Chapter 6

Antioxidant Activity of Sulfated Seaweeds Polysaccharides by Novel Assisted Extraction

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Additional information is available at the end of the chapter

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Abstract

Seaweeds have an extremely numerous of species in the world and been able to be divided into several developmental systems. Broadly, three types of seaweeds can be defined according to their color: brown seaweeds, green seaweeds, and red seaweeds. Thousands of years ago, mankind used seaweeds as food and medicine. Seaweed extracts are gaining increasing attention due to their unique composition and the potential for widespread use in industry. A variety of novel (green) extraction techniques have been devised for converting seaweed biomass into seaweed extracts, such as enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), and ultrasound-assisted extraction (UAE), which are capable of extracting seaweeds' biologically active compounds without causing degradation. Seaweed extracts contain compounds, such as carbohydrates, proteins, minerals, oils, fats, and polyunsaturated fatty acids and abundant bioactive compounds, such as antioxidants, pigments, and sulfated seaweed polysaccharides (SWP), as well as antibacterial, antifungal, anti-inflammatory, antioxidation, antitumor, antiviral, and hypolipidemic effect. The purpose of this article is to describe the antioxidant activity of SWP of brown seaweeds, green seaweeds, and red seaweeds by novel assisted extraction.

Keywords: antioxidant activity, assisted extraction, sulfated seaweed polysaccharides

1. Introduction

Edible seaweeds are a good source of antioxidants, dietary fibers, essential amino acids, vitamins, phytochemicals, polyunsaturated fatty acids, and minerals [1]. Most seaweed polysaccharides (SWP) are derived from natural sources, such as agarose, alginates, carrageenan, fucoidan, porphyran, and ulvan. Seaweed polysaccharide is a very important biological macromolecule in marine life. Seaweed polysaccharides exhibit a wide range of structure and are

still underexploited and should therefore be considered as a novel source of natural products for pharmaceutical discovery [2].

Seaweeds are plant-like organisms that generally live attached to rock or other hard substrata in coastal areas. They can be classified into three different groups, empirically distinguished since the mid-nineteenth century on the basis of thallus color: brown seaweeds (Phaeophyceae), green seaweeds (Chlorophyceae), and red seaweeds (Rhodophyceae) [3, 4]. Among marine resources, seaweeds, which are sometimes known as algae, are well-known natural sources of polysaccharides. SWP are well-known natural seaweed sources of polysaccharides which have considerable numerous bioactive compounds with significant biological features. SWP are most common in the seaweed cell walls, and their number and chemical structure are varying according to the specific seaweeds' species [5]. Bioactive SWP extracted from seaweeds can be classified into three types. The major SWP found in brown seaweeds were fucan and fucoidan; green seaweeds were sulfated rhamnans and ulvan; red seaweeds were galactan and carrageenan [6]. It displays several physicochemical and biological features of potential interest for agricultural, chemical, food, and pharmaceutical applications [7].

The purpose of this chapter is to understand the potential applications of SWP in antioxidant activity of brown seaweeds, green seaweeds, and red seaweeds by enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), and ultrasound-assisted extraction (UAE), also named green-assisted extraction in recent literary.

2. Novel assisted extraction

Marine plant materials are increasingly being used to isolate and purify bioactive compounds; and then recent studies have reported on the antioxidant potential of SWP of seaweeds [8, 9]. Traditional techniques involve application of solid-liquid extraction (SLE) simply by means of solvent application and leaching. A domestic application of conventional solvent extraction (CSE) is quite familiar to everybody in daily life from making of coffee or tea at home. SLE encompasses conventional methods: Soxhlet extraction (SE), percolation, and maceration extraction (ME). These techniques have been utilized for more than a century for the separation of SWP. However, certain disadvantages pertaining to CSE render its application quite uneconomically due to excessive consumption of energy, polluting solvents, and time. These underlying drawbacks have triggered research that explores more cost-effective and greener techniques for the extraction of SWP from a wide range of seaweed matrices [10]. They include EAE, MAE, PLE, SFE, and UAE techniques [11]. The advantages and disadvantages of these novel assisted extraction methods are shown in **Table 1**.

