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Seaweed proteins as a novel protein alternative: Types, extractions, and functional food applications

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ABSTRACT

Seaweeds, as a dietary protein source originated in Asian countries and later expanded towards France, Chile, etc. Food applications have been narrowed down due to complications in extraction. Therefore, products are not yet available in the market. Extraction of other phytochemicals along with seaweed proteins provides value addition in food products. Therefore, a trend has emerged to extract protein from edible seaweeds with many health beneficial applications. Also, consumption of many animal proteins like meat are now becoming a threat on humans due to infectious viral diseases. Hence, seaweed proteins are emerging as a global alternative source of protein.

KEYWORDS

Seaweed proteins; dietary protein; extraction; beneficial applications

Introduction

Generally, seaweeds are used as human or animal foods to gain nutrients like vitamins, minerals and polysaccharides or for their functional properties.[1] Their protein value is rarely discussed due to lack of awareness. But seaweeds are excellent source of proteins. Today, seaweeds have become an eminent protein source.[2] According to Zemke-White and Ohno,[3] it has been predicted that 2,000,000 t dry weight of seaweeds arriving from China, France, Korea, Japan and Chile had covered 90% of global seaweed production per year. The geographical distribution of algae indicates substantial differences in degree of endemism and species richness in different regions. The study evaluated by Liuzzi et al.[4] shows that biodiversity is getting diminished when moving towards lower latitudes with the highest species richness in Tierra del Fuego and southern Patagonia (54–45S), and the lowest species richness along the coasts of Rı´o Negro and Buenos Aires provinces (41–36S) in Argentina. Fortunately, greatest diversity of algae were observed in both temperate and tropical seas in the world.[4,5] Pigments in marine algae create a huge diversity among different species of algae.[6–8] For example, Brotosudarmo et al.[9] had identified chlorophyll a, β-carotene, fucoxanthin, zeaxanthin and 35 other pigments from the protein extracts of *Kappaphyccus alvarezii* and *Padina australis*. These pigments have shown antioxidant potential that provide ever-increasing demand for utilization of marine pigments in human health and nutrition. Soy beans and leguminous plants exhibit higher protein contents among plant protein sources. Similarly, some seaweeds have exhibited significant amount of proteins compared to other protein sources.[10,11]The study evaluated by Gorissen et al.[12] revealed that some commercially available plant protein sources like hemp, lupin, oat, corn, brown rice, pea, potato, wheat and soy beans had crude protein contents of 51%, 61%, 64%, 65%, 79%, 80%, 80%, 81% and 61–91% (dw) respectively. Also, animal protein sources like egg, whey protein and casein have shown protein content of 51%, 72–84% and 67–78% (dw) respectively.[12] Morgan et al.[13] has been reported that *Palmaria palmata*, an edible red seaweed can hold 35% of proteins (dry mass). Previous studies revealed that the two red algae;

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Macrocystis pyrifera (crude protein; 62%dw) and *Chondracanthus chamissoi* (crude protein; 45%dw) have demonstrated higher protein contents than some terrestrial plant sources.[14]Seaweeds have shown excellent amino acid composition than leguminous plant. For example, *Palmaria palmata* had contained higher amount of essential amino acids like methionine (2.7–4.5 g amino acid/100 g protein) than leguminous plant (1.2–1.4 g amino acid/100 g protein).[1,15,16] Also, Lourenço et al.[17] reported that algae like *Ulva, Undaria* or *Entormorpha* have been demonstrated protein contents corresponding with common vegetables. Crude protein content and amino acid profile of seaweeds provide more evidences on advantageous effects of consuming seaweed proteins as a protein source in human diet.[18] The widely used technique to measure the true protein content is the "Nitrogen to protein conversion factor of 6.25" which is formulated upon total amino acids content.[18]According to Heidelbaugh et al.,[18] N- protein conversion factor technique is the most precise method for protein determination which all nitrogen in the sample are considered to be in the form of protein. Interpretation of seaweeds' nitrogen and protein has run across diverse disciplines ranging from human and animal health to plant growth.[19–21]Therefore, nitrogen to protein conversion factor justifies the quantity and quality of protein.[22]According to the analysis of previous literature, this universal factor has overestimated the true protein content by 43%.[23]Considerable amounts of non-protein nitrogenous substances, such as nucleic acids, pigments, ammonia and other inorganic nitrogen are found in seaweeds.[24] Therefore, the nitrogen to protein conversion factor used in determining the protein content probably occupies a lower value than the traditional conversion factor of 6.25.[23] According to previous reports, the estimated conversion factors that give more justifiable protein content have been determined.[25] As suggested by Lourenço et al.[17] conversion factors for red, green and brown algae were 4.59, 5.13 and 5.38 respectively. According to analysis of 103 algae species by Angell et al.,[23] an overall "mean N-protein factors" has been estimated as 4.76. This factor used for various seaweed varieties have not been depend on geographic regions (temperate, tropical and polar), three phyla and whether the seaweed was wild harvested or cultivated.[23] All these results demonstrate that there should be specific conversion factors for different seaweeds separately to quantify the protein content accurately. Thereby, it could obviously reduce the economic loss in seaweed industry since quality products could be produced. The capability of seaweeds as a promising protein source can also be dropped down by its high water activity since it reduces the yield. Fresh seaweeds are highly susceptible to damages due to high moisture content (70–90%).[26,27] Abdollahi et al.[27] suggested that application of proper preservative methods is a deciding factor that determines final quality of the product specially when processing in large industries. Drying is the commonly used method to prevent fresh seaweeds from deterioration. But it has been reported that drying at high temperature can also leads to negative impacts on nutritional value of the seaweeds.[27,28] Generally, drying at 60°C or above reduces total phenol content and antioxidant activity of plant leaves.[26,29] However, total phenol content of *Himanthalia elongata* has been reduced when drying at 25° for long time.[30] Therefore, appropriate processing temperatures have to be used to reserve the protein quality and quantity.

Generally, proteins perform techno functional properties of food products providing emulsifying properties, texture modification, and whipping properties generated by the ability to absorb both fat and water (Amphoteric).[31]Moreover, these features contributed to the sensory properties of the final food product.[31–33] The functional properties of a protein concentrate depend on its physicochemical characteristics, which include molecular weight, amino acid composition, net charge, and surface hydrophobicity. Extraction conditions like pH, temperature, physical treatments (osmotic shock, microwaves, pulsed electric field, etc.) and enzymatic treatments also affect the physicochemical properties of a protein extract since these influenced on protein solubility, yield, and purity of the extract.[31,34] Previous studies have reported that drying conditions affect the properties of proteins differently. A study has reported the effect of two drying methods (oven and freeze-drying) on three *Sargassum* species. Accordingly, the ability of protein extraction and digestibility have been facilitated by oven-drying method (60°C, 15 hours) while freeze-drying(−70°C, 5 days) had manifested good physico-chemical properties.[35] Also, stabilization methods used in postharvest period had greatly

influenced on solubility and precipitation yield of proteins extracted from *S. latissima* using alkaline treatment/isoelectric focusing.[27] Higher protein solubilization and total protein yields have been observed under freeze dried (−80°C, 4 days) and oven dried(40°C,overnight) postharvest methods due to inactivation of protein inhibiting agents like phenol.[27] Sun drying and freezing at −80°C methods have elicited lower protein yields.[27] These results indicate how important to study about the behaviour of properties of algal protein during processing. Kazir et al.[25] stated that due to increasing population and lack of land and fresh water resources, the oceans furnish an irresistible sourcing for nutrients. Most of algae species have high protein content which accompanied with their seasonal growth throughout the year providing high yield protein.[25] Products prepared using seaweed proteins have become limited mainly due to the absence of feasible extraction methods which give higher yields. Also, it is essential to evaluate their digestibility under human gastric and intestinal conditions.[25]

