CHAPTER NINE

Current knowledge and challenges in extraction, characterization and bioactivity of seaweed protein and seaweed-derived proteins

Hugo Pliego-Cortés[a,*], Isuru Wijesekara[b], Marie Lang[a], Nathalie Bourgougnon[a] and Gilles Bedoux[a]

[a]Laboratoire de Biotechnologie et Chimie Marines, EA3884, UBS, IUEM, F-56000 Vannes, France
[b]Department of Food Science & Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka
*Corresponding author: E-mail: hugo-skyol.pliego-cortes@univ-ubs.fr

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Abstract

Given the current interest to reach sustainable protein supplies, seaweed proteins represent a potential source for pharmaceutical, nutraceutical, cosmeceutical, or food and feed applications. Some species are reported to contain high quantities of protein with original amino acid composition, which has been comparable to the values reported for other plant-based proteins. Further, seaweeds are rich in several sorts of valuable proteins, such as peptides, enzymes, phycobiliproteins, glycoproteins, cell wall-attached proteins and mycosporine-like amino acids. However, some challenges remain to be addressed concerning protein yield. The extractability is affected by the presence of the tough polysaccharide-rich cell wall and the occurrence of phenolic compounds. In addition, because the protein content depends on species, harvesting season, location, and growing conditions, aquaculture systems are proposed as an alternative to scaling up seaweed biomass and increasing protein production. An update to the current knowledge of the seaweed protein extraction is addressed over the conventional procedures, which have been improved using assisted and alternative methods, including enzymatic (EAE), microwave (MAE), ultrasound (UAE), pulsed electric fields (PEF), accelerated solvent extraction (ASE) and membrane filtration. The identification and characterization of protein could be done through electrophoresis, chromatography or spectrometry. The potential bioactivities such as antioxidant, antiviral, anti-inflammatory, or anticancer from seaweed-driven proteins, including novel glycoproteins and lectins, are discussed.

1. Introduction

1.1 Current knowledge in seaweed proteins

Macroscopic algae or seaweeds are an extraordinarily diverse group of marine organisms living in a wide range of habitats under highly variable environmental conditions. To ensure their survival they have developed complex metabolic pathways and producing molecules that represent a vast potential for use as natural products (Harnedy & FitzGerald, 2011). Seaweeds are mainly categorized in to three major groups such as green seaweeds (Chlorophyta), brown seaweeds (Phaeophyceae) and red seaweeds (Rhodophyta), depending on their chemical composition (*i.e.* pigments).

Seaweeds are considered a rich source of proteins, as well as nutraceutical and functional food ingredients for human and animal nutrition (Wijesekara & Kim, 2015), since they are also good sources of vitamins, minerals, dietary fibers, lipids and polyphenols. Seaweed contains several types of valuable proteins such as peptides, enzymes, glycoproteins, lectins, mycosporine-like amino acids, and phycobiliproteins in red seaweeds.

The protein content in seaweeds can reach up to 50% of dry weight (dw). For example, in the human edible red seaweeds *Porphyra* sp. ~44% dw (Marsham, Scott, & Tobin, 2007), *Palmaria palmata* 35%, and *Chondrus crispus* 19.5% (Fleurence, Morançais, & Dumay, 2018), while the green *Ulva* sp. 23%, and the brown *Undaria pinnatifida* 19% dw (Vieira et al., 2018). Generally, the protein concentration is higher in red species (20%–47% dw) than green (9%–26% dw) and brown (3%–15% dw) (Fleurence et al., 2018). However, seaweeds may contain non-proteinic nitrogen, obtained from nitrates, pigments or nucleic acids, and resulting in an over-estimation of their protein content, which usually is estimated by the general Nitrogen-to-Protein conversion factor of 6.25. Therefore, a Seaweed-Nitrogen-to-Protein (SNP) conversion factors of 5.0 is proposed based on the analysis of 103 species of seaweeds, across 44 studies including three phyla, multiple geographic regions and a range of nitrogen contents (Angell, Mata, de Nys, & Paul, 2016b). Specific SNP conversion factor for brown, red and green seaweeds of 5.38, 4.59 and 5.13 are also reported (Barbarino & Lourenço, 2005).

The composition of seaweed protein presented all amino acids, especially glycine, alanine, arginine, proline, glutamic, and aspartic acids. Total amino acids content have been reported up to 40 mg/100 mg dw (Table 1). Particularly, the content of Essential Amino Acids (EAA) may represent about 50% of total amino acids, as in *Fucus spiralis* 63.5%, *Porphyra* sp. 56.7%, *Osmundea pinnatifida* 41.6% (Paiva, Lima, Patarra, Neto, & Baptista, 2014), or *Rhodymenia pseudopalmata* 43% (Pliego-Cortés, Caamal-Fuentes, Montero-Muñoz, Freile-Pelegrín, & Robledo, 2017). The seaweed amino acid analysis have demonstrated profiles similar to ovalbumin (52.4% EEA) and leguminous plant (41.62% EAA), being comparable to those of the FAO/WHO requirement pattern (Fleurence et al., 2018; Paiva et al., 2014). In this sense, seaweeds have emerged as important field of research in food science and technology, because of their interesting nutritional value. The quality of proteins for human or animal nutrition, therefore, depends drastically on their digestibility and the availability of EAA. It is true that animal protein is generally considered complete because of their high

Table 1 Amino acid content and ratio AAA/NEAA of selected seaweeds species.

Species	Ratio EAA/NEAA	Total AA (mg/100 mg of dry weight)	References
Rhodophyta			
Chondrus crispus	1.58 ± 0.05	31.2 ± 1.7	Vieira et al., 2018
Gracilaria sp.	1.74 ± 0.05	35.5 ± 1.2	Vieira et al., 2018
Porphyra spp.	1.32 ± 0.04	38.8 ± 0.9	Vieira et al., 2018
Porphyra spp.	0.54	17.2	Astorga-España et al., 2016
Gracilaria domingensis	0.90	7.6 ± 0.0	Gressler et al., 2010
Gracilaria birdiae	0.90	9.1 ± 0.0	Gressler et al., 2010
Chlorophyta			
Ulva spp.	1.32 ± 0.05	41.3 ± 1.5	Vieira et al., 2018
Ulva spp.	0.56	19.2	Astorga-España et al., 2016
Enteromorpha sp.	0.55	15.8	Astorga-España et al., 2016
Pheophyceae			
Ascophylum nodosum	1.06 ± 0.01	36.6 ± 1.2	Vieira et al., 2018
Fucus spiralis	1.08 ± 0.01	40.2 ± 1.2	Vieira et al., 2018
Undaria pinnatifida	0.87 ± 0.01	44.2 ± 1.2	Vieira et al., 2018
Laminaria filiformis	0.90	11.3 ± 0.0	Gressler et al., 2010
Laminaria intricata	0.80	6.7 ± 0.0	Gressler et al., 2010

AA, Amino acids; *EAA*, Essential amino acids; *NEAA*, Non-essential amino acids.

content in EAA, however it is recommended to limit its consumption due to the development of cardiovascular disease and diabetes (Bleakley & Hayes, 2017). Algal protein could be complemented with other food proteins to achieved a high quality intake of proteins (Fleurence et al., 2018). Amino acids also play a key role in taste aspects, aspartic acid and glutamic acids, are the two most represented amino acids in all species of seaweeds, and are important for the development of flavors. Glutamic acid is particularly responsible for the taste sensations of "umami" (Pangestuti & Kim, 2015). Seaweed proteins are valuable ingredients for animal feed as well (Angell, Angell, de Nys, & Paul, 2016a). The enzymatic hydrolysis of protein by-products from *Gracilaria fisheri* yielded a roasted seafood-like flavoring. This could be useful in the food industry to replace flavoring agents from animal protein sources (Laohakunjit, Selamassakul, & Kerdchoechuen, 2014). The protein concentrate of *Kappaphycus alvarezii* contained 62.3%

of proteins represents an inexpensive source of protein, and could be incorporated into several value-added food products (Suresh Kumar, Ganesan, Selvaraj, & Subba Rao, 2014).

