase were found with small amounts of protease and amylase. Enzymatic pretreatment of macroalgae (*Cystoseira trinodis*) using enzymes produced (fermentation broth) by *Dendryphiella arenaria* was shown by Hifney, Fawzy, Abdel-Gawad, and Gomaa (2018) to increase the recovery of low molecular weight fucoidan and alginate and also enhance the antioxidant potential.

4.1.2. Novel pretreatment techniques

Mechanical disruption methods alter seaweed cell structure and influence the extractability of target compounds. Mechanical disruption pretreatments lead to alterations of the biomass cell structure, increase the surface area and penetration of the solvent into the matrices. However, the use of harsh shear force, temperature and pressure conditions may not be suitable for extraction of certain valuable components and can lead to their degradation. Mechanical disruption pretreatments generally involve high energy input in the form of heat, pulses, waves, and shear force, however this increased energy input may result in higher extraction yields. Mechanical disruption pretreatments can be used alone or combined with other pretreatments to improve extraction processes and reduce energy use.

Mechanical disruption can be achieved by bead milling, high-pressure homogenization, and hydrodynamic cavitation. Bead milling is a basic cell disruption process which has been widely used at both lab and large plant scales due to its high efficiency. Bead milling exposes samples to beads moving with high speed which disrupt the cells. In some cases, a stirrer is also included, which agitates the sample and makes it more efficient (Fig. 3a). The bead mill has been shown to facilitate the extraction of lipids from both dried and wet microalgal cells (Günerken et al., 2015), which avoids drying of microalgae cells for lipid extraction. In another study, bead milling was shown to enhance the extraction of protein from *Ulva* and *Gracilaria* seaweed compared to alkaline and ultrasound treatment. Bead milling resulted in a sufficient content of protein yield compared to other methods investigated with a condition of 3 cycles of 60s with 6500 rpm and a break of 120s between each cycle (Kazir et al., 2019).

Compression puffing is another physical pretreatment method which modifies cellular matrices by the simultaneous application of heat and pressure leading to the modification of physicochemical properties. Compression puffing pretreatment of *Sargassum glaucescens* followed by hydrothermal extraction enhanced the extraction of fucoidan. It was reported that the disruption of cells that occurred during compression puffing pretreatment improved the extraction of fucoidan compared to hydrothermal treatment alone (Huang, Wu, Yang, Kuan, & Chen, 2016).

Application of novel technologies as a pretreatment prior to drying e.g. ultrasound, microwave, pulse electric field have been reported to enhance process efficiency. Ultrasound assisted drying of *Ascophyllum nodosum* has been demonstrated to reduce drying time, increase energy efficiency and improve color retention (Kadam, Tiwari, & O'Donnell, 2015). In another study, ultrasound treatment under vacuum (USV) was reported to accelerate the dehydration rate of *Phaseolus vulgaris* (Tekin, Başlar, Karasu, & Kilicli, 2017). It was reported to reduce the drying time by 1 h and also showed higher phenolic compounds compared to control samples. When ultrasound was employed as a pretreatment, followed by acid/alkali treatment, it resulted in a decrease in the extraction time for protein from seaweed (Kadam, Álvarez, Tiwari, & O'Donnell, 2017). Ultrasound pretreatments have been reported to enhance the extraction of compounds in several studies (Table 1).

Use of microwaves as a pretreatment has been reported to enhance extraction of bioproducts. Álvarez et al. (2017) reported that microwave pretreatments after homogenization and prior to solid-liquid extraction enhanced the extraction of polyphenols, sugars and fibres, from grape pomace. They observed that the polyphenol yield increased by 57% and

that bioactivity was also enhanced. Similarly (Uquiche, Jeréz, & Ortiz, 2008), reported that pretreatment using microwaves, followed by pressing increased the extraction yield of oil from Chilean hazelnuts (*Gevuina avellana* Mol). Microwave pretreatments for 240 s at 400 W enabled recovery of 45.3% of the initial oil content compared to 6.1% from untreated samples. The enhanced recovery was attributed to the rupture of the cell walls by microwaves, which facilitated the release of oil. Limited studies have been reported on the use of microwave pretreatments for extraction of bioactive compounds from seaweeds (Table 1). However, microwave pretreatments have been used in seaweed applications for production of biogas (Montingelli, Benyounis, Stokes, & Olabi, 2016) and bioethanol (Yuan & Macquarrie, 2015c).

Pulse electric field (PEF) pretreatments can also be employed to improve extraction efficiencies in terms of yield and quality of the extract. Electroporation is the main mechanism associated with disruption of cell membranes leading to the formation of pores in cell membranes which increases permeability (Bryant & Wolfe, 1987). This increased permeability facilitates the diffusion of solvent into the cell membranes leading to enhanced extraction of target compounds and reduced extraction time (Toepfl, Mathys, Heinz, & Knorr, 2006). Vorobiev & Lebovka (2015) reported that PEF pretreatment before mechanical expression in fruit juice from solid foods such as rapes, apples and sugar beets resulted in higher yields. PEF pretreatment before maceration in wine making was demonstrated to improve polyphenolic yield from grape wine (El Darra et al., 2016). A study carried out on microalgae *Chlorella vulgaris* and *Spirulina platensis*, showed that PEF pretreatment of 15 kV/cm and 100 kJ/kg enhanced the extraction of carotenoids by up to 525 and 150%, respectively, compared to conventional ball milling homogenization alone (Töpfl, 2006).