It has been revealed that bioavailability can be inhibited by the seaweed cell wall due to its complex matrix structure. Internal factors like high fibre content also entrap the absorbability of plant proteins into the human intestine.[36] Previous data assessments show that the content of soluble fibre is higher than the insoluble fibre content of seaweed. For example, diets containing *Porphyra tenera* and *Undaria pinnatifida* had reduced the bioavailability in rats.[37] This implies that dietary fibre inhibits the protein digestion by blocking the access of proteolytic enzymes.[38] Polysaccharides which mainly in the algal cell wall interact with proteins also reduces proteolysis of seaweed proteins.[39] The relative digestibility of *Palmaria palmata* (Dulse) was reported as 56% with reference to the hydrolysis (digestibility) of casein as 100%.[39]Presence of carbohydrates like xylan may have restricted the hydrolization of Dulse proteins.[40] According to previous studies, physical and enzymatic treatments have resulted with increased bioavailability of protein. Among different algal protein extraction methods, enzymatic hydrolysis of proteins generates precursors of bioactive peptides which can be produced during in vitro hydrolysis of proteins by relevant proteolytic enzymes like pepsin, protease, bromelain, alkalase etc.[41,42] The seaweed proteins were hydrolysed by the enzymes to form amino acids which were readily absorbed by human body. Proteins from *Ulva sp. and Gracilaria sp*. that have been extracted using different solutions have reached at least 89% of proteolysis when undergoes with gastro-intestinal digestion.[25]It has been reported that physical treatments like oven dying increases the *In vitro* protein digestibility than freeze drying.[26] Microalgae protein can be hydrolysed by pepsin to obtain bioactive peptides.[43] Many studies have reported that marine bioactive peptides have been applied in food, nutraceutical and pharmaceutical industries because of their therapeutic potential in treatments or prevention of diseases using antihypertensive, antioxidative, anticoagulant, and antimicrobial components.[44,45]

Types of seaweed proteins

The amount of proteins in seaweeds differs according to the species, maturity, and geographical location.[46] Generally, brown, green, and red algae have shown 3–15%, 9–26%, and 10–47% of crude protein, respectively.[39,47] Since we rarely discover documented history on algal proteins' structure and biological properties, producers find difficulties in producing novel products. Seaweed proteins can be identified mainly as phycolectins, glycoproteins, enzymes, phycolythrins, and mycosporine-like amino acids. Glycoproteins in the cell wall perform physiological function as the major protein type in most of the seaweeds.[11]

Glycoproteins

According to Spiro[48] "Glycoproteins (GP) can be simply defined as proteins to which carbohydrate is covalently attached" through glycosylation. Usually, these two were linked using N-glycosyl linkages or O-glycosyl linkages, while some glycoproteins contain both links.[14,49]Glycoproteins are embedded in the seaweed cell wall matrix and supported in adhesion.[2] Arabinogalactan

proteins (AGPs) and hydroxyproline-rich glycoproteins (HRGPs) are two types of cell wall attached glycoproteins.[50] Previous studies have revealed that the cell walls of green seaweed contains AGPs and hydroxyproline-rich glycoproteins.[50] AGPs have been compromised by terrestrial plant development with the evolution from algae to flowering plants.[51] Previous studies have shown the amount of AGPs and HRGP in *Codium fragile* was low.[52,53] It is important to study the structure of these proteins. AGPs have a carbohydrate component and a protein fraction.[54] Accounts for around 90% (dry weight basis) that carbohydrate fraction mainly contains arabinose and galactose residues (90% dry weight basis), while protein fraction is composed of hydroxyproline residues (approximately 10% dry weight basis).[54] Furthermore, glycoprotein that has been isolated from *Codium decorticatum* contains 36.24% carbohydrate. The carbohydrate portion is formed by monosaccharides: rhamnose, galactose, glucose, and mannose with a mole ratio of 38:30:26:6.[49] This has been formed by protein and carbohydrate with (1→4)-linked β-galactose and β-linked glucose residues.[49] Glycoproteins function well in both algal cells itself and in the human body when consumed as a food ingredient. Both carbohydrate and protein fractions contribute towards these functions and maintain structure–function relationships that offer good biological characteristics of GP.[49] It has been reported that algal glycoproteins exhibit miscellaneous biological properties, such as cell proliferative, hepatoprotective effect, anticancer, anti-inflammatory, anti-Alzheimer's, antiviral, and antioxidant activities.[49] Glycoproteins isolated from the green algae *Codium decorticatum* have shown anticancer activities specially against human breast cancer cells (MCF-7, Siha and A549 cells have been inhibited at 60, 75 and 55 μg/mL concentrations of GP respectively after 24 h incubation).[49,55,56] Also, few studies have reported the antioxidant (Superoxide dismutase activity- 53.45%), anti-Alzheimer's (Acetylcholinesterase and Butyrylcholinesterase inhibition activities of GPs stated by IC50 values were 63.56 and 99.03 μg/mL), hypoglycaemic (Inhibitory effect was detected as IC50 values of 0.11 and 0.29 mg mL^{-1} in yeast and rat intestinal α-Glucosidase inhibitory activity assays respectively) and anti-inflammatory (85.27% inhibition in the In vitro COX inhibition assay at a concentration of 500 μg/mL of glycoprotein) activities of glycoproteins obtained from brown algae *Undaria pinnatifida* that is available mostly in East Asia as a food ingredient.[57–59] Antioxidant (DPPH radical scavenging activity and Superoxide dismutase activity were 85% and 94% respectively) and DNA protection actions have been identified from the glycoprotein isolated from brown algae *Saccharina japonica*.[60]

Lectins

Lectins or agglutinins, are proteins that have the affinity to bind with carbohydrate proteins and are found in a wide range of organisms.[61] Lectins have been classified as mannose-, galactose-, N-acetyl-glucosamine-, fucose-, and sialic acid-binding lectins according to their affinity to bind with sugars.[62] Mannose-specific seaweed lectins from red algae have been characterized in previous studies. According to the study of Barre et al.,[62] mannose-specific lectins of seaweeds have been classified in to five groups depending on the structural scaffold, namely, the griffithsin lectin family, oscillatoria agardhii agglutinin homolog (OAAH) lectin family, the legume lectin-like lectin family, the Galanthus nivalis agglutinin (GNA)-like lectin family, and the MFP2-like lectin family, and these lectins have elicited anticancer properties and anti-HIV properties.[62] Chaves et al.[63] reported two isolectins that have been isolated from the marine red alga *Solieria filiformis* SfL-1 and SfL-2. The primary structures of SfL-1 and SfL-2 consist of four tandem-repeat protein domains with 67 amino acids each and have been similar to OAAH-family lectins.[63] These isolectins are composed of two β-barrel-like domains formed by five antiparallel β-strands.[63] The mixture of these isolectins (SfLs) has shown an anticancer effect against MCF-7 cells. According to previous studies, a lectin isolated from *Solieria filiformis* has exhibited antidepressant-like action also, anti-nociceptive and anti-inflammatory activities have been shown in lectins isolated from *Caulerpa cupressoides var. lycopodium*.[64,65] Also, a novel lectin has been isolated from the green algae *Halimeda renschii*; namely HRL40, had shown potential inhibitory action on influenza virus (A/H3N2/Udorn/72).[66] This lectin has been formed by combining

a quaternary protein of a 11,641 Da polypeptide with a disulphide bond to a carbohydrate which its binding profile similar to antiviral Type I high-mannose specific lectin.[66] From the phycolectins that have been distinguished among algae, most of them were thermostable and low molecular weight proteins.[11] Also, have shown inclination toward oligosaccharides or glycoproteins, and divalent cations have not been essentially required for the structural coherence or biological activity.[11] Lectins are useful for: detection of alternatives in glycan synthesis with the ability of preventing diseases; blood group typing and definition of secretor status; quantification of aberrations of cell surface glycan presentation, e.g., in malignancy; cell markers for diagnostic purposes including infectious agents like viruses, bacteria, fungi, parasites.[61] Lectins from four marine algal species were examined for interaction with human platelets by Matsubara et al..[67] These results have indicated that the algal lectins are a new group of inhibitors and may be useful to study glycoconjugates on platelet membranes and to design novel platelet aggregation inhibitors.[67] The lectin designated hypnin A, from the red algae *Hypnea japonica*, inhibited adenosine diphosphate (ADP)- or collagen-induced human platelet aggregation in a dose-dependent manner.[67]