1.2 Types of proteins

1.2.1 Peptides

Peptides are generally known to be protein fragments containing 3 to 40 amino acids. These fragments are generated from the parent protein through digestion process in the gastro-intestinal tract, but can also be artificially produced during fermentation or other processes like enzymatic hydrolysis (Bleakley & Hayes, 2017; Pangestuti & Kim, 2015). Thus, it is expected that the seaweed treatment by enzymatic hydrolysis could generate enriched peptide extracts. The production of algal peptides is a particularly developed sector because of the diverse biological activities. Indeed, if the specific amino acid sequences are not necessarily active in the protein, they become active after release in the form of peptides (Pangestuti & Kim, 2015). Successful production of bioactive peptides - as describe later in bioactivity section - originating from hydrolyzed proteins of *P. palmata, Solieria chordalis*, *Ulva lactuca* and *Saccharina longicruris* are reported (Bondu et al., 2015). While in *U. pinnatifida*, 10 dipeptides were characterized and, four of them, Tyr-His, Lys-Tyr, Phe-Tyr, and Ile-Tyr, presented strong bioactivity (Suetsuna, Maekawa, & Chen, 2004). An active peptide identified as Pro-Ala-Phe-Gly, purified from *Enteromorpha clathrata* proteins, showed resistance against gastrointestinal proteases (Pan, Wang, Jing, & Yao, 2016).

1.2.2 Enzymes

Seaweed are also rich sources of enzymes. Alkaline phosphatase is a Zn-containing metalloproteinase that catalyzes the non-specific hydrolysis of phosphate monoesters. This enzyme is widely distributed in seaweeds and have been purified and characterized from *Ulva pertusa* (Yang, Wang, Bao, & An, 2003). Alternative oxidases (AOX) proteins has been described for electron flow through electron transport chain and regulation of mitochondrial retrograde signaling pathway, in the green *Caulerpa cylindracea* two AOX encoding genes were described, which support the capacity of this species for invasion of new environments (Ünlü, Ünüvar, & Aydın, 2019). The fibrinolytic enzymes has been isolated from the green alga, *Codium latum* (Matsubara, Hori, Matsuura, & Miyazawa, 1999), and *Codium fragile* (Choi, Sapkota, Park, Kim, & Kim, 2013), which is a trypsin-like serine protease with a high substrate specificity. The bifunctional enzyme Rubisco,

which is known to catalyze carbon dioxide fixation and oxygenation, was reported in *S. chordalis* (Bondu et al., 2015) and *K. alvarezii* (Tee, Yong, Rodrigues, & Yong, 2015).

1.2.3 Glycoproteins and lectins

Glycoproteins (GP) are carbohydrate-binding proteins, in which glycan is conjugated to peptide chains by two types of primary covalent linkages, N-glycosyl linkages and O-glycosyl linkages; some glycoproteins have both N-linked and O-linked forms. The carbohydrate is attached to the protein through glycosylation, which is a co-translational or post-translational modification (Senthilkumar & Jayanthi, 2016). According to Yoshiie et al. (2012), the high-mannose type N-glycans were linked to GP expressed in different species of seaweeds. GP are located on the cell wall, on the cell surfaces or they are secreted, and their functions in plants include recognition, intercellular interactions, and adhesion (Stiger-Pouvreau, Bourgougnon, & Deslandes, 2016). Currently, a few GP have been isolated from seaweeds (Table 2), and their structures and functional roles are yet to be investigated. In *Ulva* sp. three glycoprotein-rich fractions were obtained, UvGP-1 (hot water extraction), and UvGP-2-DA and UvGP-2-DS (cold-water extraction). GP-1 and GP-2-DA showed higher protein content (24.9% and 33.4%, respectively) than neutral sugars, while GP-2-DS contains a higher amount of neutral sugars (28.5%) than proteins (Wijesekara et al., 2017). While, in *U. lactuca* the high recovery of carbohydrates (42%) and protein (12.3%) in the extracts suggested the presence of GP (Abdel-fattah & Sary, 1987). Senthilkumar and Jayanthi (2016), isolated and partially characterized a GP from *Codium decorticatum* with a molecular mass of ~48 kDa, and Surendraraj, Habeebullah, and Jacobsen (2015), produces GP from the pH precipitated fractions after enzymatic extracts from the brown algae *Fucus serratus* and *Fucus vesiculosus*.

Lectins are proteins or glycoproteins of non-immune origin, containing at least one non-catalytic domain that binds reversibly to specific mono- or oligo-saccharides without modify the carbohydrates that they bind. Lectins precipitate glycoproteins and agglutinate cells (Peumans & Van Damme, 1995). In seaweeds, lectins are involved in gamete recognition and reproductive cell fusion (Ingram, 1985). Seaweeds are good sources of novel lectins (Table 2), such as griffithsin, a mannose-specific lectin isolated from the red alga *Griffithsia* sp. (Mori et al., 2005), or SfL-1 and SfL2 from *Solieria filiformis* (Chaves et al., 2018), or HRL40 from *Halimeda renschii* (Mu, Hirayama, Sato, Morimoto, & Hori, 2017). Singh and Walia (2018), and

Table 2 Glycoproteins and lectins extraction, identification and bioactivity in selected seaweeds species.

Species	Glycoprotein (GP) or lectin name	Extraction and purification	Identification	Mass (kDa)	Bioactivity	References
Chlorophyta						
Capsosiphon fulvescens	Cf-hGP	Sodium acetate Methanol Lectin wheat germ agglutinin resin	SDS-PAGE Immunoblotting	–	Reduce endoplasmic reticulum stress-induced cognitive dysfunction	Oh & Nam, 2019
Ulva sp.	UvGP-1 UvGP-2-DA UvGP-2-DS	Distilled Water TCA $(NH_4)_2SO_4$	SDS-PAGE MALDI-TOF	–	– Non cytotoxicity	Wijesekara et al., 2017
Codium decorticatum	GLPCD	Distilled Water $(NH_4)_2SO_4$ Ion exchange	SDS-PAGE FT-IR, CD, ^{1}H NMR, MALDI-TOF	~48	Anticancer activities on cell lines, MCF-7, Siha, and A549	Senthilkumar & Jayanthi, 2016
Codium decorticatum	GLPCD	$(NH_4)_2SO_4$ Ion exchange	SDS-PAGE	~48	Activity properties against human MDA-MB-231 breast cancer cells	Thangam et al., 2014
Halimeda renschii	Lectin HRL40	Phosphate buffer $(NH_4)_2SO_4$ gel-filtration Ion exchange	SDS-PAGE ESI-MS	46.5	Anti-Influenza Virus Activity	Mu et al., 2017
Caulerpa cupressoides var. lycopodium	Lectin CcL	Tris–HCl Ion exchange	–	–	Anti-nociceptive and anti-inflammatory	da Conceição Rivanor et al., 2014
Ulva lactuca	GP fraction G	Distilled Water NaOH DEAE-cellulose	Chromatographic paper	–	–	Abdel-fattah & Sary, 1987

(*Continued*)

Table 2 Glycoproteins and lectins extraction, identification and bioactivity in selected seaweeds species.—cont'd