High-pressure homogenization has been employed for the extraction of lipids from *Chlorella saccharophila* (Mulchandani, Kar, & Singhal, 2015). Extraction of fucoidans from *Nemacystus decipiens* using high pressure homogenization in a pressure range of 40–100 MPa, as a pretreatment followed by hydrothermal processing was reported by (Li, Luo, Yuan, & Yu, 2017). HPH resulted in 16.67% yield of fucoidans at 70 MPa for 2 cycles followed by hydrothermal extraction. Fig. 4 shows the structural changes before and after high pressure treatment.

Hydrodynamic cavitation involves the formation of cavities in a suspension where it leads to formation and collapse of microbubbles (Fig. 3c) (Lee & Han, 2015). These bubbles are formed when the pressure drops below the vapor pressure of the suspension and collapses when the pressure exceeds the vapor pressure. The collapse of the

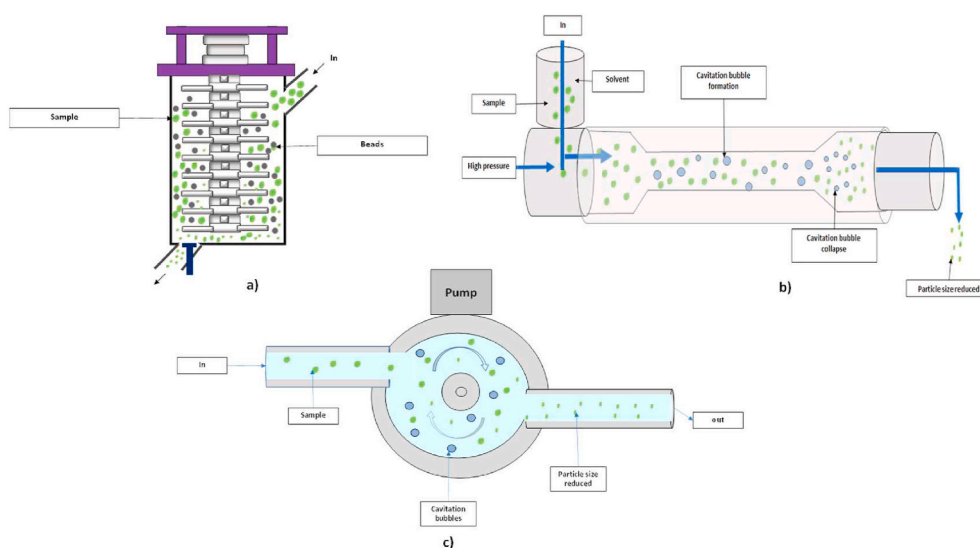


Fig. 3. Different types of shear-force disruption instruments: a) Lab scale bead milling system b) Lab scale high-pressure homogenization MN250A, and c) ROTOCav hydrodynamic cavitators

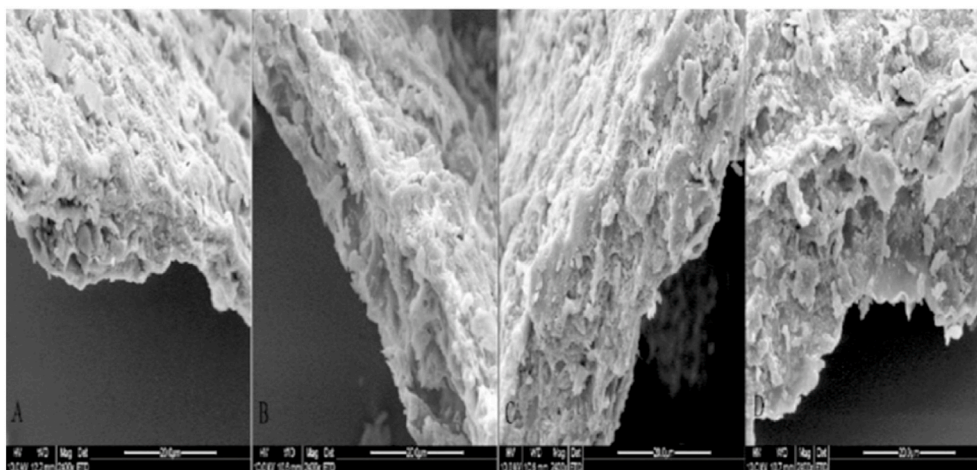


Fig. 4. Scanning electron micrographs of *N. decipiens* power: (A) untreated sample; (B–D) sample obtained after homogeneous processing at 40 MPa, 70 MPa and 100 MPa, respectively, 2 cycles. Magnification: 2400-fold. (Li et al., 2017).

microbubbles produces shock waves and momentarily increases pressure (100–5000 atm) and temperature (500–15,000 K), which mechanically disrupts the algal cells (Lee & Han, 2013). Abrahamsson (2016) reported that hydrodynamic cavitation pretreatment improved the production of methane from *A. nodosum* compared to traditional steam explosion.