2.1. EAE

Enzymes can be derived from animal organs, bacteria, fruit extracts, fungi, or vegetable. All these known enzymes are classified according to six basic groups. These categories are organized according to how the enzyme works on a molecular level. These six types of

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Novel assisted extraction methods	Advantages	Disadvantages	References
EAE	1. Non-consumed during reaction 2. High conversion yield 3. Nontoxic and biodegradable 4. High selectivity 5. High specificity 6. Utilization in soft conditions 7. Large-scale production	1. Stability 2. Cost	[23]
MAE	1. Decreased in extraction time 2. To avoid the loss of volatile substances during microwave 3. Less solvent is required because no evaporation occurs 4. No hazardous fumes during acid microwave since it is a closed vessel	1. High pressure used poses safety risks 2. The usual constituent material of the vessel does not allow high solution temperatures 3. Addition of reagents is impossible sine it is a single-step procedure 4. Vessel must be cooled down before it can be opened to prevent loss of volatile constituents	[24–26]
PLE	1. Better for increased operating temperature 2. Increase selectivity 3. Precision and reproducibility 4. Reduces oxidation risk 5. Relatively simple compared to SFE 6. Shorter extraction time 7. Reduced solvent consumption 8. Possibility for automation	1. Thermal degradation for thermolabile compounds is a cause for concern 2. Selectivity is mainly affected by varying the solvent type 3. Post-extraction cleanup step is still necessary	[27]
SFE	1. Enhanced extraction efficiency 2. Tunability of the solvent strength 3. Low organic solvent consumption 4. Preservation of bioactive properties 5. Organoleptic properties of the extracts 6. Inline integration with sample preparation and detection methods	1. High-capital investment 2. Large number of variables to optimize 3. Strong dependence on matrix analyte interactions 4. Difficulties in scale-up 5. Difficulties in technology transfer 6. Difficulty in implementing continuous extraction processes 7. Difficulty of extracting more polar compounds	[27]
UAE	1. Easy to use 2. Short time of extraction 3. Small amount of solvent	1. No good recoveries for most PCB congeners 2. Expensive system	[28]

Table 1. Advantages and disadvantages of novel assisted extractions.

enzymes are as follows: hydrolases, isomerases, ligases, lyases, oxidoreductases, and transferases. Hydrolases are the most common type, followed by oxidoreductases and transferases. Enzymes are ideal catalysts that can assist in the extraction of complex bioactive compounds of natural origin by degrading the plant cell walls and membranes. Consequently, they increase plant cell wall permeability, and thus higher extraction yields of bioactive compounds are achieved [12].

Seaweed cell walls are composed of a diverse array of fibrillar, matrix, and crystalline polymers, that is, sulfated and branched polysaccharides, interacting with proteins, various ions, and water. It is necessary to break down seaweed cell walls with enzymes that can be used to remove the cell wall specifically under optimal temperature and pH conditions and then get the desired bioactive compounds [11], depending on what organism you work with, which can be agarase [6], Alcalase [13], carragenanase [14], Celluclast [15], Kojizyme [16], Neutrase [17], Termamyl [18], Ultraflo [19], Umamizyme [20], Viscozyme [21], and xylanase [22].

2.2. MAE

MAE is a novel technique that has many advantages including high extraction efficiency, low solvent consumption, high-purity extracts, and shortened extraction time, which make it well suited for the extraction of bioactive compounds from plant materials [29]. The mechanism of microwave volumetric heating involves the inherent ionic conduction and dipolar relaxation inside a dielectric material. Microwave irradiation induces the rapid elevating temperature of solvent to accelerate the diffusion of pure solvent into plant matrix, as well as the dissolution of the targeted compound into solvent [30]. Microwave energy penetration causes quick elevation of temperature to build the internal pressure inside the cell of plant material. The high interior pressure may destroy the cell wall of plant material to easily release bioactive compounds into solvents [26]. High temperature would cause the dehydration of cellulose and reduce its mechanical strength in MAE, which promotes the solvent to penetrate into the cellular channels and subsequently increase the extraction yield [31].

