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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 the extraction process, 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. The combinations of different cell disruption technologies and extraction methods can enhance the extractability of compounds with higher purity and contribute towards improved process efficiency.

Keywords: **Cell disruption, pre-treatment, green extraction technologies, bioactives, seaweed**

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 the 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, & Krivoshapkina, 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 beetroot, eggplant (Vinson, Hao, Su, & Zubik, 1998), mangrove trees (Dahibhate, Saddhe, & Kumar, 2019), tea (da Silva, et al., 2017), 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-Ul-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 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 (Cikoš, 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 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).

Use of green solvents and novel extraction technologies have 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, Smyth, & O'Donnell, 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 the last decades due to the 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). The 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). These along with carotenoids, vitamin 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 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, 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.

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, 2015(b)). 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 (Cajnko, 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 fucodinase 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 technique 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*

255 followed by hydrothermal extraction enhanced the extraction of fucoidan. It was reported that
256 the disruption of cells that occurred during compression puffing pretreatment improved the
257 extraction of fucoidan compared to hydrothermal treatment alone (Huang, Wu, Yang, Kuan,
258 & Chen, 2016).

259 Application of novel technologies as a pretreatment prior to drying e.g. ultrasound,
260 microwave, pulse electric field have been reported to enhance process efficiency. Ultrasound
261 assisted drying of *Ascophyllum nodosum* has been demonstrated to reduce drying time,
262 increase energy efficiency and improve colour retention (Kadam, Tiwari, & O'Donnell,
263 2015). In another study, ultrasound treatment under vacuum (USV) was reported to accelerate
264 the dehydration rate of *Phaseolus vulgaris* (Tekin, Başlar, Karasu, & Kilicli, 2017). It was
265 reported to reduce the drying time by one hour and also showed higher phenolic compounds
266 compared to control samples. When ultrasound was employed as a pretreatment, followed by
267 acid/ alkali treatment, it resulted in a decrease in the extraction time for protein from seaweed
268 (Kadam, Álvarez, Tiwari, & O'Donnell, 2017). Ultrasound pretreatments have been reported
269 to enhance the extraction of compounds in several studies (Table 1).

270 Use of microwaves as a pretreatment has been reported to enhance extraction of
271 bioproducts. Álvarez, et al. (2017) reported that microwave pretreatments after
272 homogenization and prior to solid-liquid extraction enhanced the extraction of polyphenols,
273 sugars and fibres, from grape pomace. They observed that the polyphenol yield increased by
274 57% and that bioactivity was also enhanced. Similarly, (Uquiche, Jeréz, & Ortíz, 2008)
275 reported that pretreatment using microwaves, followed by pressing increased the extraction
276 yield of oil from Chilean hazelnuts (*Gevuina avellana* Mol). Microwave pretreatments for
277 240 s at 400 W enabled recovery of 45.3% of the initial oil content compared to 6.1% from
278 untreated samples. The enhanced recovery was attributed to the rupture of the cell walls by
279 microwaves, which facilitated the release of oil. Limited studies have been reported on the
280 use of microwave pretreatments for extraction of bioactive compounds from seaweeds (Table
281 1). However, microwave pretreatments have been used in seaweed applications for
282 production of biogas (Montingelli, Benyounis, Stokes, & Olabi, 2016) and bioethanol (Yuan
283 & Macquarrie, 2015(c)).

284 Pulse electric field (PEF) pretreatments can also be employed to improve extraction
285 efficiencies in terms of yield and quality of the extract. Electroporation is the main
286 mechanism associated with disruption of cell membranes leading to the formation of pores in
287 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 and 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 15kV/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 decipients* using high pressure homogenisation 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 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 to 370 °C) and pressure (100 to 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 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, Eijsink, & 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 Na_2CO_3, and different explosion treatments were investigated at 140-190°C for 15-20s 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 15s and it was observed that even short duration treatment (20s) caused complete thallus destruction and liquefaction of the algae. The gel strength, apparent modulus of elasticity and melting

353 temperature of the agar obtained by steam explosion were lower than the values obtained
354 from samples without pretreatment or with alkali pretreatment, but were still better compared
355 to the values obtained from commercial agarose samples. The yield of agar obtained with the
356 steam explosion of Na_2CO_3 soaked algae was higher than other conventional methods
357 (Murano, et al., 1993). Steam explosion was proposed as a technology for extracting
358 phycocolloids. Despite the positive results obtained, limited studies have been reported
359 related to the extraction of the wide range of bioactive compounds from seaweeds.