4.2. Extraction techniques

4.2.1. Hydrothermal liquefaction

Hydrothermal liquefaction converts wet biomass into crude extract under specific conditions of temperature (280–370 °C) and pressure (100–250 bar) (Chiaramonti, Prussi, Buffi, Rizzo, & Pari, 2017). During this process, water is used as the main solvent and when the above-mentioned conditions exist hydrolysis of biomass occurs whereby large molecular weight compounds are depolymerised into smaller molecules. This process has been reported for use with microalgae,

where a temperature of around 200 °C was required for lipid extraction (Yoo, Park, Yang, & Choi, 2015). Hydrothermal liquefaction of *Laminaria saccharina* in the presence of KOH was reported to improve the extraction efficiency of mannitol and laminarin. The authors reported that the optimum conditions for the bio crude yield were a mixing ratio of 1:10 (biomass:water), 350 °C and 15 min residence time without catalyst (Anastasakis & Ross, 2011). Hydrothermal liquefaction has been employed to obtain valuable products such as biocrude, sugars and minerals from seaweed biomass at industrial scale (Barreiro et al., 2013).

4.2.2. Steam explosion

A high pressure steam explosion technique is required to treat hard lignocellulose material for bioresource fabrication (Fig. 5) (Shafiei, Kabir, Zilouei, Horváth, & Karimi, 2013). Generally, algal biomass is heated to 180–240 °C using steam for a certain period and consecutively depressurised to achieve ambient conditions. Repetition of these

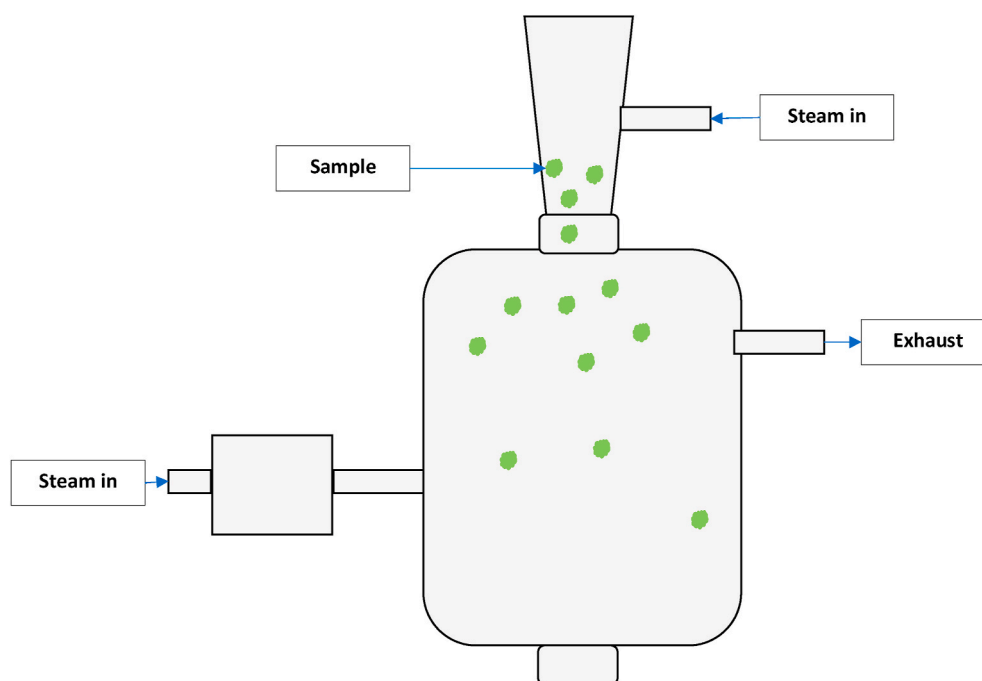


Fig. 5. Steam explosion equipment for lab-scale experiments. The lid had an inlet of steam, a temperature measurement device, and a larger vent used for release of pressure. The autoclave was put in an insulated outer beaker to more easily maintain the desired temperature.

treatments causes an explosion and cell wall damage which facilitates release of cell contents (Nurra et al., 2014). Steam explosion is mainly used for treating seaweeds for biogas production (Vivekanand, Eijssink, & Horn, 2012), for the production of bioethanol (Yanagisawa, Kawai, & Murata, 2013), and for extraction of bioactive compounds extraction from seaweeds. In one such study, *Gracilaria verrucosa* thallus was subjected to steam explosion treatment and resultant changes in its structure were observed by TEM (transmission electron microscope) and SEM (scanning electron microscope). The authors also analyzed the chemical composition of the seaweed and the agar yield extracted. They observed that the detachment of adjacent cells occurred and that the cuticle of surface layer showed extremely transformed regions with a spongy appearance. They concluded that the extraction of agar was improved and the agar obtained had low sulfate content and molecular weight (Talarico, Guida, Murano, & Piacquadio, 1990). Steam explosion as a pretreatment was also used in the extraction of agar from *Gracilaria dura*. Samples were soaked in 1M Na₂CO₃, and different explosion treatments were investigated at 140–190 °C for 15–20 s and the results were compared to samples without any pretreatment and with a NaOH based alkali pretreatment. The optimum conditions for the steam explosion treatment were 150 °C and 15 s and it was observed that even short duration treatment (20 s) caused complete thallus destruction and liquefaction of the algae. The gel strength, apparent modulus of elasticity and melting temperature of the agar obtained by steam explosion were lower than the values obtained from samples without pretreatment or with alkali pretreatment, but were still better compared to the values obtained from commercial agarose samples. The yield of agar obtained with the steam explosion of Na₂CO₃ soaked algae was higher than other conventional methods (Murano et al., 1993). Steam explosion was proposed as a technology for extracting phycocolloids. Despite the positive results obtained, limited studies have been reported related to the extraction of the wide range of bioactive compounds from seaweeds.