Mycosporine-like amino acids

In seaweeds, there are secondary metabolites called mycosporine-like amino acids (MAA); having low molecular weight, water-soluble molecules absorbing UV (Ultra Violet) radiation in the wavelength range 310–365 nm.[68] MAAs denature at high temperatures and is water-soluble due to their amphoteric properties.[11] They function as sunscreen to protect against UV radiation serve as antioxidant molecules, accumulate as compatible solutes following salt stress, etc.[68] MAAs have been commercially explored as sun care products for the protection of skin and other non-biological materials, e.g., as photo stabilizing additives in plastics, paint, and varnish.[61] A large number of derivatives have been tested in skin care products.[61] Moreover, a product called Helioguard® 365 that contains mycosporine-like amino acids from the red alga *Porphyra umbilicalis* has been commercialized.[61] The MAA called "Porphyra-334" extracted from *Porphyra vietnamensis* has also exhibited sunscreen protection due to its ability to prevent harmful UV radiation.[69] This MAA has a 5.11-fold greater sunscreen protective ability than the commercial product "Aloe vera gel" available in the Indian market.[69]

Phycobiliproteins

Phycobiliproteins, which are "located in the chloroplast stroma" are the main proteins found in red seaweeds.[70] These proteins behave like sensors, which absorb energy in the visible spectrum.[71] Phycobiliproteins are a major pigment in Rhodophyta. They are phycoerythrin, phycocyanin, allophycocyanin, and phycoerythrocyanin. *R*-phycoerythrin is an oligomeric water-soluble chromoprotein.[72] Phycobiliproteins are not found in photosystems of the lipid bilayer and attached to cytoplasmic surface of thylakoid membranes called phycobilisomes.[11] These phycobiliproteins have exhibited poor fluorescence ability.[11] Techniques such as ammonium sulfate precipitation and chromatography (ion exchange, gel filtration, etc.) have been used to purify these proteins. R-phycoerythrin is applied in immunology, cell biology, and flow cytometry and as a colorant in the cosmetics industry and foods as well.[72] Furthermore, the seaweed proteins have soluble and insoluble protein fractions.[23] The water-soluble proteins extracted using *Ulva armoricana* showed higher digestibility by trypsin or chymotrypsin compared to *Palmaria palmata* and *Sargassum* sp.[73] The protein quality and its digestibility are two important parameters, which provide itself to function inside the human body.[74] Different extraction methods are discussed in Table 1.

When studying the three major types of seaweed protein extraction methods; enzymatic hydrolysis (Cellulase, xylanase, κ-carrageenase, etc.), physical processes (i.e Osmotic stress, high shear force, aqueous treatment, potter homogenization, etc.), and chemical extraction (i.e., acid-alkaline treatment), the highest extractable protein yield have been elicited by enzymatic hydrolysis. For example, the extractable protein yields of *Palmaria palmata* using enzymatic extraction, physical process, and chemical extraction have been reported as 67%, 40%, and 24%, respectively.[77] Enzymatic digestion

Table 1. Different types of seaweed proteins and their extraction methods.

Seaweed	Name of the protein	Protein yield %	Extraction method
Ulva sp. (Green algae)[2]	Glycoproteins(GP)	"UvGP-1" (0.54) "UvGP-2 DA"(0.52) "UvGP-2-DS"(1.98)	Water TCA $(NH_4)_2SO_4$
Ulva lactuca (Green algae)[75]	GP fraction G	ND	Distilled water Water NaOH DEAE cellulose
Saccharina japonica (Brown algae)[60]	Glycoprotein	0.27	Distilled water and ethanol
Solieria Filiformis(Red algae)[63]	Lectins "SfL-1" "SfL-2"	ND	Phosphate buffer, $(NH4)_2SO_4$
S. filiformis(Red algae)[64]	Lectin "SfL"	ND	Tris-HCl buffer, $(NH4)_2SO_4$
Capsosiphon Fulvescens(Green algae)[76]	"Cf-hGP"	ND	Sodium acetate, Methanol: chloroform: distilled water Purified by lectin wheat germ agglutinin resin
Undaria Pinnatifida (Brown algae)[57]	"UPGP"	ND	Distilled water Ethanol

*ND Not detected

appears to be an effective treatment for R-phycoerythrin extraction from the red seaweed *Palmaria palmata*.[78] The study of Dumay et al.[78] revealed that under enzymatic optimization conditions, R-phycoerythrin extraction yield (12.36 g kg^{-1} dw) was 62 times greater than without enzymatic treatment (0.20 g kg^{-1} dw). Also, it has been 16 times greater even without optimization (Yield = 3.28 g kg^{-1} dw).[78] This provides an additional economic value for R-phycoerythrin production.

Amino acid composition of algal proteins

The quality and nutritional value of algal proteins are based on their amino acid composition. Humans need all essential amino acids for their growth. Amino acid composition of foods varies greatly. Foods being labelled as "high quality" protein sources consisted of higher amounts of essential amino acids like lysine, methionine, etc.[79] Interestingly, all essential amino acids are available in most seaweed proteins.[39,80]The by-product obtained during agar extraction from *Gracilaria fisheri* is rich in proteins and essential amino acids.[81] Accordingly, 60.29 g/100 g of total amino acids (including free and bound forms) have been obtained by the acid hydrolysis of the proteins in the seaweed by-product.[81] *Caulerpa lentillifera* and *Ulva reticulata* proteins were of high quality because the essential amino acids represented almost 40% of total amino acids and the essential amino acid profiles were closed to those of egg and soya protein, except for relative lack of data on tryptophan, methionine and cysteine.[82] Previous studies have reported that "aspartic" and "glutamic" amino acids occupy a large scaffold in most of the seaweed proteins (Table 2).[17] According to Table 2, amino acid content is high in green algae than red and brown algae.[86,87] It implies that amino acid composition varies with the species. The amino acid composition changes during the sampling period of the year.[2,73] Hence, the protein composition varies with the season of the year. The amino acid composition of 19 tropical seaweeds have been studied by Lourenco et al.[17] to evaluate the variation of amino acids among six chlorophytes, four phaeophytes and nine rhodophytes. Among the six green algae evaluated in this study; *Caulerpa fastigiata, Caulerpa racemose, Codium decorticatum, Codium spongiosum, Codium taylorii* and *Ulva fasciata*, the highest total amino acid content has been elicited by *Caulerpa fastigiata* (98.4 as percentage of amino acid/100 mg of algal protein).[17] The highest content of methionine has been reported from *Codium taylorii* (i.e 2.0 as percentage of amino acid/ 100 mg of algal protein).[17] From the four brown algae species; *Chnoospora minima, Dictyota menstrualis,*

Table 2. Amino acid composition of some seaweeds (in g amino acid/100 g protein).