360 **4.2.3 Pulsed electric field**

361 Pulsed electric field (PEF) applies an electrical field across the cell wall that results in cell
362 breakdown. The number and size of resultant pores is directly related to the electric field
363 pulse and strength applied (Fig. 6a) (Günerken, et al., 2015). PEF is widely used in
364 microalgae cell disruption but recent studies shown that PEF may also be used for seaweed
365 biomass. Recently PEF was investigated as a pretreatment process for protein extraction from
366 *Ulva* sp. PEF treatments (50 pulses of 50 kV) were applied over an electrode gap of 70.3 mm
367 on fresh *Ulva and* resulted in a 7-fold increase of total protein compared to osmotic shock.
368 Also the isolated protein gave better antioxidants than the protein standards (Robin, Kazir, et
369 al., 2018). The same research group used PEF with *Ulva* to extract the ash materials. They
370 reported that PEF improved the ash yield and significantly enhanced the extraction of major
371 minerals such as K, Mg, Na, P and S compared to the normal pressing method of extraction
372 (Robin, Sack, et al., 2018).
373

374 **4.2.4 Ultrasound assisted extraction**

375 Ultrasound waves are mechanical waves which propagate by compression and rarefaction,
376 and can pass through solid, liquid and gas media. This mode of propagation causes regions
377 of negative pressure in the liquid. Vapor bubbles are formed when the pressure exceeds the
378 tensile strength of the liquid, which undergo implosion under strong ultrasound fields, this
379 phenomenon is called cavitation (Kadam, Tiwari, & O'Donnell, 2015) and the ability of
380 ultrasound to cause this cavitation, depends upon several factors including, ultrasonic
381 frequency and intensity, properties of the medium such as surface tension and viscosity and
382 the ambient conditions including temperature and pressure (Tiwari, 2015). The implosion of
383 the cavitation bubbles further generates macroturbulence, high velocity interparticle
384 collisions, and perturbations in microporous particles of the biomass. The cavitation
385 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, et al., 2015), carrageenan and alginate (Youssouf, et al., 2017), fucoidan, phlorotannins and alginate (Flórez-Fernández, López-García, González-Muñoz, Vilariño, & 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.

Recent studies show that ultrasound can be used as a pretreatment to enhance the drying kinetics of *A. nodosum* seaweed. An ultrasound intensity of 6.00-75.78 Wcm^{-2} (20 kHz probe) was applied for 10 min, followed by hot air convective drying (50 °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 to 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 the microwave energy absorption by polar solvents including water, methanol etc. which is influenced by the dielectric properties of 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, 2015(a)) 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 (Tc: 31 °C, Pc: 7.38 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 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 CO_2 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 CO_2 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 CO_2 with 5% ethanol gave an improved yield of fucoidan: *S. japonica* (1.35%) and *S. oligocystum* (0.55%) compared to supercritical CO_2 alone. In another study supercritical CO_2 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 (Fig. 10) show 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). The extraction of p*hlorotannin (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 hydrolyse 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. 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

546 ultrasonic waves depending upon frequency and power (O'Donnell, Tiwari, Bourke, &
547 Cullen, 2010). (Wu, Zhu, Diao, & Wang, 2014) worked on the recovery of crude
548 polysaccharides from pumpkin with conventional extraction, UAE, UAEE and EAE. They
549 reported that the UAEE method showed a synergistic effect and the highest extraction yield
550 with a maximum crude polysaccharide recovery of 4.33 ± 0.15% compared to EAE, UAE
551 and conventional extraction alone.

552 MAEE has been studied for essential oil extraction from *Isatis indigotica* seeds (Gai, et al.,
553 2013) and pumpkin seeds (Jiao, et al., 2014). Cheng, et al. (2015) investigated the feasibility
554 of MAEE for the extraction of polysaccharides from *Schisandra chinensis* Baill.

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

565 4.2.10 Green impact of non conventional extraction technologies

566 Use of non conventional extraction technologies can help overcome some of the challenges
567 and limitations of conventional extraction methods such as long extraction times, use of large
568 quantities of solvent, high energy input and degradation of labile compounds. The wide range
569 of pretreatment and extraction methods outlined in this review demonstrate the principles of
570 green extraction techniques which include (i) innovation by selection and use of renewable
571 resources; (ii) use of green/alternative solvents; (iii) energy reduction; (iv) zero-waste
572 approach; (v) streamlined extraction processes; and (vi) residue free extracts (Chemat, et al.,
573 2019). The byproducts or left over biomass following conventional extraction of target
574 compounds are generally discarded because of the presence of chemical residues. Adoption
575 of green extraction techniques facilitates byproduct utilisation and recovery of the
576 compounds from residual biomass.

577 **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.

Conflict of Interest

The authors declare that they have no competing interests.

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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	*Ulva sp.* and *Gracilaria sp.*	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	*N. decipients*	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	*L. saccharina*	Hydrothermal liquefaction	Water	25°C min^{-1} Biomass/water (5-20)%, 250-370°C, Residence time 12-	Max. bio crude (19.3%), obtained from 1:10 biomass-water ratio (350 °C), 15 min residence without catalyst.	(Anastasakis & Ross, 2011)

				120min, Catalyst (0-100)% KOH	Sugars in aqueous phase included laminarin and mannitol	
Steam explosion						
Agar	*Gracilaria verrucosa*	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	*Garcilaria dura*	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_2CO_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	*Ulva sp*	Pulsed Electric Field	Fresh biomass with water	PEF treatment at 247 kJ/kg, 50 kV (50 pulses), 70.3 mm electrode gap, 140 g	7-fold increase in total protein extracted compared to osmotic shock samples	(Robin, Kazir, et al., 2018)

				fresh Ulva		
Ultrasound						
Phenolics, uronic acid and fucose and	*A. nodosum*	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, Smyth, et al., 2015)
Fucoidan	*Sargassum muticum*	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 sulphate content in extract increased during first 25 min of treatment, gave maximum antitumoral activity	(Flórez-Fernández, et al., 2017)
Carrageenan and alginates	*Sargassum binderi* and *Turbinaria ornate*	Ultrasound	Alginate: 2% NaOH	Alginate: 150 W ultrasound, algae/water ratio 10 g/l, 90 °C, pH 12, 30 min.	Extraction time decreased without affecting chemical structure and molar mass distribution	(Youssouf, et al., 2017)
	Kappaphycus alvarezii and *Euchema denticulatum*		Carrageenan: (water)	Carrageenan: pH 7, 15 min		