4.2.3. Pulsed electric field

Pulsed electric field (PEF) applies an electrical field across the cell wall that results in cell breakdown. The number and size of resultant pores is directly related to the electric field pulse and strength applied (Fig. 6a) (Günerken et al., 2015). PEF is widely used in microalgae cell disruption but recent studies shown that PEF may also be used for seaweed biomass. Recently PEF was investigated as a pretreatment process for protein extraction from *Ulva* sp. PEF treatments (50 pulses of 50 kV) were applied over an electrode gap of 70.3 mm on fresh *Ulva* and resulted in a 7-fold increase of total protein compared to osmotic shock. Also the isolated protein gave better antioxidants than the protein

standards (Robin, Kazir, et al., 2018). The same research group used PEF with *Ulva* to extract the ash materials. They reported that PEF improved the ash yield and significantly enhanced the extraction of major minerals such as K, Mg, Na, P and S compared to the normal pressing method of extraction (Robin, Sack, et al., 2018).

4.2.4. Ultrasound assisted extraction

Ultrasound waves are mechanical waves which propagate by compression and rarefaction, and can pass through solid, liquid and gas media. This mode of propagation causes regions of negative pressure in the liquid. Vapor bubbles are formed when the pressure exceeds the tensile strength of the liquid, which undergo implosion under strong ultrasound fields, this phenomenon is called cavitation (Kadam, Tiwari, & O'Donnell, 2015) and the ability of ultrasound to cause this cavitation, depends upon several factors including, ultrasonic frequency and intensity, properties of the medium such as surface tension and viscosity and the ambient conditions including temperature and pressure (Tiwari, 2015). The implosion of the cavitation bubbles further generates macro-turbulence, high velocity interparticle collisions, and perturbations in microporous particles of the biomass. The cavitation occurring near the solid-liquid interfaces directs a fast moving stream of liquid through the cavity at the surface. These microjets result in surface peeling, erosion, and particle break down therefore enhancing the release of bioactive compounds from the matrices (Kadam, Tiwari, & O'Donnell, 2015). Effects of ultrasound include fragmentation, erosion, capillarity, detexturation and sonoporation (Chemat, Rombaut, Sicaire, et al., 2017). Ultrasound reduces extraction time, solvent use and processing costs. Ultrasound can be used in combination with technologies such as extrusion, microwave, supercritical fluid extraction, and also in processes involving ultrasound-assisted Clevenger distillation, ultrasound-assisted Soxhlet extraction and continuous ultrasound-assisted extraction (Chemat, Rombaut, Sicaire, et al., 2017). Ultrasound can be applied via a probe or an ultrasound bath (C. Wen et al., 2018). Various ultrasound machines are shown in Fig. 6 (b) ultrasound bath, (c) ultrasound probe system. Use of ultrasound has been investigated for extraction of various biomolecules from seaweed, for example agar (Din et al., 2019), protein (Kadam et al., 2017), laminarin (Kadam, Tiwari, & O'Donnell, 2015), carrageenan and alginate (Yousouf et al., 2017), fucoidan, phlorotannins and alginate (Flórez-Fernández, López-García, González-Muñoz, Vilarino, & Domínguez, 2017) etc. A combination of ultrasound with other treatments such as enzyme extraction (Casas, Conde, Domínguez, and Moure (2019)) and with microwaves (Alboofetileh, Rezaei, Tabarsa, Rittà, et al., 2019) has also been investigated.

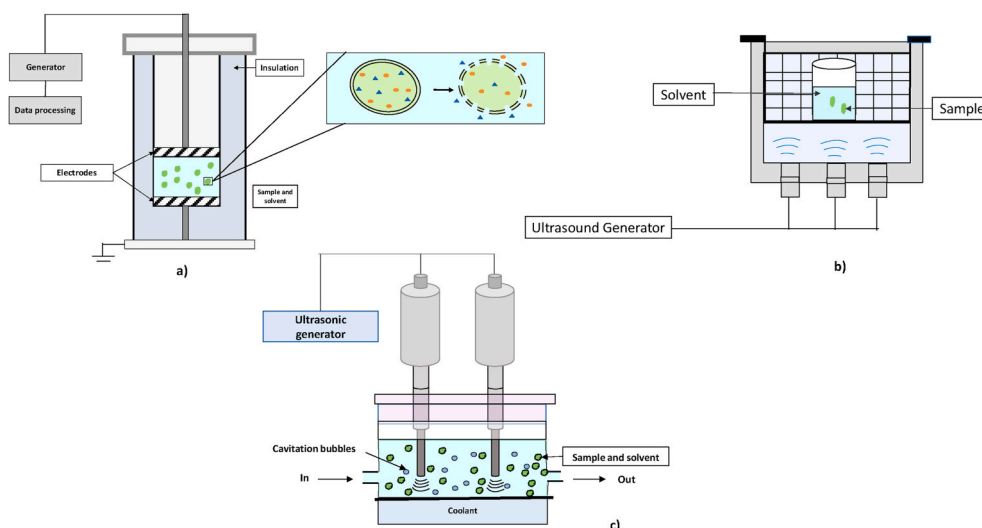


Fig. 6. a) Pulsed electric field system- ELEA PEFPILOT b) Ultrasound water bath and c) UIP 2000hdT – the new digital 2000 Watts industrial ultrasonicator.