No.	Amino acids (AA)	*Caulerpa lentillifera* (Green algae)[82]	*Ulva reticulate* (Green algae)[82]	*Kappaphycus alvarezii* (Red algae)[83]	*Gracilaria salicornia* (Red algae)[83]	*Turbinaria ornata* (Brown algae)[84]	*Durvillaea antarctica* (Brown algae)[85]
	Essential AA						
1	Threonine	6.38	5.41	2.49	2.25	0.15	5.84
2	Valine	7.03	6.30	2.49	2.20	0.23	9.97
3	Lysine	6.63	6.02	1.51	-	0.20	4.22
4	Isoleucine	5.01	4.23	2.14	1.98	0.18	8.05
5	Leucine	8.00	7.90	2.34	2.16	0.26	15.88
6	Phenylalanine	4.93	5.26	2.11	1.79	0.19	9.97
7	Methionine	-	-	1.69	1.61	0.05	3.89
	Non essential AA						
8	Aspartic	11.56	12.50	3.33	-	0.53	4.17
9	Serine	6.14	6.39	2.68	2.90	0.10	5.38
10	Glutamic	14.39	12.98	11.67	2.79	0.58	17.87
11	Glycine	6.87	6.49	2.97	2.18	0.22	18.36
12	Arginine	7.03	8.65	2.40	2.40	0.19	4.83
13	Histidine	0.65	1.08	1.60	2.29	0.07	2.26
14	Alanine	6.87	8.09	2.93	2.51	0.23	9.57
15	Tyrosine	3.88	3.62	1.81	1.74	0.05	4.45
16	Proline	4.61	5.08	-	-	0.17	7.95
17	Cystin	-	-	-	-	0.00	0.78

Padina gymnospora and *Sargassum vulgare*, the highest total amino acid content and methionine content have been recorded from *Padina gymnospora* (97.2 as percentage of amino acid/100 mg of algal protein) and *Sargassum vulgare* (2 as percentage of amino acid/100 mg of algal protein) respectively.[17] In the same study it has been revealed that among the nine red algae; *Acanthophora spicifera, Aglaothamnion uruguayense, Cryptonemia seminervis, Gracilaria domingensis, Gracilariopsis tenuifrons, Laurencia flagellifera, Plocamium brasiliense, Porphyra acanthophora* and *Pterocladiella capillacea*, the highest total amino acid content and methionine content have been obtained by *Porphyra acanthophora* (104.2 as percentage of amino acid/100 mg of algal protein) and *Gracilariopsis tenuifrons* (1.3 as percentage of amino acid/100 mg of algal protein) respectively.[17]The content of aspartic acid and glutamic acid in red and green seaweeds were significantly higher than that of brown seaweed.[17]

These impacts occur with different amino acid content in algae. More aspartic and glutamic acid contents together produce a distinctive flavor in seaweed; its savoury flavor (umami flavor).[83] Meanwhile, serine can provide a sweet taste that results in the complex flavor of seaweed.[83] Furthermore, Holdt and Kraan[88] reported that the taste of nori (dried or edible seaweed often used in Japanese cookery) is the result of a large number of different amino acids, including alanine, glutamic acid, and glycine. The study by Lumbessy et al.[83] has reported that *K. alvarezii* and *G. salicornia* seaweeds are rich in aromatic amino acids (i.e., threonine) and have limited sulphur amino acids (i.e., lysine). This suggests that *K. alvarezii* and *G. salicornia* seaweeds can act as a complementary protein source for humans as well as animals. When evaluating the amino acid composition of *Gracilaria sp*. and *Ulva sp*. protein concentrates obtained using alkaline treatment and their profiles have shown similarities with egg albumin, meanwhile they have been in compliance with FAO/WHO recommendations.[25] Amino acid composition of different seaweeds are shown below in Table 2.

The direct way for quantifying the "true protein content" in seaweeds is basically represented by sum of the amino acid residues after hydrolysis.[18,86] The "amino acid residue" means the proteomic amino acid fraction that actually remained after hydrolysis (as when copolymerized in polypeptide chains).[86] Amino acids are analysed using chromatography techniques like Ultra performance liquid chromatography (UPLC) and Reversed phase high performance liquid chromatography (RP-HPLC).[86,89]

Extraction of seaweed proteins

The interest in protein extraction from seaweeds has now sparked up.[39,45,77,90–92] Due to the high protein content in seaweeds, these were available as functional food ingredients. A major obstacle for protein extraction is the complex polysaccharide matrix in the cell wall.[2,91,93] Hence, food applications are restricted. Higher percentages of phenols and phlorotannins also decrease the protein extractability[94,95] and digestibility of the proteins.[26,95–97]

Due to the low protein extraction yield, poor technical clarifications such as low gel electrophoresis resolution and inadequate further analysis of algal protein may occur restrictions in utilization of seaweed proteins.[2] According to the study of Harrysson et al.,[98] results of protein analysis varied with the expected value due to interference of compounds like pigments, carbohydrates, and salts. Efficiency of direct extraction procedures had been influenced by seaweeds' chemical and morphological properties.[99] For example, tough leathery brown seaweeds were shown to be more resistant to certain extraction procedures compared to seaweed with soft thalli.[99]

Extraction Methods

Protein extraction procedures differ by many factors. The volume of water and exposure time used for the extraction of water-soluble proteins, pre-treatment mode (fresh/dried, milled, freeze-dried etc.), buffer type, and time duration used, solubility of proteins, the method of precipitation (e.g., centrifuge time and force)[15,26,90,99] are some of them. Traditional protein extraction methods (aqueous, acidic, and alkaline) have shown lower yields (24% to 59%).[100] Although novel protein extraction methods (ultrasound-assisted extraction, pulsed electric field, and microwave-assisted extraction[101]) have exhibited higher extraction yields, these have not yet been fully developed to an economically feasible scale.[102] Enzyme-assisted extraction (EAE) can be suggested as a beneficial protein extraction method[100] because it provides many advantages like high yield and conserves significant protein qualities.[103] The study of Vasquez et al.[102] reveals that carbohydrase enzyme 'cellulase' had intensify the protein extraction yields from the brown seaweed *Macrocystis pyrifera* and the red seaweed *Chondracanthus chamissoi*. According to this study, enzymes like cellulase, pectinase and α-amylase etc. had hydrolysed seaweed polysaccharides.[102] These extracts had resulted with better antioxidant and antihypertensive activity which can be used as potential nutraceuticals or functional ingredients in food industry.[102] Same study shows that proteins extracted from *M. pyrifera* and *C. chamissoi* have been increased by 4.7 and 1.5 times respectively using Enzyme assisted extraction compared to the non-enzymatic extraction.[102] In comparison with other methods, carbohydrases have been reported an increase in extraction yields for protein extraction from seaweeds.[15,77] According to the study of Fleurence,[15] carrageenase and cellulase had elicited 10 times higher extraction performance on *Chondrus crispus* while agarase and cellulase also had increased 3 times higher efficiency in *Gracilaria verrucosa* compared to non-enzymatic process. Alkaline treatment along with carbohydrases have resulted a higher yield in protein extraction from *Palmaria palmata* since algal protein solubility is high in alkaline pH.[77,104] However, carbohydrases like carrageenase and agarase which are specific to polysaccharides were known to give less qualitative performance[77] on proteins than nonspecific carbohydrases since these hydrolyse cellulose and other polysaccharides partially without disturbing the commercial viability of the seaweeds.[102] Maximum protein extraction yield would be reached when provided with optimum physio-chemical conditions and suitable selection of specific enzyme.[105–107]

Apart from the above extraction methods various liquid systems such as distilled water,[23,39,73] buffers, alkaline solutions,[45,93] urea,[108] lysis solutions,[45] and phenol-based extraction systems[91,108,109] have been used to extract proteins from seaweeds. Further, partial characterization of the glycoprotein-rich fractions can be carried out by the matrix-assisted laser desorption ionization-time of flight/mass spectrometry (MALDI-TOF/ MS) analysis.[2]

Among various liquid systems, the most effective results have been demonstrated by phenol-based extraction.[90,91,108] Higher extractable proteins have been reported with lysis solution systems compared to water and buffer.[2] Today, there is an immense trend for green extraction systems in the food

and pharmaceutical industries. For example, water and buffer extractions.[110] In water extraction, the addition of distilled water and holding it for a few hours creates osmotic shock and facilitates protein extraction from seaweeds.[93,99] The combination of physical methods and iso-electric precipitation for extraction of seaweeds proteins is depicted in Fig. 1.