Phenolic and carbohydrates	*S. muticum*	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)
Polyphenols, phlorotannins and antioxidants	*Fucus serratus*, *Fucus vesiculosus*, *Fucus spiralis*, *H. elongata*, *Halidrys siliquosa*, *Laminaria digitata*, *L. saccharina*, *Laminaria hyperborea*, *A. nodosum*, *Alaria esculenta* and *Pelvetia caniculata*	UAE and conventional extraction method	30, 50 and 70% ethanol	Optimisation using F. vesiculosus, 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 to 2.2-fold) in all seaweeds compared to conventional extraction	(Ummat, et al., 2020)
Fucose sulphated	*A. nodosum*	UAE, MAE or	Maceration with 0.1 M	UAE (500 W, 20kHz), MAE (2450 MHz) or	Maximum yields of compounds achieved	

polysaccharides, total soluble carbohydrate and antioxidants		UMAE	HCl for 10 min	UMAE (US; 500W, 20kHz and MW 2450 MHz) for 2 and 5 min	using UMAE	(Garcia-Vaquero, et al., 2020)
Fucose and glucan	*L. digitata*, *L. hyperborea* and *A. nodosum*	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	(Garcia-Vaquero, Rajauria, Tiwari, Sweeney, & O'Doherty, 2018)
Phenolics and antioxidant activity	*Hormosira banksii*	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	*A. nodosum*	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	(Yuan & Macquarrie, 2015(c))

					power than conventional. Molecular weight and sulfate content of fucoidan increased with decreasing extraction time.	
Fucoidan	*F. vesiculosus*	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, & Teixeira, 2011)
Phlorotannin and antioxidant	*Ecklonia radiata*	Microwave assisted enzymatic	Buffer solution	Microwave-assisted Viscozyme extraction for 5 to 30 min	Extraction time (5- 30 min), most effective process. High phlorotannins contents and antioxidant activities	(Charoensiddhi, et al., 2015)
Fucoidan	*Nizamuddinia zanardinii*	Viscozyme, alcalase, cellulase, flavourzyme, ultrasound, microwaves, subcritical water, alcalase-	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 E. coli.	(Alboofetileh, Rezaei, Tabarsa, Rittà, et al., 2019)

		ultrasound (EUAE), and simultaneous ultrasound-microwave (UMAE) and conventional hot water extraction.			Growth. Fucoidans extracted from enzyme-US, US-microwave and subcritical water showed inhibition against P. aeruginosa (2 mg/mL)	
Sulfated polysaccharides	*Ulva prolifera*	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-holding capacity. 0.05 M HCl, 150 °C: best foaming properties 0.1M HCl, 150 °C: highest antioxidant activity	(Yuan, et al., 2018)
Phytosterols and phytol	*Undaria pinnatifida* and *Sargassum fusiforme*	Microwave assisted extraction	Saponification using ethanolic solution of KOH	1.5 mol/l ethanolic KOH, 2g homogenised sample	Microwave was found to be an efficient extraction method. MW and high speed counter current	(X.-H. Xiao, Yuan, & Li, 2013)

					chromatography combination was efficient in separation and purification of compounds.	
Sulfated polysaccharides	*Ulva spp. and Monostroma latissimum*	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.	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.	(Tsubaki, Oono, Hiraoka, Onda, & Mitani, 2016)
Subcritical water						
Fucoidan	*N. zanardinii*	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)	*S. japonica*	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, & Chun, 2018)

Phenolics	*S. japonica*	Ionic liquid-assisted subcritical water (IL+ SWE)	0.25 M [C4C1im] [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	*K. alvarezii*	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)	*S. japonica*	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	*S. japonica*	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	*Porphyra umbilicalis, Ulva lactuca* and *Saccharina latissima*	a) Sonication b) pH-shift protein extraction	a) Water b) Water	a) 1-hour sonication, followed by stirring and protein precipitation by ammonium sulfate	pH-shift method showed highest protein concentration.	(Harrysson, et al., 2018)

		c) accelerated solvent extraction (ASE) to extract lipids and phlorotannin and carbohydrates before protein	c) 70% food grade acetone in water	b) sample to water 1:6 (w/v), homogenisation, 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	*A. nodosum, F. vesiculosus, F. serratus*	Accelerated Solvent extraction, using different solvents	80% ethanol/20% H2O	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	*F. serratus,* *G. gracilis,*	Solid- liquid extraction, PLE	Cold water	Cold water, shaker 24 hr, filtered twice	SLE with Cold water extracts showed max TPC from F. serratus.	(Heffernan, et al., 2014)