Species	Glycoprotein (GP) or lectin name	Extraction and purification	Identification	Mass (kDa)	Bioactivity	References
Rhodophyta						
Solieria filiformis	Lectins SfL-1 SfL-2	Phosphate buffer $(NH_4)_2SO_4$ Ion exchange	SDS-PAGE LC-MS/MS ESI Protein and peptide sequencer	27.5 27.9	Anticancer effects on Human breast adenocarcinoma and dermal Fibroblasts	Chaves et al., 2018
S. filiformis	Lectin SfL	Tris-HCl buffer $(NH_4)_2SO_4$ Ion exchange	—		Antidepressant-like action	Abreu et al., 2018
Hypnea musciformis	Lectin HML	Phosphate buffer $(NH_4)_2SO_4$ Affinity and ion exchange	SDS-PAGE	10	Antimicrobial of biofilm formation from bacteria and yeast	Vasconcelos et al., 2014
Gracilaria ornata	Lectin GOL	Tris-HCl buffer $(NH_4)_2SO_4$	SDS-PAGE	17	effect on the development of the insect cowpea weevil	Leite et al., 2005
Griffithsia sp.	Lectin GRFT	$(NH_4)_2SO_4$ HPLC HPSEC	SDS-PAGE MALDI-TOF MS ESI-MS	12.7	Antiviral activity against HIV-1	Mori et al., 2005
Eucheuma serra *Galaxaura marginata*	Lectins ESA GMA	Phosphate buffer Ethanol	—	—	Antibacterial activity against marine *vibrios*	Liao et al., 2003

Pheophyceae						
Undaria pinnatifida	UPGP	Distilled water Ethanol	SDS-PAGE FT-IR	~10	Anti-Alzheimer's and anti-inflammatory activities	Rafiquzzaman et al., 2015
Fucus serratus *Fucus vesiculosus*	GPs	EAE HCl	SDS-PAGE		Antioxidant	Surendraraj et al., 2015
Saccharina japonica	SJGP	Distilled water Ethanol HPLC	SDS-PAGE FT-IR	~10	Probiotic properties Antioxidant and DNA protection	Kim et al., 2015 Kim et al., 2012
Laminaria japonica	LJGP	Distilled water Ethanol $(NH_4)_2SO_4$	SDS-PAGE	~10	antiproliferative effects on HT-29 colon cancer cells	Go et al., 2010
Hizikia fusiformis	HFGP	Distilled water Ethanol $(NH_4)_2SO_4$	SDS-PAGE	–	Chemoprotective effects against acetaminophen-induced liver injury	Hwang et al., 2008

–, data no reported; *EAE*, Enzyme-Assisted Extraction.

Barre, Simplicien, Benoist, Van Damme, and Rougé (2019), have reviewed their structure and potential biomedical applications extensively.

1.2.4 Cell wall-attached proteins

Arabinogalactan proteins (AGPs) belong to the hydroxyproline-rich glycoproteins (HRGP), they are highly glycosylated proteins reported in the cell wall of few species of seaweeds. AGP are implicated in terrestrial plant developmental process but their roles in seaweed has been yet poorly known (Popper et al., 2011). The genome sequence of *Ectocarpus siliculosus* encodes AGP protein backbone motifs shown that differs considerably from land plants in a gene context, while AGP on the cell surfaces of the brown seaweed embryos *F. serratus*, was confirm by positively stained with β-Glucosyl and β-Galactosyl Yariv reactive (Hervé et al., 2016). In AGP, the carbohydrates represent between 90 and 98% (*w/w*), composed mainly of arabinose, rhamnose and glucuronic acid residues (Tan et al., 2012), while the protein backbone typically contains repeating motifs rich in hydroxyproline/proline, alanine and serine/threonine (Gorres & Raines, 2010). The presence of HRGP was confirmed by immunolabeling and by the β-Glc Yariv reactive in the green seaweeds *Codium vermilara* (Fernández, Ciancia, Miravalles, & Estevez, 2010) and *C. fragile*, in which an arabinose-rich fraction showed a furanosic α-arabinosyl structure (Estevez, Fernández, Kasulin, Dupree, & Ciancia, 2009).

1.2.5 Phycobiliproteins

Phycobiliproteins (PBPs) are the main light-harvesting pigments in red seaweeds and the only water-soluble algal pigments (Fig. 1A and B), representing up to 20% dw. PBPs are grouped into four classes: phycoerythrin (PE), phycocyanin (PC), phycoerythrocyanins (PEC), and allophycocyanin (APC). PE is the main pigment, and it is divided into R- for Rhodophyta (R-PE) and B- for Bangiales (B-PE). Biliproteins are ensemble of α and β subunits, and only R-PE possess an extra γ subunit (Dumay, Morançais, Munier, Le Guillard, & Fleurence, 2014). Isolation of PE has been reported in many species, for example *Gelidium pusillum* (Mittal, Sharma, & Raghavarao, 2019), *Grateloupia turuturu* (Denis, Massé, Fleurence, & Jaouen, 2009; Le Guillard et al., 2016), *R. pseudopalmata* (Pliego-Cortés et al., 2017).

1.2.6 Mycosporine-like amino acids

Mycosporine-like amino acids (MAAs) are small-sized (<400 Da) secondary metabolites with strong absorption of UVR, including A and

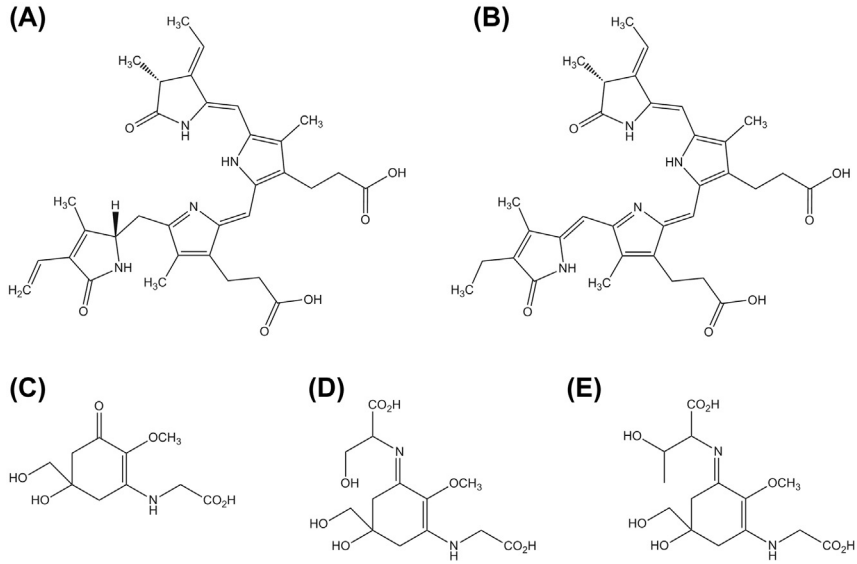

Fig. 1 Chemical structures of (A) phycoerythrobiline, (B) phycocyanobiline (based on ChemSpider ID 16741434), and the mycosporine-like amino acids: (C) mycosporine-glycine, (D) shinorine, and (E) porphyra-334.

B (292–365 nm), colorless, and stable in changing conditions of light, temperature, and pH (Fig. 1C to E). Their main functions are UVR-protection and antioxidant activities (Carreto & Carignan, 2011). More than 20 MAAs have been identified in seaweeds, and their capacity to synthesize a large variety of MAAs facilitates their adaptation to stressful environments (Pliego-Cortés et al., 2019; Wada et al., 2015). A full description of MAAs is given in Chapter 8 by Bedoux et al.

2. Challenge: variability and availability of biomass sources

2.1 Wild harvested versus cultivated

According to literature, high levels of variability in protein content have been reported due to seasons, environmental factors, or harvesting locations (Table 3). This represents an important challenge, since protein quantity and quality cannot be the same through the year. Generally higher amounts of proteins are reported in winter, such as in *Gracilaria tikvahiae* (Madden, Mitra, Ruby, & Schwarz, 2012) collected in winter (~30% dw) compared to spring (~17% dw) or in *P. palmata* (Galland-Irmouli

Table 3 Protein content, extraction and quantification of selected seaweeds species according to the season, source and geographical location.