Microwave is an electromagnetic radiation with wavelengths ranging from 1 m to 1 mm, with frequencies between 300 MHz (100 cm) and 300 GHz (0.1 cm), which can be transmitted as the wave [32]. When microwave passes through the seaweed medium, its energy may be absorbed and converted into thermal energy. Heating using microwave energy is based on two principles: (1) ionic conduction refers to the electrophoretic migration of the charge carriers (e.g. ions and electrons) under the influence of the electric field produced by microwave [33] and (2) dipole rotation happens when the dipolar molecules attempt to follow the electric field in the same alignment [34].

There are two main types of MAE systems available for industrial and commercial applications for natural product extraction: (1) the closed-vessel system and (2) the open-vessel system [10]. In the closed-vessel system, extraction is carried out under controlled conditions of temperature and pressure. This is generally employed for extractions under extremely high-temperature conditions. Diffused microwaves from a cavity magnetron radiate in all directions to interact with plant samples placed in extraction vessels in a closed-vessel chamber. Owing to the even dispersion of microwaves, this technique is also known as the multimode system [35]. In the open-vessel system, also known as the monomode system, the extraction vessel is partially exposed to microwave radiation (focused radiation). A circular metallic waveguide directs the focused microwaves toward the extraction vessel inside the microwave (monomode) cavity. This interaction promotes the initiation of mass transfer between the solute and extractant upon solvation [36].

2.3. PLE

PLE is another novel assisted extraction technique for natural product extraction from food and botanical sample matrices. The Dionex Corporation was the first to introduce the PLE technique as an accelerated solvent extraction (ASE) technology (ASER®) in 1995 [37]. PLE is called accelerated solvent extraction (ASE), enhanced solvent extraction (ESE), pressurized fluid extraction (PFE), or pressurized solvent extraction (PSE). When the solvent used is water, it is common to use other terms, such as high-temperature water extraction (HTWE), hot liquid water extraction (HLWE), hot water extraction (HWE), pressurized hot water extraction (PHWE), subcritical water extraction (SWE), or superheated water extraction (SWE) [38].

The principle of the PLE is based on using elevated temperatures (50–200°C) and pressures (50–150 atm) to extract analytes from solid or semisolid samples within short periods of time (5–15 min). PLE is similar to Soxhlet extraction, except that during the extraction process, the solvent condition inside the PLE cell approaches the supercritical region which results in more efficient extractions. Depending on the temperature at which the extraction is performed, PLE allows the sample to become more soluble and achieve a higher diffusion rate, while the elevated pressure keeps the solvent below its boiling point [39]. PLE permits high extraction efficiency with a low solvent volume (15–40 mL) and a short extraction time (15–20 min). PLE has used less solvent in a shorter period of time, and in oxygen and light-free environment, it has the potential to be a powerful tool in industry [40–42].

2.4. SFE

SFE is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent [43]. The supercritical fluid state occurs when a fluid is above its critical temperature (Tc) and critical pressure (Pc), when it is between the typical gas and liquid state. Manipulating the temperature and pressure of the fluid can solubilize the material of interest and selectively extract it. The unique physical properties of supercritical fluids have values for density, diffusivity, and viscosity between liquids and gases. Moreover, near-zero surface tension as well as low viscosities similar to gases allow supercritical fluids to easily penetrate into a microporous matrix material to extract desired compounds [44].

Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by cosolvents, such as ethanol or methanol. Since the critical temperature and critical pressure of CO_2 are only 31°C and 74 bar, extraction is done at temperatures that will not damage heat labile molecules, and the absence of oxygen minimizes oxidation. Thus, an extraction process can take 10–60 min with SFE, while it would take hours or even days with classical methods [45, 46]. On the other hand, it is to be noticed that no organic residue is found both in extract and solid residue and no thermal degradation appears, which results in very high-quality products [47].

2.5. UAE

Ultrasound is sound waves with frequencies higher than the upper audible limit of human hearing. This limit varies from person to person and is approximately 20 kHz (20,000 Hz) in

healthy young adults. Ultrasound devices operate with frequencies from 20 kHz up to several GHz [48]. Sound waves produced by an ultrasonic probe allow greater penetration of solvent into the seaweeds, and ultrasonic power also produces high-energy cavitation bubbles containing solvent vapor. The bubbles implode near seaweed walls causing very high local temperatures, pressure increase, and seaweed cell wall destruction, which eases mass transfer from cell to solvent and enhances microstreaming [49]. This effect is much stronger at low frequencies (18–40 kHz) [50].