Recent studies show that ultrasound can be used as a pretreatment to enhance the drying kinetics of *A. nodosum* seaweed. An ultrasound intensity of $6.00\text{--}75.78\text{ W cm}^{-2}$ (20 kHz probe) was applied for 10 min, followed by hot air convective drying ($50\text{ }^{\circ}\text{C}$, air velocity as 0.3 m s^{-1}) until a constant weight was obtained. It was observed that the pretreatment reduced the drying time required, with 75.78 W cm^{-2} intensity treated samples showing the shortest drying time (Kadam, Tiwari, & O'Donnell, 2015). It was also observed that the colour of the ultrasound treated samples were lighter than the control. It was also concluded that the ultrasound pretreatment reduced both the energy consumption and time required for drying of *A. nodosum*.

Fig. 7 shows SEM images of *Gracilaria gracilis* treated using different extraction technologies. Fig. 7 (c) illustrates that ultrasound probe treatment (50–60 kHz, 200 W) for different periods of time (10 s–10 min), on and off cycles (30 s and 20s) increases cell rupture over other methods such as freeze thaw, maceration, high pressure assisted and ultrasound bath extraction, and releases the chlorophyll and phycobiliprotein from *G. gracilis* (Pereira et al., 2020). Less sulfate was observed in agar extracted using a combination of sonication and ultrasound. Ultrasound as a pretreatment enhances the greenness by having the following advantages: significant reducing the process time required for extraction, digestion etc., reduces energy consumption, facilitates use of low concentration and quantities of solvents, may be carried out at room temperature and atmospheric pressure, reduces analyte loss and contamination risks, and increases productivity (Bendicho et al., 2012).

4.2.5. Microwave assisted extraction

Microwave assisted extraction (MAE) has been demonstrated for bioactives extraction from a wide range of matrices. Microwaves are electromagnetic radiation emitted in the range of 300 MHz–300 GHz. Two main frequencies (915 MHz and 2.45 GHz) are employed for microwave processing. Microwave heating is generated by ionic conduction of dissolved ions and dipole rotation of polar solvent. Rapid internal heating leads to effective cell rupture which releases the target compounds into the solvent (Vázquez-Delfín, Robledo, & Freile-Pelegrín, 2014). The efficacy of MAE depends on microwave energy absorption by polar solvents including water, methanol etc., which is influenced by the dielectric properties of the solvents.

The efficiency of microwave heating depends on the ability of the material to absorb electromagnetic energy, and energy dissipated is

measured by the dielectric loss tangent. When the dielectric loss tangent of biological material is higher than that of the solvent, the plant material can reach a higher temperature than the solvent and consequently the inside cell pressure increases, resulting in the rupture of the cell membrane and release of the target compounds into the solvent. Therefore, the compounds from plant material can be extracted more rapidly compared to conventional extraction (Vinatoru, Mason, & Calinescu, 2017).

The application of microwaves for extraction may be unsuitable for temperature sensitive bioactives extracted from biological matrices e.g. from *Hibiscus sabdariffa* (Pimentel-Moral et al., 2018) broccoli, choy-sum and cabbage (Wachtel-Galor, Wong, & Benzie, 2008). MAE has been reported for the extraction of fucoidan (Yuan & Macquarrie, 2015a) sulphated polysaccharides (Yuan et al., 2018) from seaweed. It has also been used in combination with ultrasound for extraction of fucoidan from seaweed (Alboofetileh, Rezaei, Tabarsa, Rittà, et al., 2019) (Table 1).

4.2.6. Supercritical fluid extraction

A fluid is said to be in a supercritical state when the temperature and pressure conditions are above its critical point. During this state, the properties of the fluids are intermediate between gases and liquids i.e. a density close to that of liquids which induces a solvating power like liquids, a viscosity close to gases, diffusivity intermediate between liquids and gases, which increases mass transfer between target compound and the supercritical fluid (Chemat, Rombaut, Meullemiestre, et al., 2017). CO_2 is used for over 90% of supercritical fluid extraction (SFE) applications of natural compounds (Uddin et al., 2015) because of its low critical conditions ($T_c: 31\text{ }^{\circ}\text{C}$, $P_c: 7.38\text{ MPa}$), wide availability, non-toxicity, non-flammable and non-explosive nature (Chemat, Rombaut, Meullemiestre, et al., 2017). Apart from CO_2 ethanol, hexane, methanol, pentane, butane, nitrous oxide, sulfur hexafluoride and fluorinated hydrocarbons can also be used for SFE due to their supercritical state properties. A key advantage of CO_2 is that it can be eliminated from the extract during decompression without leaving any residue (Herrero, del Pilar Sánchez-Camargo, Cifuentes, & Ibáñez, 2015). Additionally the non-oxidative nature of CO_2 favours extraction of compounds which are prone to oxidation (Essien, Young, & Baroutian, 2020). A disadvantage related to the use of CO_2 for SFE is that it exhibits a chemical behaviour similar to that of lipophilic or non-polar