Species	Source	Location	Sampling	Protein content % Dry weight	Protein extraction and quantification	References
Rhodophyta						
Rhodymenia pseudopalmata	Wild IMTA	Yucatan, Mexico	July	9.4 ± 0.4 18.7 ± 0.4	Freeze-dried BCA Lowry	Pliego-Cortés et al., 2019
Gracilaria conferta	IMTA	Haifa, Israel	March−August	17.7 ± 1.52	Dried (60 °C)	Ashkenazi et al., 2019
Hypnea musciformis	IMTA Controls	Herzliya, Israel		25.32 ± 1.25 ~5 to 10	Kjeldahl N x 6.25	
Chondracanthus chamissoi	Wild	Coquimbo, Chile	January−March	45.2 ± 4.2	Dried (60 °C) EAE, Lowry	Vásquez et al., 2019
Chondrus crispus	Wild	North-Central coast, Portugal	April−July October−November	19.5 ± 0.16 19.1 ± 0.33	Dried (52 °C), Kjeldahl N x 6.25	Vieira et al., 2018
Gracilaria sp.	Wild	Portugal	April−July October − November	24.7 ± 0.24 24.4 ± 0.24	Dried (52 °C), Kjeldahl N x 6.25	Vieira et al., 2018
Osmundea pinnatifida	Wild	Portugal	April−July October − November	24.3 ± 0.73 22.8 ± 0.33	Dried (52 °C), Kjeldahl N x 6.25	Vieira et al., 2018
Porphyra spp.	Wild	Portugal	April−July October − November	27.4 ± 0.08 28.2 ± 0.16	Dried (52 °C), Kjeldahl N x 6.25	Vieira et al., 2018

R. pseudopalmata	Cultivated	Yucatan, Mexico	30 psu 40 psu	10.5 ± 1.42 4.9 ± 0.43	Freeze-dried BCA Lowry	Pliego-Cortés et al., 2017
Palmaria palmata	Wild	Brittany, France	Winter–spring Summer– early autumn	21.9 ± 3.5 11.9 ± 2.0	Freeze-dried Kjeldahl N x 6.25	Galland-Irmouli et al., 1999
Chlorophyta						
Ulva ohnoi	Commercial cultivated	Queensland, Australia	October – November Unprocessed Protein-enriched-I Protein-enriched-II Protein-enriched-III Protein-enriched-IV	 22.2 ± 0.4 ~41% ~42% ~40% 39.5 ± 1.9	Fresh, milli-Q water, HCl, ultrafiltration, EAE, MAE. Sum of all amino acids	Magnusson et al., 2019
Ulva spp.	Wild	Portugal	April–July October – November	20.5 ± 0.49 23.3 ± 0.01	Dried (52 °C), Kjeldahl N x 6.25	Vieira et al., 2018
Pheophyceae						
Macrocystis pyrifera	Wild	Coquimbo, Chile	January–March	61.6 ± 4.7	Dried (60 °C) EAE, Lowry	Vásquez et al., 2019
Ascophyllum nodosum	Wild	Portugal	April–July October – November	6.90 ± 0.16 9.40 ± 0.08	Dried (52 °C), Kjeldahl N x 6.25	Vieira et al., 2018

(*Continued*)

Table 3 Protein content, extraction and quantification of selected seaweeds species according to the season, source and geographical location.—cont'd

Species	Source	Location	Sampling	Protein content % Dry weight	Protein extraction and quantification	References
Fucus spiralis	Wild	Portugal	April–July October – November	11.8 ± 0.16 11.7 ± 0.24	Dried (52 °C), Kjeldahl N x 6.25	Vieira et al., 2018
Saccorhiza polyschides	Wild	Portugal	April–July October – November	12.4 ± 0.01 11.8 ± 0.16	Dried (52 °C), Kjeldahl N x 6.25	Vieira et al., 2018
Undaria pinnatifida	Wild	Portugal	April–July October – November	16.5 ± 0.08 19.5 ± 0.21	Dried (52 °C), Kjeldahl N x 6.25	Vieira et al., 2018
Sargassum horneri	Wild	Fukuoka, Japan	Feb. Immature Feb. Male/Female May Male May Female	12.8 ± 1 10.7/10.4 ± 0 5.6 ± 0.2 8.4 ± 0.1	Freeze-dried Kjeldahl N x 6.25	Murakami et al., 2011

N x 6.25, Nitrogen-to-Protein conversion factor of 6.25; *psu*, practical salinity units.

et al., 1999), during winter–spring (21.9% dw) *vs.* summer–early autumn (11.9% dw). The brown algae *Ascophyllum nodosum*, *U. pinnatifida* and the green *Ulva* sp., analyzed during October–November exhibited higher protein than in April–June. The protein quality was not affected since total amino acids, EAA and EAA/NEAA ratios remain unchanged (Vieira et al., 2018). *Sargassum horneri* also showed significant differences in protein content between seasons and thalli maturity. Immature thalli collected in February showed the highest value (128 mg g^{-1}), while in May, male and female thalli showed lower content (56 and 84 mg g^{-1}), being early spring the best season to harvest the alga for human consumption, when its growth and maturity are complete (Murakami et al., 2011). Concerning the harvesting localization, the total content of AA and the ratio EAA/NEAA in *Porphyra* sp. collected in Portugal (38.8 mg AA 100 mg^{-1} dw) (Vieira et al., 2018) was higher than those harvested in Chile (17.2 mg AA 100 mg^{-1} dw) (Astorga-España et al., 2016). According to Angell et al. (2016b), a meta-analysis including 382 species, the mean protein content was lower in tropical seaweeds (14.5% dw), compared to temperate and polar (17.6% dw) by using the Nx6.25 factor. However, polar seaweed showed lower content (8.14% dw) when the total amino acid determination was used. The authors indicated the difficulty to compare the protein content between geographic regions since differences were related to the methods of protein determination. In contrast, the cultivated seaweeds showed higher protein contents in the meta-analysis, regardless of the method of protein determination.

The cultivation of seaweed could face the challenge of variability and availability of biomass, cultivation usually enhance the content of protein and also decrease the pressure of harvesting large quantities of biomass from wild populations. For instance, in Europe more than 97% of seaweed production is harvested from the wild in Norway, France and Ireland (Grote, 2019). The same *scenario* is reported in Latin America, 96% harvested from natural beds in Mexico, Peru, Ecuador (Alemañ, Robledo, & Hayashi, 2019; Vázquez-Delfín, Freile-Pelegrín, Pliego-Cortés, & Robledo, 2019). Eco-intensification of seaweed cultivation can be improved through integrated multitrophic aquaculture (IMTA). Further, biomass could be maximized using biorefinery strategies. Under an IMTA approach, thalli from *Rhodymenia pesudopalmata* showed an increase of total protein content after 3 days of cultivation (18.7% dw) in fishpond effluents rich in ammonium (NH_4^+), than thalli from wild populations (9.4% dw), and a fivefold increase

in the MAAs total content as well (Pliego-Cortés et al., 2019). The cultivation of *P. palmata* under different cultivation systems and nutrient treatments has been addressed, and proved to be a good candidate for IMTA because their high affinity for NH_4^+, which resulted in an increase of 20% of protein content in thalli fed with NH_4^+ (Grote, 2019). The IMTA systems does not just allow the production of algal biomass but also reduces negative impact on ecosystems because the capacity of seaweeds to absorb the inorganic nutrients. Recently, high protein content from *U. lactuca* (37.4% dw) and *Gracilaria conferta* (29.4% dw) produced in IMTA were used to fed sea urchins as a sole feed compared with a formulated diet. *U. lactuca* enhanced somatic and gonad growth, and better protein assimilation efficiency (81%) compare to formulated diet (59%), which was attributed to the relatively high protein and EEA (44%) content. *Ulva* produced in IMTA compared to wild harvested, was more productive and had lower biochemical composition variability. The use of *Ulva* seems to be cost effective and a more sustainable method to produce echinoderms (Shpigel, Shauli, Odintsov, Ashkenazi, & Ben-Ezra, 2018). The cultivation of *R. pseudopalmata* under different conditions of light and salinity increased the content of EAA up to 43% (Pliego-Cortés et al., 2017). Furthermore, other plausible alternative to face this challenge is to obtain complementary products to seaweed protein and seaweed derived proteins in the same biomass using a biorefinery approach.