UAE has emerged as a promising technique that fulfills the required criteria as an inexpensive green extraction technique providing higher recovery of targeted compounds with lower solvent consumption and/or faster analysis and bioactivity properties. Notable UAE features include cost-effectiveness, eco-friendliness, rapidity, simplicity, safety, and versatility, due to the reduced consumption of time, energy, and expensive organic solvents, which is in contrast to traditional extraction techniques [10].

3. Sulfated seaweed polysaccharides (SWP)

Seaweeds are the most abundant source of nonanimal in nature. SWP from some seaweeds have become very important products in the food industry and also possess biological activity of potential medicinal value, such as anti-allergy, anticancer, anticoagulant, anti-inflammation, antioxidant, and antiviral [9, 51–53]. SWP are commonly found in three major groups of seaweeds: brown seaweeds (Phaeophyta), green seaweeds (Chlorophyta), and red seaweeds (Rhodophyta). The major SWP of brown seaweeds are fucans, including fucoidan, sargassan, ascophyllan, and glucuronoxylofucan; and those of red seaweeds are galactans commercially known as agar and carrageenan. On the other hand, the major SWP of green seaweeds are usually sulfated heteropolysaccharides that contain galactose, xylose, arabinose, mannose, glucuronic acid, or glucose [54].

3.1. Brown seaweeds

There are about 1800 species of brown algae, and most are marine. In general, brown algae are larger and more species are found in colder waters. Brown seaweeds are usually grown or collected for food consumption and especially known for their health benefits and high nutritional value, such as kombu or haidai (*Laminaria japonica*), wakame or quandai-cai (*Undaria pinnatifida*), hijiki (*Hizikia fusiforme*), and mozuku (*Cladosiphon okamuranus*). The major fucoidan yielding brown seaweed genera are *Fucus*, *Sargassum*, *Laminaria*, and *Undaria* [55].

The term fucoidan is commonly applied for complex SWP, often isolated from seaweeds, mainly containing fucose residues but also many other monosaccharides [7]. Furthermore, fucoidan has a backbone built of (1→3)-linked α-l-fucopyranosyl or of alternating (1→3)- and (1→4)-linked α-l-fucopyranosyl residues, also including sulfated galactofucans with backbones built of (1→6)-β-D-galacto- and/or (1→2)-β-d-mannopyranosyl units with fucose or fuco-oligosaccharide branching and/or glucuronic acid, xylose, or glucose substitutions. There are at least two distinct forms of fucoidan: U-fucoidan, which is approximately 20% glucuronic acid, and F-fucoidan, which is >95% composed of sulfated esters of fucose [56].

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Fucoidans with greater molecular masses and higher degrees of sulfatation form solutions of higher viscosity. Adding glycerol and diols also leads to a significant increase in viscosity [57]. Rheological characteristics of fucoidan from *C. okamuranus* showed shear thinning behavior below 1.5% (W/V) but plastic behavior at 2.0% (W/V). The dynamic viscoelasticity of the fucoidan increased linearly with an increase in concentration and decreased gradually with increase in temperature [58]. Fucoidan with covalent linkage of bovine serum albumin had emulsifying properties, high solubility after heating, and high melting temperature [59]. Crude fucoidan from *Sargassum* sp. demonstrated good emulsion-stabilizing capacities, especially with cedar wood oil and xylene [60].

3.2. Green seaweeds

Green seaweeds are usually grown or collected for food consumption and especially known for their health benefits and high nutritional value, such as aonori, hirohano-hitoegusa nori, or hitoegusa-nori (*Monostroma* spp.) or green laver (*Enteromorpha* spp.). SWP from green seaweeds can be found in *Caulerpa* (sulfated galacotans), *Codium* (sulfated arabinogalactans), *Enteromorpha* (ulvans), *Monostroma* (sulfated rhamnans), and *Ulva* (ulvans) [61].