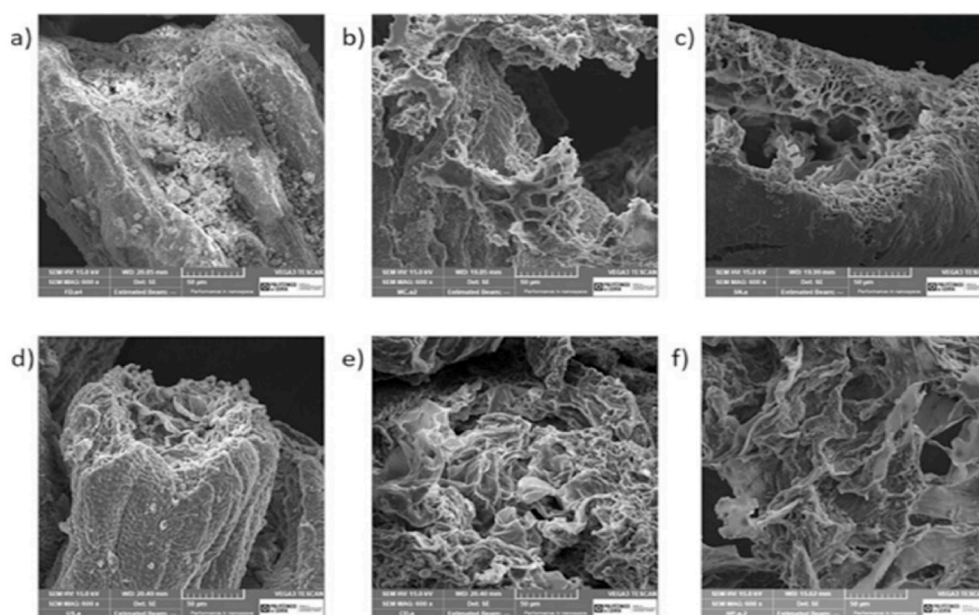


Fig. 7. SEM images of the *Gracilaria gracilis* biomass cells a) before and b)–f) after the extraction treatments (b – maceration, c – ultrasonic probe, d – ultrasonic bath, e – freeze-thaw, f – high pressure-assisted extraction) at a magnification of $600\times$. (Pereira et al., 2020).

solvents and is able to extract non-polar compounds only. In order to overcome this limitation, polar solvents such as water, methanol and ethanol can be used as co-solvents to modify the solvent polarity (Molino et al., 2020). Supercritical CO₂ has been employed for extraction of different target compounds including fucoxanthin (E. M. Balboa, Moure, & Domínguez, 2015) and fucosterol (Becerra et al., 2015) from seaweeds. Supercritical CO₂ with soyabean oil, canola oils, water, and ethanol as a co-solvent was found to be efficient for extraction of phlorotannins and carotenoids (Saravana et al., 2017) and fatty acids, phenolics and fucoxanthin (Saravana et al., 2019) (Table 1). The use of supercritical fluid as a pretreatment for rice straw was reported to facilitate cellulase enzymatic hydrolysis (Gao et al., 2010). (Men'shova, Lepeshkin, Ermakova, Pokrovskii, & Zvyagintseva, 2013) studied the effect of supercritical fluid pretreatment of brown algae (*Saccharina japonica* and *Sargassum oligocystum*) with and without 5% ethanol as a co-solvent (P = 550 bar, T = 60 °C) to extract fucoidan. They found that supercritical CO₂ with 5% ethanol gave an improved yield of fucoidan: *S. japonica* (1.35%) and *S. oligocystum* (0.55%) compared to supercritical CO₂ alone. In another study supercritical CO₂ was used as a pretreatment for deoiling *Undaria pinnatifida*, followed by hydrothermal-microwave treatment to extract fucoidan (Quitain, Kai, Sasaki, & Goto, 2013).

4.2.7. Pressurized liquid extraction

Pressurized liquid extraction (PLE) also referred to as pressurized fluid extraction (PFE), pressurized hot-solvent extraction (PHSE) or accelerated solvent extraction (ASE) is based on the use of solvents under high temperature and pressure conditions which are below their critical points. The solvents under these conditions remain in liquid state. When PLE is carried out with water as the solvent, it is known as subcritical water extraction (SWE), superheated water extraction (SHWE) or pressurized hot-water extraction (PHWE) (Essien et al., 2020; Srinivas & King, 2010). Subcritical water is defined as hot water at sufficient pressure to maintain the liquid state at critical temperature between 100 °C (the boiling point of water) and 374 °C (the critical point of water) under the critical pressure (1–22.1 MPa) (Ju & Howard, 2005). One of the most beneficial features of subcritical water is that its dielectric constant which governs the polarity of the solvent can be modified by varying temperature and pressure. For example, at ambient conditions, the dielectric constant of water is 80 which indicate that it is an extremely polar solvent. However, at 250 °C and 4 MPa water has a dielectric constant of 27 which is close to ethanol. Hence it is suitable for extraction of low-polarity compounds (Chemat et al., 2012).