Using a multi-step biorefinery process, the quality and yield of the protein content from commercially produced *Ulva ohnoi* was enhanced, by producing four fractions (PEB-I to PEB-IV) and one protein isolate (PI). The protein increased from 22% dw in unprocessed biomass to 39% in PEB-IV and 45% in the PI. Authors projected an annual production (t dw ha^{-1} $year^{-1}$) of 29 t of PEB-I, or 3.2 t of PI, and 24 t of salt, and 4.3 t of ulvan (Magnusson et al., 2019). In *U. lactuca*, a protein-enriched fraction containing 343 g protein kg^{-1} dw was achieved, and its content in EEA and *in vitro* digestibility (90%), led the authors to propose it as a protein source for monogastric animals, but also as an effective source to produce acetone, butanol, ethanol and 1,2-propanediol by fermentation (Bikker et al., 2016). A sustainable production of biomolecules from macroalgae could strongly contribute to marine bio-economy, since the recovery of proteins (11% dw) from *U. lactuca* with a high digestibility (85.8%) and good amino acid content shows their suitability for use as food supplements. Other products such as mineral salts (14%), fatty acids (1.5%), ulvan (19.9% dw) and cellulose (10.35% dw) were coproduced (Gajaria et al., 2017).

3. Challenge: protein extraction and purification

The raw biomass from seaweed after harvesting must be preserved by drying or freezing, or used in fresh as soon as possible to avoid protein degradation. The effect of drying methods (freeze-, vacuum-, solar-, and convective-drying) has been established in *Ulva* sp. The convective-drying showed the higher content of crude protein (20% dw) with the lower EAA content (41%), the highest EAA content (56%) was obtained in vacuum-drying (Uribe et al., 2018). While, the processes of freeze-dried, oven-dried, and −20°C-frozen on *Saccharina latissima* resulted in significantly higher protein yield than the −80°C-frozen, sun-dried and ensiled processes. The sun-dried had the highest EAA content and freeze-dried the lowest (51.7 vs. 40.2 mg 100 g^{-1} protein); however, a high degree of protein degradation was observed in sun-drying and −20 °C-freezing methods. These processes showed that post-harvest preservation must be carefully chosen according to the final use of the protein (Abdollahi et al., 2019). Afterward, seaweeds need to be processed for use as a high quality protein source.

The development of seaweeds as a protein source challenges the complex nature of the cell wall. The presence of polysaccharide-bound cell wall mucilage including anionic or neutral polysaccharides, and polyphenols reduces protein extractability and requires additional adapted steps for fractionation and purification. Studies in model systems using carrageenan associated to whey protein, or ι- κ-carrageenan or dextran sulfate with bovine serum albumin, have proven that polysaccharides induced strong electrostatic interactions (Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2004). Whereas, polyphenols may form reversible hydrogen bonds with proteins or oxidize to quinones, to bind irreversibly to proteins (Vilg & Undeland, 2017). Thus, the combination of extraction methods and purification techniques are necessary to improve protein yield and purity, or digestibility. This challenge becomes greater on an industrial scale, because require methods with low-time, -cost and -energy consumption (i.e. environmentally friendly).

3.1 Conventional extractions

There are various liquid systems which allowed the protein extraction, such as distilled water, buffers, acid or alkaline solution (Fleurence, Le Coeur, Mabeau, Maurice, & Landrein, 1995; Galland-Irmouli et al., 1999), urea (Contreras et al., 2008), lysis solutions (Kim et al., 2010; Wijesekara et al.,

2017), and phenol-based extraction systems (Nagai, Yotsukura, Ikegami, Kimura, & Morimoto, 2008; Wong, Tan, Nawi, & AbuBakar, 2006). Combining with physical methods, as osmotic shock, freeze/thawing or grinding, the extraction in some seaweeds have been enhanced (Table 3). Extraction with alkaline solubilization, isoelectric precipitation and frozen/thawing showed higher protein concentration in *Porphyra umbilicalis* (71%), *S. latissima* (51%), and *U. lactuca* (40%) compared to water extraction (Harrysson et al., 2018). Precipitation of solubilized proteins at pH 2 yielded 34.5% of proteins, and osmoshocking with 60 vol of water gave 59% yield in *S. latissima* (Vilg & Undeland, 2017). Glycoproteins (GP) and lectins are commonly extracted using water or buffers, followed by ethanolic, acidic or ammonium sulfate precipitation (Table 2), such as the GP from *Laminaria japonica* (Go, Hwang, & Nam, 2010). While, protein hydrolysis using 6 M HCl in either a liquid or vapor phase, allows high recovery rate for a majority of amino acids, but tryptophan is destroyed, and methionine and cysteine are also sensible, and therefore underestimated (Kazir et al., 2019). Alternate methods must be used, as alkaline hydrolysis using 4 N LiOH (Dawczynski, Schubert, & Jahreis, 2007). The content of free amino acids (amino acids not embedded into proteins) was extracted by ultrasound-assisted perchloric acid (Vieira et al., 2018).

3.2 Assisted extractions and alternative methods

Enzyme-Assisted Extraction (EAE) has been applied by using enzymes to assist and to extract proteins, and hydrolysates from seaweeds. The use of commercially enzymatic preparations is frequently used with limited practices using specific enzyme on seaweeds, such as κ-/ι-carragenase or β-agarase (Denis, Morançais, Gaudin, & Fleurence, 2009; Fleurence, Massiani, Guyader, & Mabeau, 1995). In a recent study, protein extraction from *Chondracanthus chamissoi* and *Macrocystis pyrifera* was enhanced using a cellulose cocktail (Cellic CTec3, Novozymes), producing 452 and 616 mg g^{-1} dw of protein. This cocktail contained multiple hydrolytic activities (cellulase, hemicellulase and β-glucosidase) for carrageenan, agar, alginate and cellulose. Furthermore, did not exhibit proteolytic activity (Vásquez, Martínez, & Bernal, 2019). On the contrary, the subtilisin protease (Novozymes), which raised an 8.9% dw protein extraction in *S. chordalis*, allowed the cleavage by subtilases or serine proteases by breaking down the cell wall of seaweeds through a selective degradation of structural proteins, a degradation of serine glycoproteins of the cell wall might be possible (Burlot, Bedoux, & Bourgougnon, 2016). The EAE of *Ulva armoricana*, *Sargassum muticum*,

S. chordalis and *Pyropia columbina* yielded bioactive extracts and hydrolysates (Cian, Garzón, Ancona, Guerrero, & Drago, 2015; Hardouin et al., 2013, 2016; Puspita et al., 2017). Xilanase enzyme allowed to a significant increase from 0.11 to 1.15 g kg^{-1} dw (dry ground algae) in R-PE from *P. palmata* (Dumay, Clément, Morançais, & Fleurence, 2013). These studies have shown that the EAE is free from application of organic solvents, leads to higher extraction rate of bioactive hydrolysates in reduced extraction times and costs. Different digestion enzymes have been used to release bioactive peptides from the parent proteins, chymotrypsin, trypsin and pepsin being the most commonly used.