The structural diversity of SWP found in seaweeds varies with species. Water-soluble extract polysaccharides from *Caulerpa* are mainly composed of glucans and SWP. Heteropolysaccharides (SWP) from *Caulerpa* consist of different monosaccharides, such as galactose, glucose, mannose, and xylose. Among them, galactose is the major sugar source, while glucose, mannose, and xylose are common components. The water-soluble fraction obtained from *Caulerpa sertularioides* with antimicrobial effects which grown under natural conditions contains sulfated galactans constituted of (1→3)-β-D-Gal and (1→6)-β-D-Gal units, and sulfation is observed to occur at the C-2 position of the residues [62]. A water extraction of SWP from *Codium divaricatum* with anticoagulant activity is a galactan which is highly sulfated and substituted with pyruvic acid ketals was mainly constituted of (1→3)-β-D-galactopyranose residues, branched by single (1→)-β-D-galactopyranose units, and the backbone of CP2-1 attached to the main chain at C-4 positions [63]. *Monostroma nitidum* extracted with boiling water could obtain rhamnan sulfate with antithrombin active that consists of *α*-1,3-linked L-rhamnose residues, some substituted with sulfate groups mainly at position O-2. Minor amounts were also exist internal 1,2-linked rhamnose and branched rhamnose linkages [64]. The structure of an ulvan with anticancer obtained by water extraction from *Ulva lactuca* consists of galactose, glucose, mannose, rhamnose, xylose, uronic acid, and sulfate content. This ulvan is mainly composed of disaccharide [→4)-β-d-GlcA-(1→4)-*α*-l-Rha3S-(1→] and other minor disaccharides β-GlcA-(1→2)-*α*-Xyl and β-GlcA-(→2)-*α*-Rha [65].

Dielectric properties of aqueous solutions from ulvan (*U. meridionalis*) and rhamnan sulfate (*M. lattisimum*) with H^+-form hydrocolloids possess significant improvement in hydration function [66]. Ulvan of *Ulva armoricana* and *Ulva rotundata* showed that chemical structure, macromolecular characteristics, and rheological properties were affected by both species and seasons. The proportion of high-molecular-weight ulvan was the major factor positively correlated with the gelling properties [67]. Ulvan from *Ulva fasciata* in different ionic strengths (Na^+ and Ca^{2+}) had significant effects on the stability of o/w emulsions [68]. The rheological properties and zeta-potential of the emulsions appeared to be dependent on the ulvan

concentration. The emulsifying and stabilizing mechanism of the ulvan may ascribe to its surface-active protein moiety, also to the hydrophobicity of the polysaccharide itself [69].

3.3. Red seaweeds

Red seaweeds are usually grown or collected for food consumption and especially known for their health benefits and high nutritional value, such as nori or purple lave (*Porphyra* spp.), ogo, ogonori, or sea moss (*Gracilaria* spp.) The red seaweeds *Gracilaria* and *Gelidium* are used in the manufacture of the agar, *Kappaphycus* and *Betaphycus* are now the most important sources of carrageenan, and *Porphyra* could extract porphyran for food and biotechnological applications [70].

Carrageenan are high-molecular-weight sulfated D-galactans composed of repeating disaccharide units with alternating 3-linked β-D-galactopyranose and 4-linked α-galactopyranose or 3,6-anhydro-α-galactopyranose [7]. There are at least 15 different carrageenan structures, including but not limited to κ-, ι-, λ-, μ-, θ-, β-, and ν-carrageenan [20]. Porphyran has hypolipidemic and antiallergic pharmacological property applications, is a complex SWP, and is consist of about 30% of agarose repeating units (1,3-linked β-D-galactopyranose followed by 1,4-linked 3,6-anhydro-α-L-galactopyranose), with the remaining residues being 3-linked β-D-galactopyranose and 4-linked α-L-galactopyranose-6-sulfate. The composition includes 6-O-sulfated L-galactose, 6-O-methylated D-galactose, L-galactose, 3,6-anhydro-L-galactose, 6-O-methyl D-galactose, and ester sulfate. Some of the ester is present as 1-4-linked L-galactose 6-sulfate [71].