Methods including the salting out method, isoelectric precipitation, and the accelerated solvent extraction (ASE®) method have manifested better results after pre-removal of lipids and phlorotannins.[98] Among these, the highest protein yields have been achieved using the pH-shift method applied to *Porphyra umbilicalis* and *Saccharina latissima*.[98] Moreover, fatty acids in *Ulva lactuca* and *S. latissima* also have been increased by 2.2 and 1.6 times, respectively.[98] Hence, it is advantageous to use this method in food industry.

However, selecting the best method to obtain the highest yield of seaweed protein is challenging. Previous studies have revealed that cultivated seaweeds have shown higher protein content, inattentive to protein analysis methods.[23,56] Seaweed cultivation seems sound reasonable in obtaining a yield with high protein content and helps to reduce the burden caused due to harvesting huge quantities of seaweeds from wild populations.[56] Grote[111] has reported that in Europe, including countries like Norway, France, and Ireland, wild harvested biomass has been used for their productions (more than 97%). Also, in Latin America nearly 96% of the total production has been harvested from naturally grown.[14,111,112]The protein quality, and yield could be improved through Microwave Assisted Extraction, Enzyme Assisted Extraction, and ultrafiltration extractions[113–115]Pressurized Liquid Extraction (PLE), or Accelerated Solvent Extraction (ASE), also increase the efficiency of extraction, while stabilized in the liquid state to increase mass transfer rate at temperature (50–200°C) and pressure (35–200 bar).[56,116] However, both conventional and modern extraction methods exhibit advantages and disadvantages as well (Table 3).

Biological activities of proteins

Seaweeds are well-known today for their natural bioactive substances.[125,126] For example, sulfated polysaccharides,[127] phlorotannins,[128] pigments,[128] sterols,[129] peptides, and proteins, etc.[15,130] In the algal protein extraction process, valuable phytochemicals are also co-extracted to provide augmented final products meanwhile reducing the waste.[25] Polyphenols can be considered as one such bioactive molecule which is present in algae[106,131] and is highly beneficial for human health mainly as reducing agents/antioxidants.[132] Polyphenols like Catechin, epicatechin, and gallate that have been found in *Halimeda* sp. has provided potential antioxidant properties in algae.[133] Similarly, sulfated polysaccharides like fucoidan, alginic acid, laminaran that have been found in *Turbinaria conoides* and

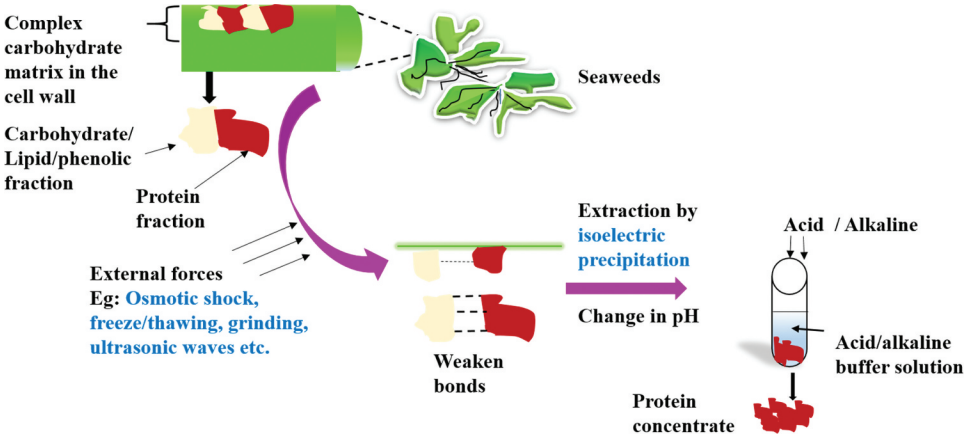

Figure 1. Extraction of seaweed proteins by combining physical methods and iso electric precipitation.[41,45,46,50].

Table 3. Extraction methods

Extraction method	Theory applied	Factors affected	Advantages	Limitations
Liquid extraction Distilled water[39, 57] Isoelectric precipitation[117] Acid/Base [45] Buffers eg: Phosphate buffer Lysis solutions(Reducing agents like 2-mercaptoethanol or N-acetyl-L-cysteine[77]	Creates osmotic shock and facilitates extraction. Liquids like ammonium sulphate, trichloroacetic acid, urea and organic solvents are added to increase protein solubilisation.[70] In isoelectric precipitation proteins are precipitated in their isoelectric point by acidifying the medium. Acids and bases also increase protein solubilisation. Use of acid prior to solubilisation with alkaline solution has been shown to aid the release of polysaccharides and proteins located in the cell wall matrix.[70] Lysis solutions contain reducing agents which facilitate dissociation of proteins from polysaccharide complex matrix by breaking S–S bonds.[26]	Time and temperature used for the treatment, type of the solvent, volume of the solvent, protein solubilisation, pretreatment (freezing,drying), pH of the liquid medium	Economically feasible in food applications. Eg: Water extraction	Nontoxic reagents have to be used in food applications. Eg: N-acetyl-L-cysteine (NAC) Yield is mainly affected by the protein solubility. Time consuming prolonged agitation is needed to extract necessary compounds.
Enzyme assisted extraction (EAE)[102]	Commercially available enzymes (eg: Cellulase, glucanase, protease)[119, 120] help in releasing proteins from polysaccharides bound to cell wall by hydrolysing them.	Enzymes which are specific to their substrate, temperature, pH	Food grade approach, preserve properties of protein, higher yield than non- enzymatic methods. Extracts exhibit better antiviral, antioxidant, anticancer like beneficial properties than non- enzymatic methods due to high co-extraction of bioactive metabolites.[119]	Higher cost for enzymes. Due to the presence of higher amount of uronic acid in the cell wall least applicable in brown seaweeds.[118]
Ultrasound/sonication assisted extraction(UAE)	Ultrasonic cavitation is the principle used here. Bubbles formed by cavitation collapse on the surface of solid particles & interparticlular collisions cause perforation of cell walls and release proteins to the media. Furthermore, the implosion of cavitation bubbles in solvent media create macro turbulences and micro-mixing.	Type of solvent, ultra- sonication time, ultrasonic frequency, temperature, pH	A green technology, Simple, less time consuming and uses less solvent than other methods, can be easily coupled with other extraction techniques, can be performed under room temperature, can prevent the oxidation and decomposition of target natural products.[115]	High cost compared to liquid extraction, possibility of disruption of other cellular compounds due to uncontrolled ultra-sonication.

(*Continued*)

Table 3. (Continued).