Protein extraction using Ultrasound-Assisted Extraction (UAE) during 2 h followed by ion exchange purification yielded a protein content of 70% in *Ulva sp*. and 86% in *Gracilaria* sp. This procedure was up-scalable and suitable to obtain a 'food-grade' product (Kazir et al., 2019). The effects of UAE in the brown seaweed *A. nodosum*, showed that an amplitude of 68.4 μm, using acid and alkali treatments yielded 43% and 57% of protein recovery, respectively. The effect of UAE can be attributed to bubble cavitation, facilitating the degradation of biological matrices (Kadam, Tiwari, & O'Donnell, 2013). A recovery of 71% and 74% of the initial material into the soluble phase rich in proteins, carbohydrates, amino acids and salts, was obtained by using EAE or UAE in *G. turuturu*. However, when these processes were combined, up to 91% of solubilized material was archived due to a synergistic effect (Le Guillard et al., 2016). Extraction of R-PE has been effective by combining these two processes in *G. turuturu* (Le Guillard et al., 2015). In *G. pusillum*, UAE followed by maceration and buffers, allowed 77%–93% of recovery for R-PE and R-PC (Mittal, Tavanandi, Mantri, & Raghavarao, 2017). Even a very high purity of R-PE with a removal of up to 95% of total sugars was achieved (Mittal et al., 2019). Iodinated proteins in *Porphyra* sp. raised extraction yields close to 100% using UAE with pancreatin hydrolysis (Romarís-Hortas, Bermejo-Barrera, & Moreda-Piñeiro, 2013).

The Pulsed Electric Field (PEF) extraction uses electric potential generated from an electric field using high voltage (kV) and different duration time in the range of microseconds to milliseconds, to disrupt cell membranes (Okolie, Akanbi, Mason, Udenigwe, & Aryee, 2019). PEF treatments in *U. lactuca* showed that the higher content of protein (15% dw) was achieved at an electric field strength of 7.5 kV cm^{-1}, with two pulses of 0.05 ms, and a specific energy input of 6.6 kW h kg_{prot}^{-1} (Postma et al., 2018). While 50 pulses of 50 kV, applied at a 70.3 mm electrode gap, resulted in an

~sevenfold increase in the total protein in *Ulva* sp. compared to osmotic shock (Robin et al., 2018). The PEF extracted juice from *U. lactuca* showed higher protein content than the control (59 vs. 23 μg mL^{-1}). The developed process has almost no thermal effects on the produced proteins (Polikovsky et al., 2016).

Membrane filtration is based on the molecular size of the compounds, which can be separated by a semi-permeable membrane to obtain different fractions, namely microfiltration, ultrafiltration, nanofiltration and reverse osmosis. For example, ultrafiltration allowed to isolate proteins between 1 and 200 kDa (Bleakley & Hayes, 2017). An ultrafiltration unit at industrial scale, employing polyethersulfone 25−30 kDa membrane showed the recovery of 100% of R-PE, 32.9% of other proteins and 64.6% of sugars from *G. turuturu* (Denis, Massé, Fleurence, & Jaouen, 2009).

Other procedures could be applied to enhance protein extraction, like Microwave Assisted Extraction (MAE), mainly used for carbohydrate extractions. This method is based on the transfer of energy to the solution, as microwaves induce the vibration of water molecules in the matrixes, disrupting the hydrogen bonds and allowing migration of dissolved ions (Kadam et al., 2013). The quality and yield of the protein in *U. ohnoi* was improved through EAE, MAE and ultrafiltration extractions (Magnusson et al., 2019). Pressurized Liquid Extraction (PLE) also known as Accelerated Solvent Extraction (ASE), combines temperature (50−200 °C) and pressure (35−200 bar) to increase mass transfer rate and reduce the viscosity and surface tension of the solvents, allowing to keep it in a liquid state (Grosso, Valentão, Ferreres, & Andrade, 2015). Under a biorefinery approach applying ASE to *U. lactuca*, *S. latissima* and *P. umbilicalis,* the protein content was lower than in the crude biomass, probably because proteins were removed during phlorotannins extraction (Harrysson et al., 2018). However, ASE technique allowed to concentrated ash and glutamate, which can be used as salt replacers and/or umami enhancers.

Purification of extracted protein also represents a challenge, especially for novel proteins, because their physico-chemical characteristics are often unknown. Indeed, the selection of methods depends on the nature and final application, and the scale of production. Single or combined methods could be used, such as anion-exchange chromatography, hydrophobic interactions, or gel permeation, sodium dodecylsulfate polyacrylamide gel electrophoresis SDS-PAGE (1-D or 2-D), and lectin or dyes affinity, for example, the Yariv reagent to bind glycoproteins.

4. Challenge: protein characterization

Characterization and/or identification of isolated proteins is possible by direct comparison with standards molecules, or with literature data. However, when compounds are unknown and standard are not available, comprehensive and systematic techniques are required.

4.1 SDS-PAGE

SDS-PAGE is performed to identify the molecular weight of dominant protein sub-units bands. The Tris-Glycine SDS-PAGE stained with silver nitrate allowed to identify that the protein profile of *Ulva* sp. varies with seasons, showing sharper protein bands during October and November than September and March (Wijesekara et al., 2017). SDS method was effective to recognize five proteins bands in *Himantalia elongata* with molecular weights of 71, 53, 43, 36 and 27 kD (Garcia-Vaquero, Lopez-Alonso, & Hayes, 2017). R-PE purified in protein extracted from *Furcelaria lumbricalis* analyzed by denaturing SDS-PAGE, allowed to identify a band near the 25 kDa region (Saluri, Kaldmäe, & Tuvikene, 2019). The periodic acid-Schiff (PAS) staining after SDS-PAGE was used to detect glycoproteins in *L. japonica* (Go et al., 2010).

4.2 Chromatography

High Performance Liquid Chromatograph (HPLC) is widely used for separation and purification of many compounds. R-PE was over 60% recovered and identified by combining HPLC coupled to photodiode array detector (PAD), and Size Exclusion Chromatography (HPSEC) in *F. lumbricalis* (Saluri et al., 2019). HPSEC reveled molecular weights from 2.6 to 3.8 kDa, in protein extracted from *A. nodosum* (Kadam et al., 2013). HPSEP and SDS-PAGE highlighted protein degradation, based on the apparent molecular weight profiles from *S. latissima* after different postharvest stabilization methods (Abdollahi et al., 2019). The analyses of amino acids through gas chromatography (GC) or LC requires a derivatization step, treated using Ortho Phtalaldehyde (OPA) or Fluorenylmethoxy chloroformate (FMOC). These reaction allow the separation of amino acids on reversed phase and their detection using UV or fluorescence detectors (Fernández-Segovia, Lerma-García, Fuentes, & Barat, 2018; Vieira et al., 2018). Recently, Kazir et al. (2019) presented the possibility to analyze the amino acid profile without derivatization using anion exchange (HPAEC-PAD).

4.3 Spectrometry

Fourier Transform Infrared (FTIR) spectroscopy provides information about the structural composition of proteins, especially for the secondary structural composition. The FRIT-ATR (Attenuated Total Reflection) spectrum of protein extracts from *M. pyrifera* and *C. chamissoi*, showed bands at 3281 cm^{-1} and 3274 cm^{-1} may corresponds to amide of the protein polypeptide skeleton because the N—H vibrations; bands at 1637 cm^{-1} and 1544 cm^{-1} could be identified as C═O vibrations of the peptide bond of proteins. While bands at 1220 cm^{-1} and 1243 cm^{-1} may corresponds to S═O vibrations from sulfated polysaccharide (fucoidan and carrageenan), and could indicate the presence of polysaccharides co-precipitated with proteins (Vásquez et al., 2019). In protein concentrate from *Kappaphycus alvarezzi*, bands at 704 cm^{-1} revealing N—H bending, and band at 616 cm^{-1} could be related to phosphate group (Suresh Kumar et al., 2014). An accurate protein identification and characterization can be done through mass spectrometry (MS), since supports mass determination, purity, mass fingerprint, sequence analysis, post-translational modifications analysis and protein-protein interactions. Electrospray ionization (ESI) and matrix-assisted laser desorption ionizations/time-of-flight (MALDI-TOF) have appeared to be important tools. MALDI-TOF/MS was used to confirm the presence of protein part in fractions rich in glycoproteins obtained from *Ulva* sp. (Wijesekara et al., 2017). The combination of RP-HPLC with inductively coupled plasma mass (ICP-MS) allowed the succeed determination of iodinate proteins such as mono-iodotyrosine (MIT) and di-iodotyrosine (DIT), with limits of detection of 1.1 and 4.3 ng g^{-1}, respectively, in the edible seaweeds *P. palmata*, *P. umbilicalis*, *Ulva rigida*, *U. pinnatifida* and *Laminaria ochroleuca* (Romarís-Hortas et al., 2013). The bioactive peptides in *P. palmata* and *S. chordalis* were identified by ESI-MS/MS online nanoscale capillary LC (RP-nano-LC), the method also allowed to identify the peptides origin (Bondu et al., 2015). The fibrinolytic enzyme codiase was determined by MS and SDS-PAGE in *C. fragile* (Choi et al., 2013).