Kappa carrageenan showed that shear stress-shear rate and viscosity curves clearly indicated sharp increases in viscosity and consistency coefficient (k), and addition of KCl was more effective in increasing viscosity [72]. Kappa-carrageenan film incorporation of plant oils increased the film thickness and plasticizing effect significantly. However, the moisture content, solubility, and tensile strength of films decreased significantly when plant oils were added [73]. Six formulations carrageenan/porphyran films from *Pyropia columbina* were homogeneous and flexible. Their moisture content, water solubility, and water vapor permeability significantly increased by glycerol content increasing could obtain more stretchable but less resistant films. When Ca^{+2} addition could mask glycerol effect in water solubility and water vapor permeability, it could stabilize the three-dimensional structure of carrageenans/porphyrans by interactions between sulfate groups, promote water retention, and open film structure [74]. Porphyran, alkali-treated porphyrin, and sulfated porphyran from *Porphyra haitanensis* demonstrate that the anticoagulant activities mainly depend on the position of sulfate and the antioxidant activities mainly depend on degree of sulfate substitution [75]. The rheological behavior of porphyran exist pseudoplastic behavior, which agrees with the Herschel-Bulkley model. The effect of temperature on the viscosity of porphyrans was increasing concentrations from 3 to 7% [76].

4. Antioxidant activity of SWP

Reactive oxygen species (ROS) is produced in the forms of H_2O_2, superoxide radical (O^{-2}), hydroxyl radical (•OH) and nitric oxide (NO) in the organisms are produced by non-enzymatic and enzymatic reactions. Moreover, endogenous antioxidant enzymes and antioxidants

in the body under synergy effect would been removed these ROS. But when the body is aging and experiences illness or fatigue, the body's free radicals may be destroyed, and excess oxygen free radicals cause a series of oxidative damage to the body. Excessive free radicals can cause irreversible damage to the body. It can cause damage to the body at molecular level, cell level, and level of tissue and organs by attacking the life molecules and various kinds of cells [77]. Oxidative stress and disease have resulted in identification of important oxidative stress biomarkers—the products of oxidation of biological molecules: DNA, lipids, and proteins. It would lead to many health disorders including cancer, diabetes, cardiovascular, inflammatory, and neurodegenerative diseases [78]. SWP from a number of seaweeds has appreciable antioxidant capability. Antioxidant activity of SWP has been determined by various in vitro methods, such as ABTS radical scavenging [79], 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging [80], ferric reducing antioxidant power (FRAP) [81], lipid peroxide inhibition [82], NO scavenging [83], and superoxide and hydroxyl radical scavenging assays [84, 85].

4.1. SWP by EAE

Enzymes are more action specific and operate at lower temperature during hydrolysis; thus, EAE is more amicable for degradation of biological materials without damaging the bioactive compounds. The extract of *Ecklonia cava* (brown seaweed) obtained using Celluclast was compared with extract obtained by organic solvents. Methanol extract, which gave the highest radical scavenging activities among the organic solvents investigated, was 20% less than the extract obtained by Celluclast [86, 87].

EAE was performed using Alcalase, cellulase, flavourzyme, and Viscozyme L applied on *Sargassum muticum* (brown seaweed), *Codium tomentosum* (green seaweed), and *Osmundea pinnatifida* (red seaweed). SWP with higher extraction yields were observed for *Co. tomentosum* EAE (for cellulase and Viscozyme L), followed by *O. pinnatifida* (except Alcalase) and *S. muticum*. A higher effect on hydroxyl-radical scavenging activity (35–50%) was observed for all SWP extracts [88].

The extracts of *U. armoricana* (green seaweed) were determined to assess the efficiency of endo-protease treatments which significantly increased the extraction yields compared to the control. The organic matter, neutral sugar, and protein contents were increased in all extracts compared to an extraction with water, up to 2.0-, 2.7-, and 1.75-fold, respectively. Free radical scavenging capacity at medium inhibition concentrations (IC_{50}) of 1.8 and 12.5 mg/mL was shown for the extracts produced with endo-protease treatments and 6.0 mg/mL for the sample resulting from the extraction with the multiple mix of glycosyl hydrolases [89]. Enzyme-assisted extraction of *Un. pinnatifida* (green seaweed) was performed using five proteases (Alcalase, Flavourzyme, Neutrase, trypsin, and Protamex) and six carbohydrases (AMG, Dextrozyme, maltogenase, Promozyme, Viscozyme, and Celluclast). SWP of *Un. pinnatifida* exhibited strong radical scavenging activity on DPPH and hydroxyl radical, and activity increased with increasing concentration [90].