Extraction method	Theory applied	Factors affected	Advantages	Limitations
High hydrostatic pressure extraction (HHP)[124]	Pressure is created by the solvent used under controlled time and temperature. This pressure leads polysaccharides to release proteins to the medium.	Time duration and temperature at which pressure is applied, pressure.	Microbiologically safe processing method. A green technology, Nonthermal technique. Therefore, minimizes decomposition of essential compounds, Increases the yield, less time consumption, less expenditure of organic solvents and energy. anthocyanins.[123]	High pressure conditions 2 can harm other sensitive compounds. At pressures higher than 400 MPa, or for longer treatment times, the polyphenol content has been decreased.[121]
Microwave-assisted extraction (MAE)[70]	Nonionizing electromagnetic waves having frequency from 300 MHz to 300 GHz were applied on sample.[122] This frequency creates a dipole rotation which molecules reorient in the direction of the electric field, causing thermal agitation.[122]As the water evaporates due to heat, the intracellular pressure increases.[122] As a result, the cell walls were broken and the high-value compounds started to leach out from the cell.[122]	Microwave frequency, time, temperature, pressure, pH	A green technology, reduce the use of chemicals, both the heat and mass gradients work in the same direction toward the outside of the cells allowing to increase the yields.[122] Reduce the time of the process.	Need to use a separate 3 method to segregate the extract from the sample. Eg: Centrifugation, filtration Higher temperatures can damage other thermo unstable compounds and structures in the cell.[122]
Sub- and supercritical fluid extraction.[125]	Supercritical carbon dioxide (CO2) or subcritical water as a solvent were used. Supercritical solvent penetrates rapidly into the solid- matrix interior and solutes from solid-matrix diffuse into the supercritical CO2. .Hence, it facilitates higher extraction yield than other methods.	Supercritical solvent used, time, temperature, pressure	Supercritical CO2 possesses a higher diffusion coefficient which assists to a more favorable mass transfer.	Diffusion of solutes 4 from the solid-matrix into the supercritical CO2 occurs slowly. Therefore, a sufficient contact time must be provided to allow the CO2 solvent to penetrate into the solid-matrix and allow solute diffusion from the solid-matrix into the CO2 solvent.

phycoerythrin and phycocyanin that were found in red algae also have shown antioxidant properties in algae.[134,135]It has been reported that glycoproteins extracted from algae have beneficial properties like preventing liver cells, antidiabetic, anti-inflammatory, antioxidant, and anti-Alzheimer's activities.[2] Dexamethasone (DEX)-induced myotube atrophy has been prevented by the peptides of *Pyropia yezoensis* called "PYP15."[56,136] Many new bioactive natural molecules and valuable metabolites from seaweeds having an economic impact have been investigated during the past few years.[61,88] Several biological activities such as antiviral,[137] antibacterial[138] antioxidant[139] have been identified in red (Rhodophyta) and brown (Phaeophyta) algae. Antioxidant and antiviral properties of proteins extracted from *Ulva armoricana* have been previously reported with the use of enzyme-assisted extraction.[72–75,118] Amino acids, which show antioxidant potential have been disclosed by enzymatic hydrolysis, hence they easily donate hydrogen to the peroxyl radical.[25,140] Peptides are the most commonly available antioxidant substances in food due to their high bioactive performance.[56,141]

Due to the high attraction of polyphenols towards proteins,[25] polyphenols present in protein extracts[142] facilitate their co-extraction, and may improve their health-promoting value. Recent studies indicated that seaweed, which contained 4.2% of polyphenol could effectively suppress body weight gain, decrease lipogenesis in the liver, and inhibit hyperinsulinemia by promoting energy regulation.[143] The ethanolic extract of red seaweeds like *Plocamium telfairiae* which contains 26.79 ± 0.08 protein, contained around 4% polyphenol and have shown anti-obesity activities.[143] Moreover, amino acid residues of algal proteins have elicited antioxidant properties.[140] Assessment of antioxidant activity in algal proteins depicts potential health benefits with regards to their valuable amino acid profile.[144] Furthermore, the co-extraction process has elicited best medicinal benefits in utilizing plant sources rather than pharmaceutical sources in food products.[145] Therefore, the antioxidant ability of seaweed protein provides added value in food processing. In addition to these, "mannose-specific algal lectins" specially from Rhodophyta has elicited anti-cancer and antiviral properties.[62] These identified proteins could be utilized to protect against human immunodeficiency virus (HIV-1), due to their broad-spectrum specificity to interact with the envelope glycoprotein and prevent the pathogenic effect of HIV-1 towards the host CD4 + T-lymphocyte cells *in vitro*.[62] The high-mannose specific lectin and its recombinants from *K. alvarezii* have a high tendency to bind with the glycoprotein gp120 of the virus envelop and has shown a strong anti-HIV activity.[56,146]

Functional food applications

The seaweed industry in Europe has branched towards France, Norway, and Ireland as main producers, while Spain, Portugal and the UK as small producers and suppliers.[46] Although the protein quantity and quality of many seaweeds have been explored,[77] their food applications have been promoted sparsely. Food applications are merely a challenge since seaweed proteins are novel.

Challenges in food applications

There are several reasons that restrict food applications of seaweed protein. Mainly, the efficiency of protein extraction has been reduced due to the more complex and heterogeneous structure of cell walls in seaweeds than those of terrestrial plants.[147] They are composed of mixtures of sulfated and branched polysaccharides which are associated with proteins.[103] Hence, treatments are needed to degrade these structures and extract proteins. However, advanced methods (EAE, Ultrasound assisted extraction (UAE), Microwave assisted extraction (MAE) etc.) which give higher extraction yields are of high cost. Also, it is a big challenge to maintain the same quality and quantity of seaweed protein throughout the year since environmental factors, or harvesting period and locations probably resulted an instability.[56] For example, higher protein contents have been reported during the winter compared to summer and autumn.[15] Therefore, this will appear to have a negative impact on seaweed food industry compared to other plant sources. Although seaweeds are popular as a healthy and safe food for humans, they are very easy to experience damage in fresh conditions because the chemical composition is dominated by water. For example, *Caulerpa* sp. were easily susceptible to

microorganisms and physical damage due to mushy nature of their thalli.[148]Therefore, food applications become limited due to the losses occurred during harvesting and storage. Lack of knowledge on protein content and functionality of different seaweeds among consumers is another reason that limits food applications. Although several studies have evaluated the functional properties of different seaweed varieties, rarely these functionalities have come in to practice.[31,149,150] Functional properties like solubility, foaming capacity and stability, water holding capacity, oil holding capacity and emulsifying ability are dependant factors of food applications of seaweeds. For example high nitrogen solubility is required for protein concentrates to be used as functional ingredients in many foods including beverages, dressings, coffee whiteners, whipped toppings and confections.[31,151] Therefore, scarcity of knowledge and experience on handing these functional properties may delay the approaches for reaching seaweed protein based food products to the market. Apart from the functional properties, presence of contaminants, anti-nutritional factors, allergens and food safety hazards associated with novel proteins also affect food applications.[31,152]

Possible solutions

Interestingly, several studies have brought solutions to this. The potential food applications given in Table 4 will provide more evidence on utilization of seaweed proteins for human consumption. Although the extraction process of protein is often affected by polysaccharides in the cell wall, production capability has been improved with the introduction of efficient methods like EAE, UAE, and MAE. In EAE, enzymes convert water-insoluble materials into water-soluble materials and this method does not adapt to any toxic chemicals.[103] In addition, cheap and food-grade enzymes are useful in the future to extract proteins commercially.[103] Currently, research studies have proved that the toxicity of many seaweed proteins has minimal impact on humans. Cytotoxicity of glycoprotein fractions isolated from selected seaweeds has been tested on cultured Vero cells, and no toxicity has been detected in *Solieria chordalis* (Rhodophyta), *Ulva sp*. (Chlorophyta) and *Sargassum muticum* (Phaeophyta).[118,154] Generally, seaweeds should be stored under freezing conditions or dried immediately after harvesting to prevent quality and nutrient loss. The strong tastes associated with seaweed may also be reduced by cooking or washing since these are allied with many beneficial micronutrients.[36]