	C. fragile, *L. digitata,*				The antioxidant activity and TPC for Solid liquid extraction were greater than Pressurised Liquid Extraction using same solvents. SLE was better in yield obtained, low capital cost and ease. F. serratus showed best yields.	
Fucoidan	*S. muticum*	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	*Sargassum glaucescens*	Compressional puffing hydrothermal	Hydrothermal extraction: Double distilled water	Puffed samples, after removal of protein, pigments and lipids were given	Compressional puffing disrupted cellular structure and enhances extraction	(Huang, et al., 2016)

		extraction	(w/v 1:10)	Hydrothermal extraction: Double distilled water (w/v 1:10), 80 °C for 1 hour	with hot water. It was simple and the samples showed antioxidant activity. Fucoidan yield found to be more than conventional method	
Isoflavones	*S. vulgare*, *Porphyra sp.*, *Undaria pinnatifida*, *Sargassum muticum*, *Chondrus crispus*, *Hypnea spinella and Halopytis incurvus*,	Sonication pretreatment followed by supercritical CO_2 fluid extraction.	SFE modifier (MeOH: H_2O 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, Šnóblová, & Štěrbová, 2010)
Enzymatic extraction						
Phlorotannin	*S. muticum*	Enzymatic pretreatment	Alcalase and viscozyme enzyme	- Alcalase : 50 °C, 7.0 pH, 0.1 M phosphate buffer	PLE alone gave highest yields.	(del Pilar Sánchez-Camargo, et al., 2016)
		Pressurized liquids	Water and ethanol sonicated for 10 min	- Viscozyme enzyme 50 °C, 4.5 pH, 0.1 M sodium acetate-acetic acid buffer, for 2 or 4 hour. PLE: static extraction	Viscozyme, 2 hour with pressurized liquids, gave higher antioxidant rich extracts compared to PLE alone. Optimum conditions were 160°C, Pressurized solvent:	

				time: 20 min, 1500 psi; 120 °C; extraction solvent (75:25 ethanol: water) (v/v).	95% ethanol	
Fucoxanthin	*U. pinnatifida*	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 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.	(Billakanti, et al., 2013)
Fermentable sugars	*Enteromorpha sp.*	Enzymatic degradation	Various acid	Nitric acid, dilute sulphuric acid, steam flashing, pretreatment followed by enzymatic	Enzymatic hydrolysis was found to be efficient	(Nahak, Nahak, Pradhan, & Sahu, 2011)

				degradation		
Supercritical carbon dioxide extraction						
Fucoxanthin, phenolic compounds	*S. horneri* and *S. japonica*	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 Sargassum horner via SC-CO_2, gave high fucoxanthin yields and better biological activities compared to S. japonica.	(Sivagnanam, et al., 2015)
Fucoidan	*Saccharina japonica* and *Sargassum*	Co solvents using supercritical CO_2	Ethanol as co-solvent	Pressure = 550 bar, Temperature = 60 °C, 5% ethanol as co-	Supercritical CO_2 with 5% ethanol gave an improved yield of	(Men'shova, et al., 2013)

	oligocystum			solvent	fucoidan	
Fucoxanthin and phlorotannin, carotenoids	*S. japonica*	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	*S. muticum*	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)

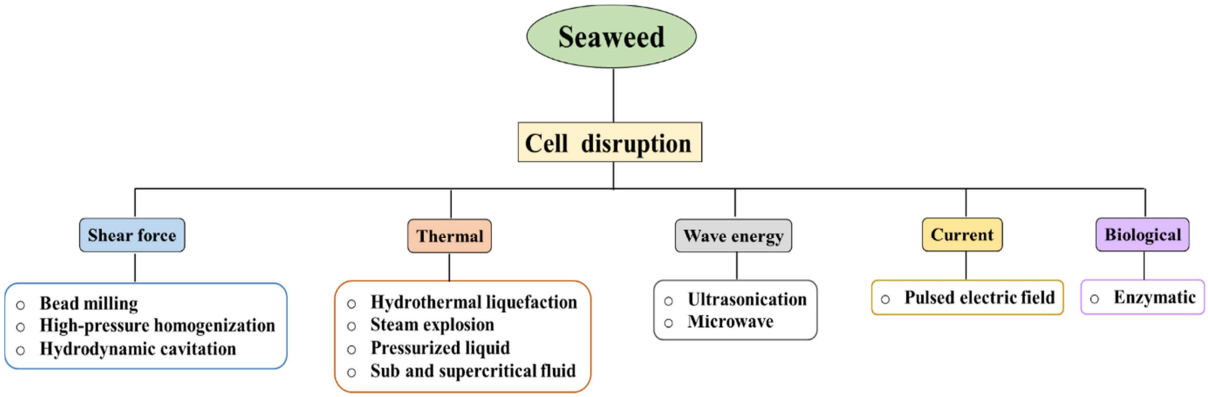

Fig 1. Classification of cell disruption methods employed in seaweed applications.