SWP were extracted from *Pterocladia capillacea* (red seaweed), and the produced fractions were hydrolyzed using different enzymes, such as Viscozyme L (a multienzyme complex containing arabanase, cellulase, β-glucanase, hemicellulase, and xylanase), β-glucanase from

Trichoderma longibrachiatum, and β-galactosidase from *Kluyveromyces lactis*. The Viscozyme hydrolysate exhibited DPPH scavenging activity of about 92% with more than 50% increase over its mother fraction [27]. Three SWP were obtained from the *Mastocarpus stellatus* (red seaweed) using Alcalase hydrolysis at three different treatments. The Alcalase enzymatic hydrolysis at 50°C provided films with reducing power and radical scavenging capacity, which did not change as a result of subsequent heat treatment at 90°C. Their viscoelastic properties of the film-forming solutions showed improved gel-like behavior when the κ/ι-hybrid carrageenan extraction at 90°C was promoted [91].

4.2. SWP by MAE

SWP (fucoidan) from *Ascophyllum nodosum* (brown seaweed) were extracted by MAE technology. Different conditions of temperature (90–150°C) and extraction time (5–30 min) were evaluated, and optimal fucoidan yield was 16.08%, obtained from 120°C for 15 min of extraction. All SWP from *A. nodosum* exhibited antioxidant activities as measured by DPPH scavenging and reducing power, among which SWP extracted at 90°C was the highest [62]. SWP were extracted from *Fucus vesiculosus* (brown seaweed) seaweed by using MAE. The SWP obtained by MAE that contained 53.8 mole% of fucose, 35.3 mole% of xylose, and 10.8 mole% of galactose presented comparable values of antioxidant activity by the DPPH, $ABTS^{+}$, and lipid oxidation inhibition methods [92].

Six representative (molecular weight 446.5, 247.0, 76.1, 19.0, 5.0, and 3.1 kDa) SWP from *Enteromorpha prolifera* (green seaweed) were extracted by MAE. All samples showed that great inhibitory effects on superoxide radical at a low concentration and high molecular weight exhibited higher inhibitory effects. Otherwise, samples with low molecular weight possessed stronger inhibitory effects on hydroxyl radical; IC_{50} of molecular weight 3.1 kDa was 0.39 mg/mL. The chelating effect of molecular weight 3.1 kDa was 77.3% at 5 mg/mL, which was twice more than initial polysaccharide [93].

Four SWP were extracted from *Durvillaea antarctica* (brown seaweed), *Sarcodia ceylonensis* (brown seaweed), *U. lactuca* L. (green seaweed), and *Gracilaria lemaneiformis* (red seaweed), respectively, by MAE. The average molecular weight of SWP of *D. antarctica*, *S. ceylonensis*, *U. lactuca* L., and *G. lemaneiformis* was 482, 466, 404, and 591 kDa, respectively. The in vitro antioxidant activity of all of the polysaccharides was evaluated using $ABTS^{+}$, hydroxyl radical, nitrite scavenging capacity, and reducing power. SWP of *U. lactuca* L. presented the highest ABTS radical scavenging activity; SWP of *U. lactuca* L., *S. ceylonensis*, and *D. antarctica* also showed a strong effect on the ABTS radical scavenging activity. SWP of *U. lactuca* L. and *S. ceylonensis* exhibited excellent hydroxyl-radical scavenging activities, about 83.33% ± 2.31% and 80.07% ± 2.17%, respectively, at 4 mg/mL. The reducing power of SWP of *D. Antarctica* was relatively more pronounced than that of the three other polysaccharides. However, the nitrite scavenging activities of the four seaweed polysaccharides were weaker than other antioxidant activities (ABTS), hydroxyl radical scavenging capacities, and reducing powers [94].