Food applications

The application of seaweed proteins as a source of animal feed has been investigated. The study evaluated by Anh et al.[19] shows that the green algae *Cladophora* spp. could replace up to 30% of protein in the diets for postlarval tiger shrimp (*Penaeus monodon*) as a protein substitute. Various food products, like soups, ready to serve foods, snacks are usually flavoured by thermally processed flavourings.[81] When sources of plant proteins like wheat,[156] soy,[157] and Brassica[158] were thermally processed, meaty or beefy flavours were generated meanwhile they act as good flavour enhancers than animal proteins.[81] Although, seafood flavour is generally produced by protein hydrolysates from marine sources, like fish, crab, prawn etc., it is difficult to maintain high quality due to their susceptibility to fat oxidation and removing excess fat being expensive.[81,159] By-products produced after agar extraction contain low fat and rich in flavour generating amino acids, such as glutamic, aspartic, lysine and arginine.[81] The study evaluated by Laohakunjit et al.[81] has suggested, the by-products of agar-extraction from *Gracilaria fisheri* are a suitable protein source to produce such flavours because they contain more protein (28%) and less fat content (0.60%). Among various proteolytic enzymes bromelain is generally used in many food products because of its stable activity over a broad pH range (pH 4.0–8.0) and the cleavage of peptide bonds with a wide range of specificity.[81]

For ages, marine algae has been used as a flavour enhancer in the preparation of soups and salads.[81] Edible green seaweed *Ulva* sp. (sea salad) is well popular in Japan as Baonori and used in Europe for soups and salad preparations.[2] A blue colored chromoprotein, which is a phycobiliprotein, has been added to foods by Japanese as a colourant.[1] This is a phycocyanin which is extracted from the microalgae *Spirulina* sp. is commonly added to chewing gum, soft drinks,

Table 4. Potential applications and extraction methods of different seaweed species collected from different places in the world.

Name	Sampling locations	Potential applications	Extraction methods	Reference
Brown algae-*Saccharina latissima, Ascophyllum nodosum, Fucus vesiculosus, Laminaria digitata*	Barents Sea & White Sea	Source of amino acids.	Acid hydrolysis	Bogolitsyn et al.[153]
Red algae-*Gracilaria fisheri*	Commercial seaweed pond in the Southern part of Thailand	A precursor to thermally processed seafood-like flavour that can be used as a flavour supplement and as a savoury flavour source for various seafood products.	Bromelain hydrolysis	Laohakunjit et al.[81]
Brown algae-*Saccharina latissima*	Island of Froya, Norway	Microbial protein produced from Brown Seaweed and Spruce Wood as a Feed Ingredient.	Enzymatic hydrolysis	Sharma et al.[20]
Green algae-*Ulva rigida* and *Ulva fasciata* & **red algae-** *Gracilaria dura*	Israeli Mediterranean Sea intertidal zone	Protein concentrates suitable for human diet (high protein content i.e. 70–86%, has a nutritional amino acid composition, easily digested & antioxidative properties).	Extracting by liquid medium. Eg: Deionized water, lysis buffer, NaOH	Kazir et al.[25]
Green algae- *Ulva armoricana* (Ulvales, Ulvophyceae)	English Channel (Brittany, France)	Production of antiviral and antioxidative extracts.	Enzymatic hydrolysis	Hardouin et al.[154]
Brown algae-*Saccharina japonica*	Japan, China and Korea	As an ingredient in food, cosmetic, and pharmaceutical products.	Extraction using different lysis buffers and detergents	Kim et al.[45]
Green algae- *Ulva lactuca* & **brown algae-** *Padina pavonica*	Buleji coast, Karachi, Pakistan	Could be utilized as food, medicines and fodder *etc.*	TCA/ Acetone method	Fareeha et al.[155]
Brown algae- *Himanthalia elongata (Linnaeus)*	Muros, A Coruña, Galicia, Spain	Known as sea spaghetti. Foaming and emulsifying properties suggest that it could be suitable for its use in the formulation of a wide variety of food products such as sausages, breads, and cakes as well as soups and salad dressing.	Ultrapure water extraction method	Garcia-Vaquero et al.[31]
Red algae-*Palmaria palmata (Dulse)*	Belle Ile (located on the French Brittany coast).	Potentially a good source of proteins (10–26% of the dry mass, fairly moderate in vitro digestibility.	Ultrapure water extraction method	Galland-Irmouli et al.[39]
Red algae-*Kappaphycus alvarezii*	Cultivation farm, Port Okha, Gujarat, Northwest coast of India	Protein concentrate (62.3 ± 1.62% proteins) could be incorporated into several value-added food products.	Treatment with de-ionized water and 2-mercaptoethanol	Kumar et al.[149]
Brown seaweed- *Macrocystis pyrifera* & **red seaweed-** *Chondracanthus chamissoi*	Tongoy Bay of Coquimbo Region, Chile	Nutraceutical or functional ingredients. Both have antioxidant activity and *M. pyrifera* protein extract exhibited a potential antihypertensive activity.	enzyme-assisted extraction	Vásquez et al.[102]

dairy products, and sushi bars.[160] Rhodophyta are also featured with purple and red colours owing to pigments like R-phycocyanin, R-phycoerythrin.[161] Currently, enrichment of bakery foods with proteins against protein malnutrition has prompted, and seaweeds are an excellent source to enrich foods[162] as protein alternatives.[163] Around the world, seaweed proteins extracted using different methods have shown different food potentials (Table 4).

Table 5. Digestibility of different protein sources (%).

Protein source	Digestibility %	Reference
Plant proteins		
Lentils (canned)	84	Sarwar and Peace[176]
Kidney bean (canned)	81	Sarwar and Peace[176]
Pinto bean (canned)	79	Sarwar and Peace[176]
Pinto bean (autoclaved)	80	Sarwar and Peace[176]
Seafarer bean (autoclaved)	84	Sarwar and Peace[176]
Black bean (autoclaved)	72	Sarwar and Peace[176]
Fababean (autoclaved)	86	Sarwar and Peace[176]
Soya protein isolate (SPI)	95	Gilani and Sepehr[177]
Soyabean meal, raw	80	Gilani and Sepehr[177]
Animal proteins		
Casein	99	Gilani and Sepehr[163]
Raw egg	90	Evenepoel et al.[178]
Cooked egg	51	Evenepoel et al.[178]
Boiled beef	27	Yin et al.[179]
Microalgae		
Nostoc sphaeroides F&M-C117	82	Niccolai et al.[180]
Arthrospira platensis F&M-C256	81	Niccolai et al.[180]
Chlorella vulgaris Allma	76	Niccolai et al.[180]
Nannochloropsis oceanica F&M-M24 C	50	Niccolai et al.[180]
Chlorella sorokiniana F&M-M49	55	Niccolai et al.[180]
Macroalage		
Rhodophyta		
Palmaria palmata	58	Marrion et al.[181]
Gracilaria verrucosa	42	Marrion et al.[182]
Chlorophyta		
Ulva lactuca	86	Wong and Cheung[183]
Phaeophyta		
Laminaria japonica	72	MišurCoVá et al.[184]
Undaria pinnatifida	69	MišurCoVá et al.[184]

Digestibility of seaweed proteins

Generally, the digestibility of plant protein is lower than animal protein.[164] Naturally occurring anti nutritional factors tannins, trypsin inhibitors restrict the digestibility of proteins. For example, tannins in legumes and cereals; trypsin inhibitors and haemagglutinins in legumes; phytates in cereals and oilseeds; gossypol in cottonseed protein products; and glucosinolates in mustard and canola protein products that reduce the digestibility of plant proteins.[165] Therefore, the digestibility of algal proteins by human proteases must be evaluated at the preliminary stage by utilizing them as a food ingredient.[25]