The use of subcritical water for enhanced extraction of fucoidan (Alboofetileh, Rezaei, Tabarsa, You, et al., 2019), phenolics (Dinh, Saravana, Woo, & Chun, 2018), carrageenan (Gereniu, Saravana, & Chun, 2018) from seaweeds has been reported. Enhanced extraction of bioactives is mainly due rupture of seaweed matrices. SEM images showed the changes in structure of *E. cottonii* and *Gracilaria* sp. after subcritical water treatment. The control samples do not show any surface cracks and had a regular and compact surface structure. After subcritical water treatment, residues of *E. cottonii* and *Gracilaria* clearly showed disruption (Machmudah, Winardi, Kanda, & Goto, 2017).

PLE techniques require small amounts of solvents compared to extraction at ambient conditions. The increase in the extraction temperature can promote higher solubility of target compounds and increased mass transfer rate. In addition, high temperature decreases the viscosity and the surface tension of the solvents, which increases penetrability into the matrix and extraction of target compounds (Ibañez, Herrero, Mendiola, & Castro-Puyana, 2012, pp. 55–98). The extraction of phlorotannin (del Pilar Sánchez-Camargo et al., 2016), polyphenol (Heffernan, Smyth, FitzGerald, Soler-Vila, & Brunton, 2014) and fucoidan (Saravana, Cho, Park, Woo, & Chun, 2016) from seaweeds has been reported (Table 1).

4.2.8. Enzyme assisted extraction (EAE)

Enzymes can hydrolyze cellular components (e.g. complex polysaccharides) to facilitate the accessibility of the target solute compounds to the solvent. Various factors influencing enzyme assisted extraction (EAE) include enzyme selection according to the target compound, hydrolysis time, pH, proportion of enzyme to substrate and solvent. However seaweed is a complex matrix which is more difficult to hydrolyze compared to plant biomass (Wijesinghe & Jeon, 2012).

The use of enzymes as a pretreatment prior to conventional extraction or in combination with novel technologies including ultrasound, high pressure, ionic liquid, microwave and supercritical fluids has been reported (Nadar, Rao, & Rathod, 2018). The use of the enzyme assisted extraction of various compounds (polysaccharides, carotenoids and polyphenols etc) from a range of matrices has been reviewed by (Nadar et al., 2018) and (Wijesinghe & Jeon, 2012). EAE has been employed for the extraction of agar (Q. Xiao et al., 2019), fucoxanthin (Billakanti et al., 2013), and in combination with ultrasound, microwave and subcritical water for fucoidan (Alboofetileh, Rezaei, Tabarsa, Rittà, et al., 2019), in combination with ultrasound for phenolic compounds and carbohydrate monosaccharides (glucose, arabinose, fucose and the sum of xylose, galactose and mannose) (Casas et al., 2019) and in combination with microwaves for phlorotannin (Charoensiddhi, Franco, Su, & Zhang, 2015) from seaweeds.

4.2.9. Combined extraction techniques

Combination of extraction techniques to exploit synergies between complementary technologies and improve extraction efficiencies has been widely investigated for extraction of bioactive compounds (Fig. 8). For example, guava seeds and pulp extracted with hot water and microwaves had a higher yield of polysaccharides compared to conventional extraction (Arasi, Rao, & Bagyalakshmi, 2016).

Both ultrasound assisted enzymatic extraction (UAEE) and microwave assisted enzymatic extraction (MAEE) combine two complementary extraction methods. In UAEE and MAEE, enzymatic hydrolysis promotes recovery of target compounds by partial disruption of cellular matrix and ultrasound or microwave treatments also assist inactivation of enzymes to terminate the reactions. In some cases enzyme activity can be enhanced in the presence of ultrasonic waves depending upon frequency and power (O'Donnell, Tiwari, Bourke, & Cullen, 2010). Wu, Zhu, Diao, & Wang (2014) worked on the recovery of crude polysaccharides from pumpkin with conventional extraction, UAE, UAEE and EAE. They reported that the UAEE method showed a synergistic effect and the highest extraction yield with a maximum crude polysaccharide recovery of 4.33 ± 0.15% compared to EAE, UAE and conventional extraction alone.

MAEE has been studied for essential oil extraction from *Isatis indigotica* seeds (Gai et al., 2013) and pumpkin seeds (Jiao et al., 2014). Cheng et al. (2015) investigated the feasibility of MAEE for the extraction of polysaccharides from *Schisandra chinensis* Baill.

The combination of UAE and MAE together (UMAE) has been demonstrated to have potential to be a cost-effective and efficient extraction technology. Wen et al. (2019) investigated the effect of conventional solvent extraction (CSE), UAE, MAE and UMAE on extraction yield of soluble dietary fibre (SDF) from coffee silver skin. They reported an SDF yield (42.7 ± 0.4%) obtained by UMAE which was 1.5, 1.9 and 1.2 times higher than the recovery rates achieved by CSE, UAE, and MAE, respectively. In another study Garcia-Vaquero, Ummat, Tiwari, & Rajauria (2020) (Fig. 9) investigated the effect of UAE, MAE and UMAE on extraction of fucose-sulphated polysaccharides (FSPs), total soluble carbohydrates and antioxidants from Brown algae, *A. nodosum*. They reported that UMAE improved the yields of compounds extracted compared to the use of UAE and MAE alone (Table 1).