SWP from *Porphyra dentate* (red seaweed) is adjusting the pH value of the ethanol solutions used as extraction solvents and then applying continuous or intermittent MAE to extract *P. dentate* solutions. The SWP content was significantly affected by ethanol concentration, pH value of the

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ethanol solution, and intermittency at a 1% significance level. It also showed high antioxidant activities by the DPPH and FRAP. Gelling property of the extracted SWP was not affected [95].

4.3. SWP by PLE

PLE was utilized to extract SWP from *Saccharina japonica* (brown seaweed). Various conditions of temperature (80–200°C), pressure (5–100 bar), and solvents (water, 0.1% sodium hydroxide, 0.1% formic acid, 70% ethanol, 50% ethanol, and 25% ethanol) were assessed; the best crude SWP yield was 8.23%, obtained from 140°C and 50 bar (sodium hydroxide). All crude SWP showed antioxidant activities as measured by DPPH radical and $ABTS^+$ radical scavenging. Crude SWP demonstrates good emulsion-stabilizing capacities, especially with vegetable oils [96].

4.4. SWP by SFE

Gracilaria mammillaris (red seaweed) from the Colombian Caribbean coast were investigated as a source of extracts with antioxidant activity, by means of supercritical CO_2 extraction with ethanol as cosolvent. A central composite design was used to investigate the effects of pressure (10, 20, and 30 MPa), temperature (40, 50, and 60°C), and cosolvent concentration (2, 5, and 8%) on antioxidant activity. The antioxidant activity of samples was evaluated by determining their capacity for protecting an edible oil against oxidation, upon accelerated oxidation trials. The extracts obtained at 30 MPa, 60°C, and 8% cosolvent showed the highest antioxidant activity, inhibiting 42.1% in the formation of TBARS after 6 days of accelerated oxidation [97].

4.5. SWP by UAE

UAE was carried out in a water bath ultrasonicator for 60 min (sonicate for 10 min and pause for 2 min) at 50°C applied on *S. muticum* (brown seaweed), *Co. tomentosum* (green seaweed), and *O. pinnatifida* (red seaweed). A higher effect on hydroxyl-radical scavenging activity (35–50%) was observed for all SWP [88]. The SWP from *Gracilaria birdiae* (red seaweed) were obtained using five different extraction conditions. Their infrared and electrophoresis analysis showed that all conditions extracted the same SWP. The total capacity antioxidant of the SWP was also affected by extraction condition, since GB2s (NaOH/sonication/proteolysis extraction) and GB1 (water extraction) showed lower activity in comparison to the other conditions. The data revealed that NaOH/sonication/proteolysis was the best condition to extract antioxidant SWP from *G. birdiae* [98].

In general, the antioxidant properties of SWP are influenced by chemical characteristics like molecular weight, degree of branching, type of monosaccharides, ratio of monosaccharides, intermolecular associations of polysaccharides, glycosidic branching, and modification of polysaccharides. It has been observed that crude polysaccharide has better antioxidant activity than purified polysaccharide components. Antioxidant activity of SWP depends on their structural features, such as degree of sulfating, molecular weight, type of the major sugar, and glycosidic branching. The rationale is that low-molecular-weight SWP may incorporate into

the cells more efficiently and donate protons more effectively than high-molecular-weight SWP. Evidence suggests that SWP are higher useful candidates when searching for effective nontoxic substances with potential antioxidant activity among various naturally occurring compounds. Therefore, SWP could be used as a rich source of natural antioxidants with potential application in the food industry as well as in cosmetic and pharmaceutical areas [99].

5. Conclusions

Seaweeds are used in many maritime countries as a source of human food, for industrial applications, and as a fertilizer. The major utilization of these plants as functional food is in Asia, particularly China, Japan, and Korea. They have the potential to be used as a source of long- and short-chain chemicals with medicinal and industrial uses. Seaweeds are a rich source of amino acids, bioactive peptides, carotenoids, dietary fiber, minerals, omega-3 fatty acids, SWP, and vitamins. Among them, SWP have the potential to significantly improve extraction efficiency. SWP include a complex group of macromolecules with a wide range of important biological activities, such as antioxidant, anticoagulant, anticancer, antiviral, anti-allergy, and anti-inflammation. Future research priorities in this area should be concentrated on overcoming the challenges of employing these novel technologies on chemical characteristics of SWP.

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