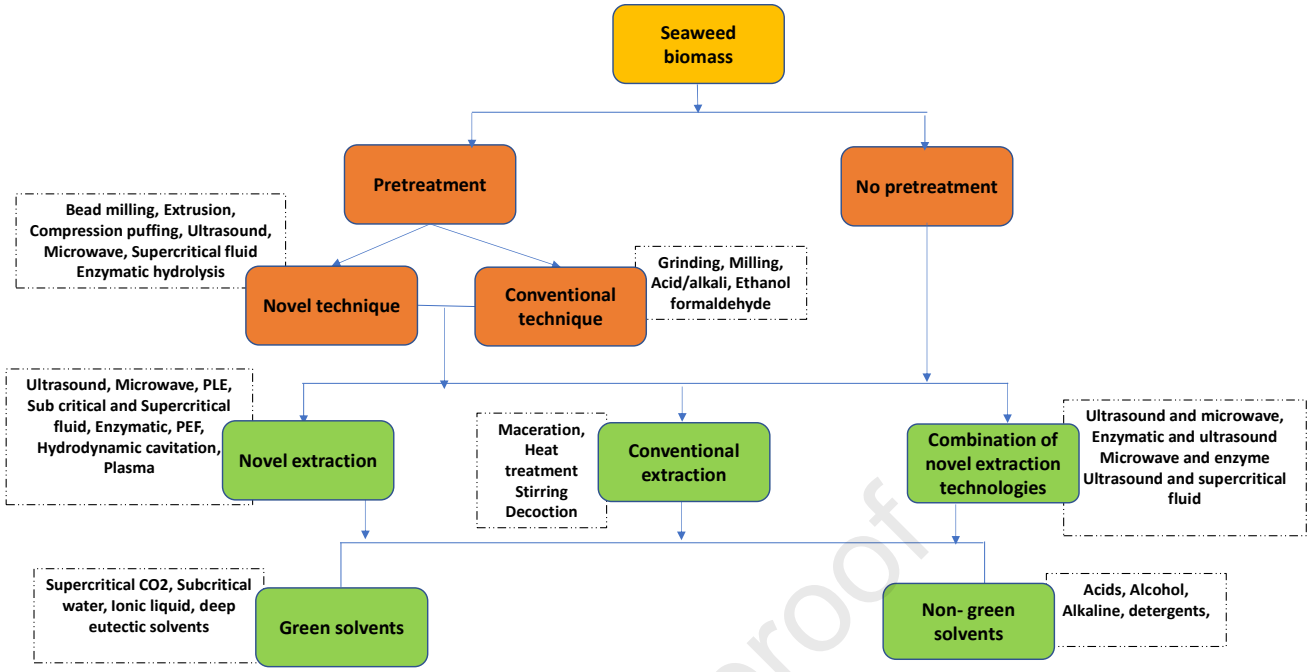

Fig.2. Overview of extraction processes for extraction of seaweed bioactive compounds.

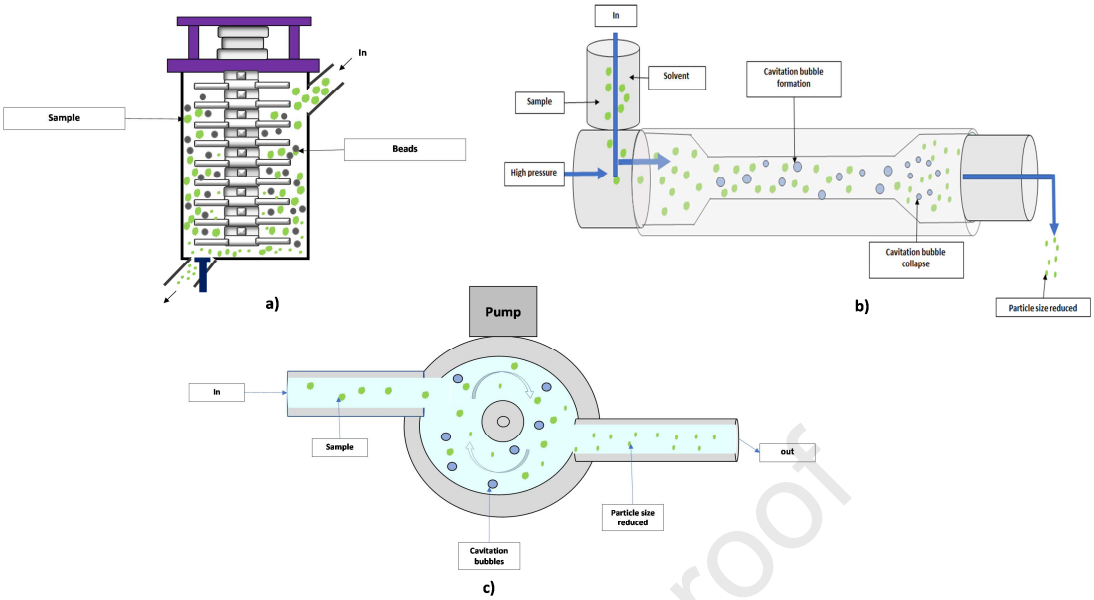

Fig. 3. Different types of shear-force disruption instruments: a) Lab scale bead milling system b) Lab scale high-pressure homogenizationMN250A, and c) ROTOCAV hydrodynamic cavitators