4.2.10. Green impact of non conventional extraction technologies

Use of non conventional extraction technologies can help overcome some of the challenges and limitations of conventional extraction

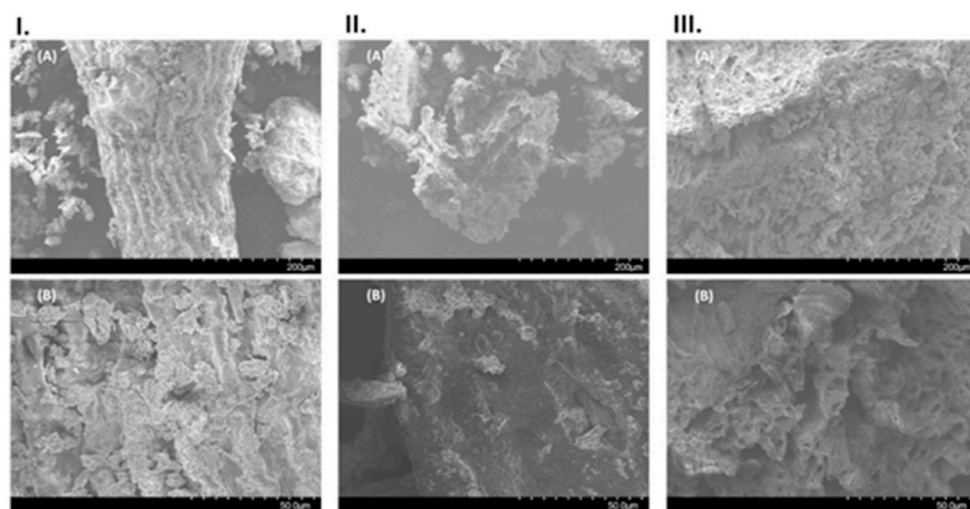


Fig. 8. Scanning electron microscopy images of (I) dried and milled *A. nodosum* biomass before extraction, (II) macroalgal residue after MAE (250 W, 2 min) and (III) macroalgal biomass after the process of UMAE (1000 W, 100%, 5 min). Scale bars (A) 200 μm (magnification: 250 $\times$) and (B) 50 μm (magnification: 1000 $\times$) (García-Vaquero et al., 2020).

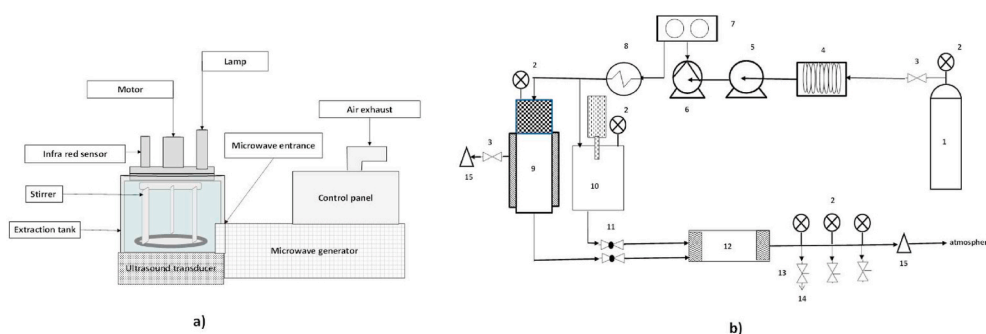


Fig. 9. Semi pilot scale extracting instruments a) Ultrasound and microwave combined process and b) Sub-supercritical carbon dioxide extraction instrument coupled with ultrasound system.

methods such as long extraction times, use of large quantities of solvent, high energy input and degradation of labile compounds. The wide range of pretreatment and extraction methods outlined in this review demonstrate the principles of green extraction techniques which include (i) innovation by selection and use of renewable resources; (ii) use of green/alternative solvents; (iii) energy reduction; (iv) zero-waste approach; (v) streamlined extraction processes; and (vi) residue free extracts (Chemat et al., 2019). The byproducts or left over biomass following conventional extraction of target compounds are generally discarded because of the presence of chemical residues. Adoption of green extraction techniques facilitates byproduct utilisation and recovery of the compounds from residual biomass.

5. Conclusions and future perspectives

Seaweeds are an abundant and renewable biomass resource from which a wide range of target compounds can be extracted such as alginate, agar, carrageenan, polyphenol, phlorotannins, carotenoids, proteins, lipids, etc. These target compounds have a wide range of applications in the food, nutraceutical, pharmaceutical, biotechnology and cosmetic sectors. The cellular structure of seaweed is complex and the target compounds are difficult to extract. Therefore, the use of an efficient extraction technique is of utmost importance. Traditional extraction methods have been widely studied and commercially employed despite their limitations. Several studies have shown that the use of pretreatments can improve the extraction yield. Novel extraction

technologies such as MAE, UAE, EAE and supercritical fluid extraction are currently being employed as pretreatments followed by conventional or novel extraction techniques.

Despite all the advantages of novel green extraction processes outlined in this review, conventional methods still dominate industrial applications in the marine sector. This is mainly due to, (i) costs associated with the implementation of high-tech, expensive, sophisticated techniques; (ii) limited scientific knowledge on novel extraction methods; (iii) non uniformity of reporting of novel extraction techniques and control parameters in reported studies and (iv) scale up challenges associated with novel extraction technologies.

Declaration of competing interest

The authors declare that they have no competing interests.

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