5. Biological activities

5.1 Antioxidant activity

Seaweeds have been developed efficient and complex defense systems of antioxidants to protect themselves against oxidation. The antioxidant activity evaluated by the Oxygen Radical Absorbance Capacity (ORAC) of

extract rich in proteins obtained by EAE, was higher in *M. pyrifera* than *C. chamissoi* (83 vs. 35 μmol Trolox Equivalent (TE) g^{-1} protein) (Vásquez et al., 2019). While the extracts containing 20% protein from *Ulva* sp. and *Gracilaria* sp., showed 10−20 times higher antioxidant capacity than β-Lactoglobulin (β-Lg), bovine serum albumin (BSA), and potato protein isolates, evaluated by the ferric reducing antioxidant power (FRAP) assay and ORAC. The activity of the protein was attributed to cysteine, methionine, tyrosine, phenylalanine, and tryptophan (Robin et al., 2018), and to the presence of 0.7%−1.0% dw of polyphenols (Kazir et al., 2019). These amino acids, including histidine were also reported in *R. pseudopalmata* cultivated under stress conditions of light and salinities. These results suggested that they are involved in antioxidant protection (Pliego-Cortés et al., 2017). Peptides <1 kDa from *S. chordalis* exhibited higher antioxidant activities (2,2-diphenyl-1-picrylhydrazyl (DPPH), FRAP and ORAC), than the parent proteins (Bondu et al., 2015). Due to high bioactive of peptides, they are the most commonly occurring antioxidant substances in food (Admassu, Gasmalla, Yang, & Zhao, 2018). Antioxidant activity of seaweed proteins and their types of proteins have been previously reported (Admassu et al., 2018; Harnedy & FitzGerald, 2011; Pangestuti & Kim, 2015; Wada et al., 2015).

5.2 Antivirus activity

The high-mannose specific lectin and its recombinants from *K. alvarezii* showed a potent anti-HIV activity. This lectin has strong affinity to bind to the viral envelope glycoprotein gp120 (Hirayama, Shibata, Imamura, Sakaguchi, & Hori, 2016). Very low concentrations (EC_{50} 0.043 and 0.63 nM) of lectin Griffithsin GRFT, isolated from the red alga *Griffithsia* sp. were needed to inhibit the cytopathic effects of laboratory strains and clinical primary isolates of HIV-1 on T-lymphoblastic cells, and macrophage-tropic (M-tropic) strains of HIV-1. GRFT also aborted cell-to-cell fusion and transmission of HIV-1 infection. The lectin produced with success by expression of a corresponding DNA sequence in *Escherichia coli*, being suitable for large-scale production and provide an effective and economical prophylaxis strategy for HIV infection (Mori et al., 2005). Since then, recombinant GRFT has been produced in diverse organisms, such as *Nicotiana benthamiana* plant and/or *E. coli*, which showed a strong *in vitro* activity against Hepatitis C Virus (HCV) (Meuleman et al., 2011). GRFT also showed good bioavailability after subcutaneous injection, with a significant *in vivo* efficacy in reducing HCV viral titers in a mouse model system with

engrafted human hepatocytes (Takebe et al., 2013). The recombinant GRFT combined with carrageenan obtained from Gelymar (Puerto Montt, Chile), showed higher activity against herpes simplex virus 2 (HSV-2) and human papillomavirus (HPV) compared to placebo (Levendosky et al., 2015).

A wide range of antiviral spectrum against influenza A and B virus has been obtained from lectins. ESA-2 from the red seaweed *Echeuma serra*, showed the highest activity at EC_{50} 0.8 nM (Sato et al., 2015). HRL40 from the green algae *H. renschii*, inhibited the infection of influenza virus A strain A/H3N2/Udorn/72 (Mu et al., 2017). While, KAA-2 from *K. alvarezii*, effectively inactivated swine origin H1N1 influenza virus. Lectins inhibits the virus entry into the cells by binding to viral envelop glycoproteins (Sato, Morimoto, Hirayama, & Hori, 2011).

5.3 Antimicrobial activity

Antibacterial peptides (>10 kDa) isolated from *S. longicruris* exhibited activity against the bacterium *Staphylococcus aureus*. The identified peptides came from precursors such as a protein similar to ubiquitin, histones, a hypothetical leukine rich repeat protein and a ribosomal protein, which might be associated with the innate immune defenses of this brown seaweed (Beaulieu, Bondu, Doiron, Rioux, & Turgeon, 2015). Bacterial inhibition obtained from *H. elongata* extracts, was almost 100% against *Salmonella abony* and *Listeria monocytogenes*, using protein and carbohydrates model food systems. A bacteriostatic effect was observed preventing further growth of the bacteria (Cox, Hamilton Turley, Rajauria, Abu-Ghannam, & Jaiswal, 2014). Likewise growth reductions of *S. aureus*, *S. epidermidis*, and *Pseudomonas aeruginosa*, was caused by the lectins, BSL from *Bryothamnion seaforthii*, and HML from *Hypnea musciformis* (Vasconcelos et al., 2014). This later red seaweed also showed antifungal activity on human pathogenic yeasts *Candida albicans* and *C. guilliermondii* using a protein fraction rich in lectin (Cordeiro, Gomes, Carvalho, & Melo, 2006). While, extracts from *E. serra* and *Pterocladia capillacea,* rich in lectins, inhibited markedly the growth rate of *Vibrio vulnificus*. Futher, the antibacterial activity from *P. capillacea* was improved when algae was grown under higher irradiance, severe nutrient stress and temperature of 20 °C (Liao, Lin, Shieh, Jeng, & Huang, 2003).

5.4 Anti-inflammatory activity

Seaweed proteins are potential anti-inflammatory candidates. The *Pyropia yezoensis* peptide (PYP1) showed a potent inhibition activity on the release

of pro-inflammatory cytokines, and induced the proliferation of intestinal epithelial cells IEC-6 (Lee et al., 2015). In a recent study, the synthesized peptide Cyclophilin, from *P. yezoensis* (pyCyp), activated the epidermal growth factor receptor (EGFR) signaling pathway, which is involved in the regulation of cell growth, proliferation, and survival of IEC-6 cells (Jung, Choi, Lee, Choi, & Nam, 2019). The anti-inflammatory activity of UPGP, a purified glycoprotein from *U. pinnatifida*, was evident since inhibited COX-1 and COX-2 enzymes (IC_{50} 53 and 193 μg mL^{-1}) in the cyclooxygenase assay, whereas inhibition of nitric oxide (NO) production reached 94.2% in lipopolysaccharide (LPS) activated RAW 264.7 macrophages (Rafiquzzaman et al., 2015). The protein hydrolysate from *Porphyra columbina* inhibits the production of pro-inflammatory cytokines by macrophages under LPS stimulation (Cian, López-Posadas, Drago, Sánchez de Medina, & Martínez-Augustin, 2012). A potentially valuable tool for studies the complex event of inflammation was observed using the lectin CcL, from *Caulerpa cupressoides* var. *lycopodium,* which revealed that the anti-inflammatory effect involves the inhibition of cytokines secretion (IL-1b, TNF-a, IL-6), and COX-2 in dextran or histamine-induced rat paw edema model (de Queiroz et al., 2015). Similarly, CcL showed a potential anti-inflammatory effects in the temporomandibular joint disorders (da Conceição Rivanor et al., 2014).