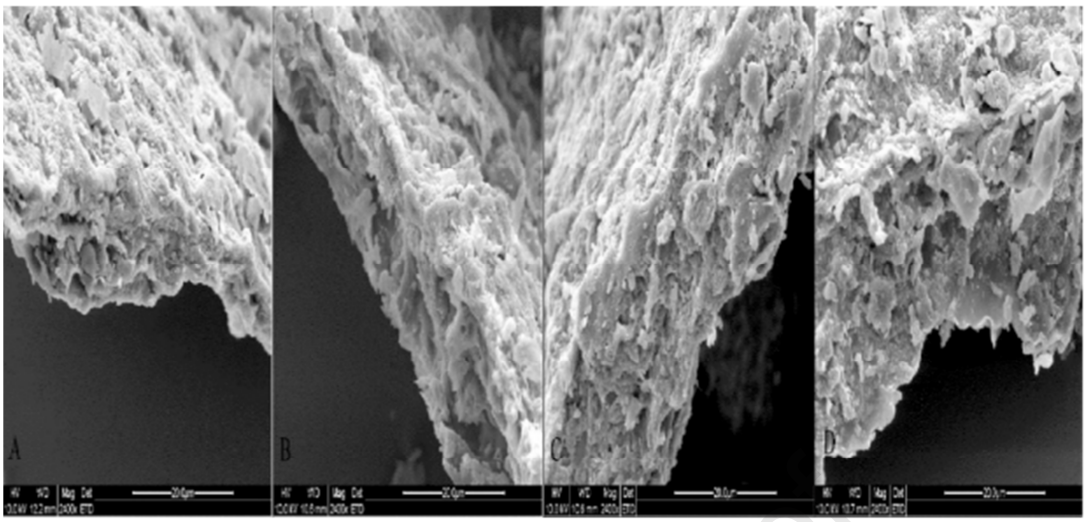

Fig. 4. Scanning electron micrographs of *N. decipients* power: (A) untreated sample; (B–D) sample obtained after homogeneous processing at 40 MPa, 70 MPa and 100 MPa, respectively, 2 cycles. Magnification: 2400-fold. (Li, et al., 2017).

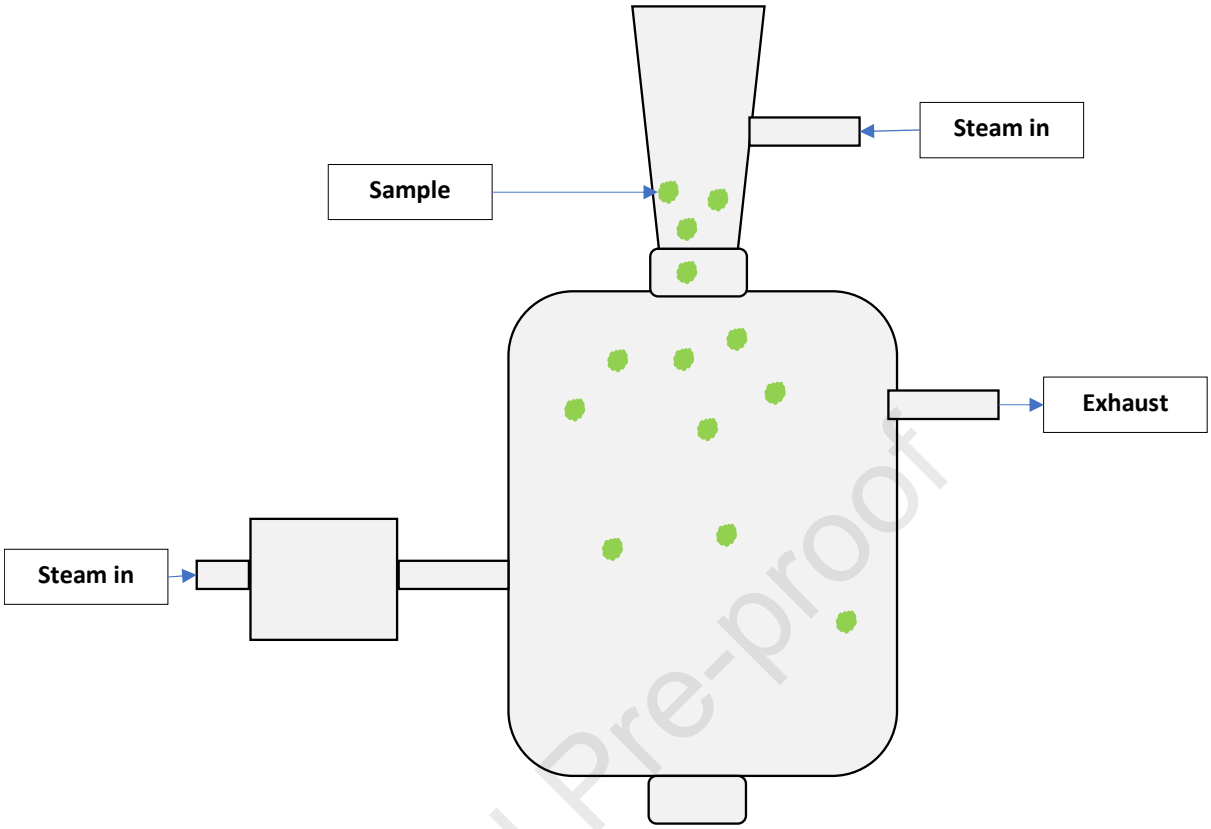

Fig. 5. Steam explosion equipment for lab-scale experiments. The lid had an inlet of steam, a temperature measurement device, and a larger vent used for release of pressure. The autoclave was put in an insulated outer beaker to more easily maintain the desired temperature.

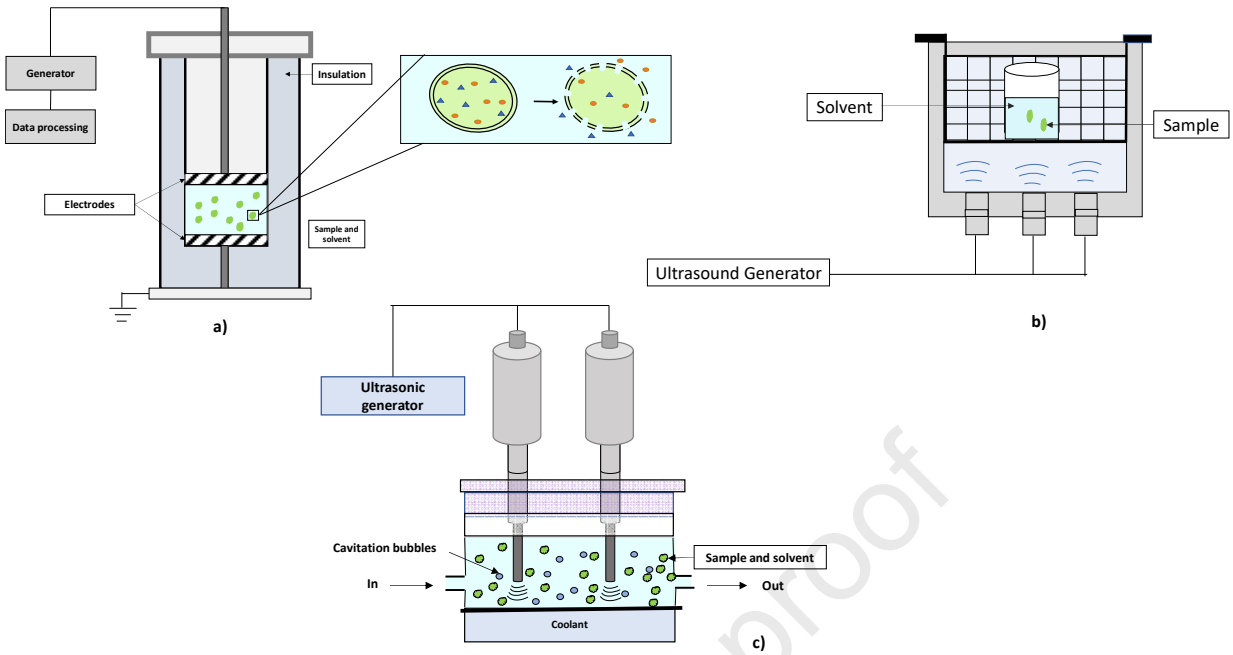

Fig. 6. a) Pulsed electric field system- ELEA PEFPILOT b) Ultrasound water bath and c) UIP 2000hdT – the new digital 2000 Watts industrial ultrasonicator

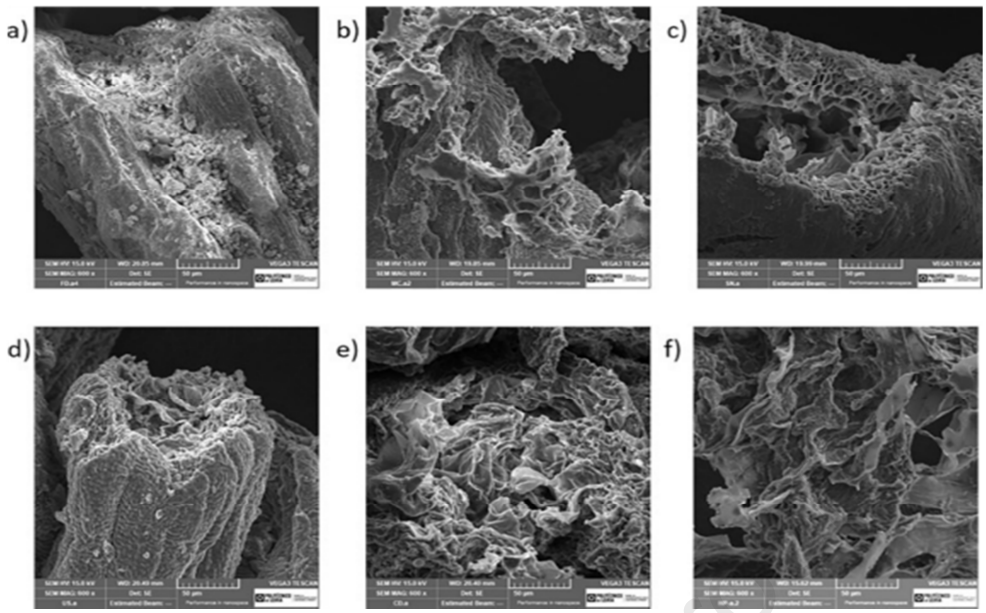

Fig. 7. SEM images of the *Gracilaria gracilis* biomass cells a) before and b)-f) after the extraction treatments (b – maceration, c – ultrasonic probe, d - ultrasonic bath, e – freeze-thaw, f - high pressure-assisted extraction) at a magnification of 600×.(Pereira, et al., 2020).