5.5 Anti-hypertensive activity

Cardiovascular diseases are affliction that affect human increasingly, one of the most at risk is hypertension, which can be regulated by the action of two key enzymes, namely Angiotensin Converting Enzyme (ACE) and Renin Converting Enzymes (Admassu et al., 2018). Many studies show the activity of seaweed-derived proteins in the downregulation of these enzymes. An extract of *M. pyrifera* containing 1.72 μg protein mL^{-1}, showed an ACE inhibition of 38.8% (Vásquez et al., 2019). The protein hydrolysates from *P. palmata*, showed that ACE inhibitory activity was independent from the time of harvesting (Harnedy, Soler-Vila, Edwards, & FitzGerald, 2014). Nine ACE-inhibitory peptides derived from phycobiliproteins were identified, and the oligopeptide LRY (Leu-Arg-Tyr) demonstrated particularly high inhibitory activities, with an IC_{50} of 0.044 μM (Furuta, Miyabe, Yasui, Kinoshita, & Kishimura, 2016). The activity of ACE-inhibitory peptide Pro-Ala-Phe-Gly extracted from *E. clathrata,* showed an IC_{50} value of 35.9 μM. Moreover, their work confirmed that this product was resistant to gastrointestinal protease, making it a potential pharmaceutical or

nutraceutical product (Pan et al., 2016). Most recently, two peptides obtained from *Caulerpa lentillifera*, the oligo-peptides FP-5 (Phe-Asp-Gly-Ile-Pro) and AA-7 (Ala-Ile-Asp-Pro-Val-Arg-Ala), exhibits IC_{50} of 58.9 and 65.8 μM respectively (Joel, Sutopo, Prajitno, Su, & Hsu, 2018). Phycoerythrins, obtained from red seaweeds, also showed ACE inhibitory activity (Furuta et al., 2016).

5.6 Anti-cancer activity

The anti-cancer activity from glycoproteins (GP) have been reported in *C. decorticatum*, which revealed a significant inhibition of cell growth in breast, cervical and lung cancer cells, the cell membrane damages was induced by releasing the LDH enzyme producing apoptosis (Senthilkumar & Jayanthi, 2016). The *U. pinnatifida* glycoprotein UPGP, showed hypoglycaemic activity against yeast and rat intestinal α-glucosidase (Rafiquzzaman et al., 2015), while in *Capsosiphon fulvescens,* Cf-GP induces apoptosis signaling in gastric cancer (AGS) cells, through the inhibition of β-catenin, Wnt-1 and transcription factors (Kim, Kim, & Nam, 2012) and downregulated the expression of growth-related proteins, especially integrin (Kim, Kim, & Nam, 2013a). Cf-GP could be used for the development of functional foods and therapeutic agents, since this glycoprotein also inhibit cell invasion and proliferation (Kim, Kim, & Nam, 2013b). Another glycoprotein, LJGP, from the brown alga *L. japonica*, inhibited the proliferation of several cancer cell lines (AGS, HepG2 and HT-29), HT-29 were the most sensitive, reaching 60% inhibition, and apoptosis of colon cancer cells were observed (Go et al., 2010). R-PE induced apoptosis of AGS cancer cells by arresting the SGC-7901 cell, due to the expression of the CDC25A protein, and reduced the formation of Cyclin-CDK complex (Tan et al., 2016). R-PE showed *in vitro* activity against A549 and HepG2 cancer cell lines, the cell shrinkage, membrane blebbing, and nuclear DNA fragmentation were observed as characteristic apoptotic features (Senthilkumar et al., 2013). The use of R-PE subunits (α, β, γ) were an attractive option for improving the selectivity of photodynamic therapy (PDT) in mouse cancer tumor cell S180 and human liver carcinoma cell SMC 7721 (Bei, Guang-Ce, Chen-Kui, & Zhengang, 2002). The mixture of two *S. filiformis* lectin (SfL) isoforms (SfL-1 and SfL-2) inhibited 50% of viability in human breast adenocarcinoma (MCF-7) cells, and 34% of inhibition in Human Dermal Fibroblasts (HDF), and also could potentially exert antitumor activity because the ability to bind to high-mannose oligosaccharides present in the MCF-7 cells (Chaves et al., 2018). The lectin *E. serra* agglutinin (ESA), induced apoptotic cancer cell death,

such as in human osteosarcoma cells (OST) and murine osteosarcoma cell line (LM8), by decreasing cell viability of 54% and 41%, respectively (Hayashi et al., 2012). The inhibition of human colon cancer (Colo201) cell growth and diminishing tumor growth implanted in nude mice by treated with ESA in Span 80 vesicles as drug delivery system was observed (Omokawa et al., 2010).

5.7 Others biological activities

The anti-Alzheimer activity was reported for the glycoprotein UPGP purified from *U. pinnatifida*, which effectively inhibits the enzymatic activity of acetylcholinesterase (AChE), butarylcholinesterase (BChE) and β−secretase (BACE-I) enzymes involved in the Alzheimer's disease, with IC_{50} values of 63.5, 99.0, and 73.3 $\mu g\ mL^{-1}$, respectively (Rafiquzzaman et al., 2015). Protection against dexamethasone (DEX)-induced myotube atrophy has been reported from *P. yezoensis* peptides PYP15 (Lee, Choi, Choi, & Nam, 2019), and crude protein PYCP (Lee, Choi, Choi, & Nam, 2018). The former inhibited the insulin-like growth factor-I receptor (IGF-IR) and the Akt-mTORC1 signaling pathway, and the latter increased the body weight, calf thickness, and muscle weight in mice. The bioactivity against neurodegenerative diseases associated with age have been studied. The oral administration (15 $mg\ kg^{-1}\ day^{-1}$) of hydrophilic green algae *C. fulvescens* glycoproteins (Cf-hGP), during four weeks, promoted the activation of the synaptosomal BDNF-ERK1/2 signaling in the dorsal hippocampus due to the inhibition of the endoplasmic reticulum (ER) stress-induced cognitive dysfunction (Oh & Nam, 2019). Similarly, microinjections (1 $\mu g\ kg^{-1}$, 0.54 nmol) of phycoerythrin-derived peptide of *P. yezoensis* (PYP) attenuated the glucose-regulated protein 78 (GRP78) expression in rat prefrontal cortex caused by perfluorooctane sulfonate (PFOS, 10 mg/kg) a stable fluorosurfactant, which causes ERs in the brain (Oh, Kim, & Nam, 2018). While the supplementation of *Hizikia fusiformis* glycoprotein in fish diet improved the immunity in juvenile fish olive flounder, because the interleukin IL-2 and IL-6 levels increased significantly in fish fed with glycoprotein compared to those in the control (Choi, Kim, Han, Nam, & Lee, 2014). An antidepressant-like effect, was reported from *S. filiformis* lectin (SfL), the results showed no sychostimulant and anxiolytic-like effects in mice treated with intravenous injections (Abreu et al., 2018). Lectin also has the potential to be used as a bio-pesticide, the *Gracilaria ornata* lectin significantly affected the development of *Callosobruchus maculatus* larvae, a common pest of stored cowpea seeds (Leite et al., 2005).

6. Concluding remarks

Seaweed have been identified as excellent sources for protein and seaweed-derived proteins since their quality, bioactivities, and availability; IMTA practices following by biorefinery strategies are potential alternatives to face the protein variability and availability from wild harvesting. Seaweed proteins and seaweed-derived proteins are yet to be explored and they are promising candidates to incorporate in the food, pharmaceutical and cosmeceutical products. Moreover, they are an excellent material for the animal feed industry. The molecular structures of these proteins need to be elucidated, the global advances in high-resolution instruments and bioinformatics support these analyses for reliable results, such as the increased attention focused on metabolomics and proteomics.

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