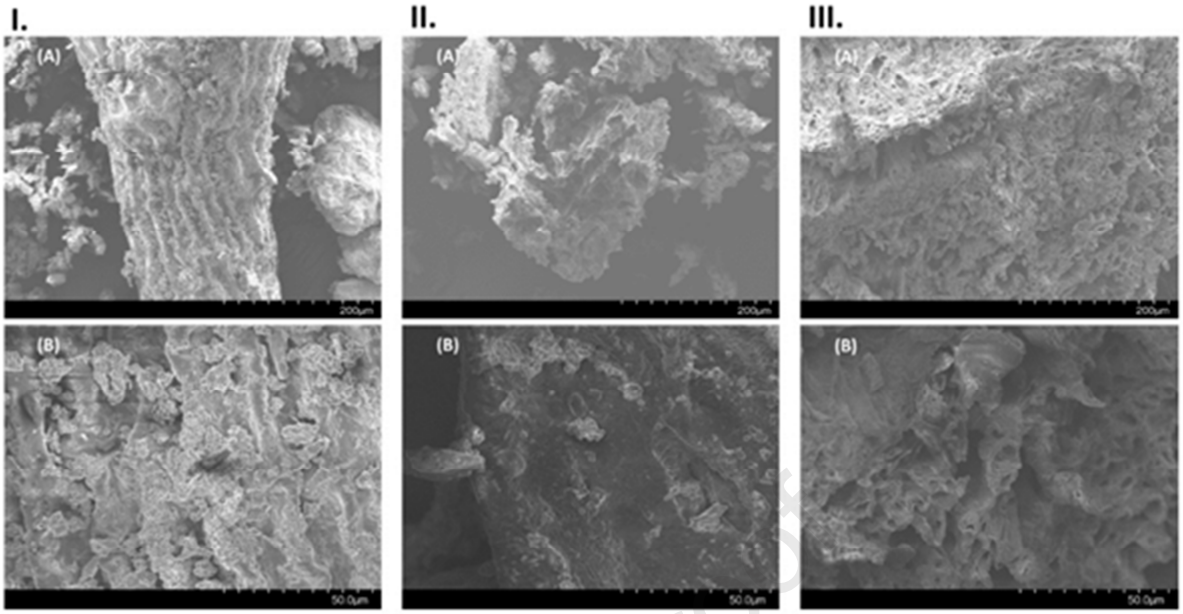

Fig 9. Scanning electron microscopy images of (I) dried and milled A. nodosum biomass before extraction, (II) macroalgal residue after MAE (250 W, 2 min) and (III) macroalgal biomass after the process of UMAE (1000 W, 100%, 5 min). Scale bars (A) 200 µm (magnification: 250×) and (B) 50 µm (magnification: 1000×).

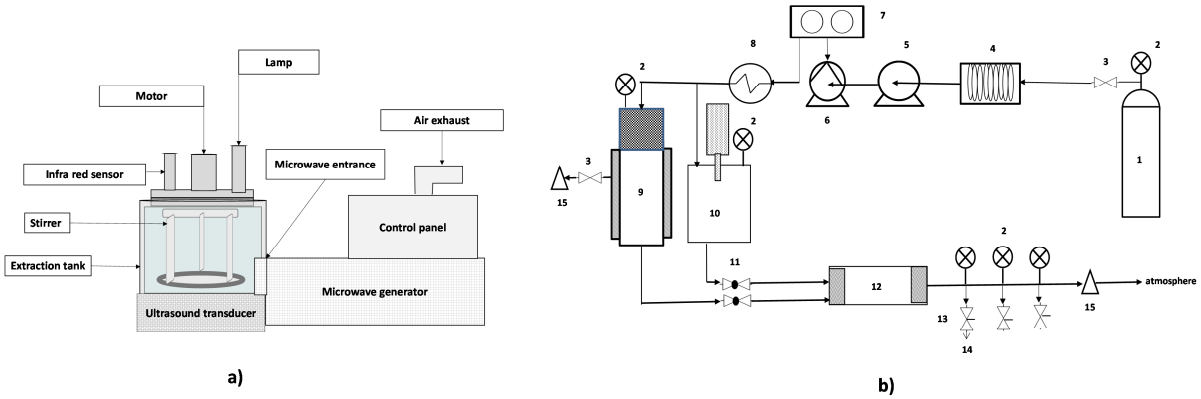

Fig. 10. Semi pilot scale extracting instruments a) Ultrasound and microwave combined process and b) Sub-supercritical carbon dioxide extraction instrument coupled with ultrasound system

Highlights

1. **Comprehensive assessment of pre-treatment technologies**
2. **Novel extraction techniques for bioactives from seaweeds**
3. **Mechanism of pre-treatment and extraction techniques**
4. **Factors influencing extraction of bioactives from seaweeds**