Several studies have reported, "in vitro digestibility of algal proteins" that have been extracted under strong alkaline conditions.[15,74] According to the study evaluated by Fujiwara-Arasaki et al.[74] algal protein digestion has been carried out by three enzymes, pepsin, pancreatin, and pronase. The relative digestibility (Expressed as a percentage of casein digestibility base, i.e. 100%) of proteins extracted from *Porphyra tenera* was 70% in the presence of pronase and 56% with pepsin and pancreatin.[74] The relative digestibility of alkali-soluble proteins extracted from *Ulva pertusa* by three enzymes pepsin or pancreatin or pronase is 17%, 66% and 95%, respectively.[74] Similarly, the relative digestibility of proteins extracted from the brown algae *Undaria pinnatifida* by above three enzymes are 24, 48 and 84 respectively.[1,74] Digestibility of red seaweeds by pepsin, pancreatin and pronase have been relatively lower than green and brown seaweeds.[39,74] The results evaluated by Kazir et al.[25] using casein as the digestion standard have depicted that significant amounts of *Ulva* protein and *Gracilaria* protein have been digested by pepsin at the end of the gastric phase(47.8% and 68.1% have been hydrolysed respectively). In the same study, trypsin and chymotrypsin also had hydrolysed 89.4% of the *Ulva* protein and 100% of the *Gracilaria* protein at the end of the gastric phase.[25]Removing anti-nutritional factors using the two methods; fermentation processes and

enzymatic maceration increase the digestibility and improve the nutritional value of algal protein.[166] Protein digestibility is inhibited by several factors. For example, fibres extracted from *Laminaria japonica* and *Undaria pinnatifida* had shown notable restrictive impact (55 and 21%) on pepsin activity.[167] The variations in protein fraction digestibility of green seaweed *Ulva armoricana*, has been associated with glycoprotein content which mainly affected by seasons,[73] while other algae are rich in lectins that could affect protein digestibility.[168] However, these studies reveal that the availability of higher quantities of polysaccharides in seaweeds has become the major reason for low protein digestibility.[169] Compared to animal proteins, moderate digestibility has been recorded in red algae proteins. High fiber content of red algae in general acts as blocking agents to digestive enzymes which adhere on substrates thereby reduce the activity of proteolytic enzymes.[11] Other compounds like polyphenols or trypsin inhibitors also inhibit the digestibility of seaweed proteins.[170]

Discussion

At present, the global food system has moved towards the consumption of plant protein sources mainly due to the inability to access sufficient dietary protein requirements of people. Also, many zoonotic flu pandemics like H1N1 have been circulated around the world due to unhygienic food practices with meat consumption. Seaweed proteins, a novel meat alternative, can be considered as a major plausible protein source among other alternative sources. Novel seaweed extraction methods direct pathways to utilize algal protein in food, pharmaceutical and cosmetic products. Novel protein extraction technologies like EAE, UAE, HHP, MAE, and supercritical fluid extraction have higher efficiencies and yields while performing as green technologies. After extraction of proteins, identification of different proteins is important. Electrophoresis is a simple and relatively inexpensive technique used to characterize proteins in a sample. Co-extraction of carbohydrates, polyphenols with proteins may provide beneficial health properties, which conversely interfere in the identification and purification of proteins.[70]

Protein identification and purification

Sodium dodecyl sulfate (SDS)-PAGE gel electrophoresis, which allows protein separation by mass, is a common method used for protein characterization.[171] Here, the extracted protein fraction that runs on a Polyacrylamide-based discontinuous gel is influenced by structure and charge, and proteins are separated solely on the basis of differences in their molecular weight.[171]Chromatographic separation is another identification mode in which separation of peptides occurs on the basis of differences in their hydrophobicity.[70] High-Performance Liquid Chromatograph (HPLC) is extensively applied to detect amino acid profile and purification of many compounds.[14] Generally, HPLC requires a derivatization step prior to the purification or separation of amino acids.[14] Derivatization reagents that commonly used were 9-fluorenylmethyl chloroformate (FMOC-Cl), ortho-phthalaldehyde (OPA), phenyl isothiocyanate (PITC), 1-fluoro-2,4-dinitrobenzene, 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide, and dansyl chloride.[172] For example, the reversed-phase high-performance liquid-chromatographic method has been used for analysis of the amino acids in edible seaweeds *Himanthalia elongata, Laminaria ochroleuca, Undaria pinnatifida, Palmaria sp*. and *Porphyra sp*.[172] Those amino acids produced had been derivatized with phenyl isothiocyanate. The amino acid content of the algae analysed ranged from 22.4 ± 1.9 to 138.0 ± 5.6 mg g^{-1} dry weight.[172] Spectrometry is another identification method which mainly provides the structural composition of proteins. Fourier Transform Infrared (FTIR) spectroscopy is the commonly used spectrometric method. This helps to detect non protein compounds in the protein extracts.[102] FTIR method has been used to characterize proteins of *Macrocystis pyrifera* and *Chondracanthus chamissoi* extracted from enzyme-assisted extraction.[102] Accordingly, the bands represented at 3302 $cm^{-1}$and 3321 cm^{-1} in both seaweeds had been corresponding to O-H in polysaccharides/polyphenol or N–H present in proteins.[102] For the identification of proteins in *C. chamissoi*, the bands that were detected at 1637 cm^{-1} and 1524 cm^{-1}

had been identified as C = O, N–H, or C–N that probably had been corresponding to amides.[102] Also, the bands at 3281 cm^{-1} and 3274 cm^{-1} found in the protein extract in both seaweeds probably had been N–H stress vibrations corresponding to amide of a protein polypeptide.[102]

Future trends

Extraction of the soluble protein fraction of seaweed is essential since these were digested and absorbed by the human intestine. However, the protein content is mainly affected by geographical location, seasonal variation, and variety of microalgae. Hence, these factors may appear more essential in food applications. The study of Banach et al.[152] stated that in 2016, over 30 million tons (fresh weight) of farmed seaweed was reported to be produced globally, countries including China (47.9%), Indonesia (38.7%), the Philippines (4.7%), the Republic of Korea (4.5%), Japan (1.3%), and Malaysia (0.7%). Also, the European Union (EU) exported 101,594 tons of seaweed, of which 4,607 tons were for human consumption, while imports totalled 178,467 tons, of which 15,184 tons were for human consumption.[152]According to statistical analysis, in 2018 global soybean production accounted for over 360 million tonnes.[173] Although soya bean production is extremely high compared to seaweed, it requires millions of hectares of terrestrial land for cultivation and large quantities of herbicides. Unlike terrestrial plant sources, seaweed grows naturally in large amounts without the addition of artificial fertilizers. Development of macro-algae cultivation in the sea is a sensible option to increase total production in the country since it diminishes the requirements for both free inlands and freshwater for irrigation.[25,174] When utilizing seaweed proteins as a protein alternative, it is worth revealing the toxicity levels of algal proteins before consumption. Only a few studies have reported the cytotoxicity of seaweed. According to a study by Wijesekara,[2] glycoproteins extracted from *Ulva* sp. harvested from France had shown no cytotoxicity in Vero cells at a concentration of 500 mg dw ml^{-1}. However, further analysis to detect the toxicity of seaweed proteins has to be encouraged. Based upon available findings, the total number of deaths and illnesses reported due to the consumption of seaweed is very small.[175] The most serious reports of illness and death have come from direct consumption. For example, in Pacific Rim countries, just three genera (*Caulerpa, Gracilaria, and Acanthophora*) were found as toxic and harmful seaweeds.[175] Many factors including seaweed type, physiology, season, harvest and cultivation environment, geography, processing etc. can affect the presence of hazards.[152,175] Also, it has been reported that cultivation of seaweeds near industrialized or anthropogenic activities may negatively influence and can increase the likelihood of hazards in seaweed.[175] Therefore, collecting data on hazards will be helpful in future studies. Since many studies are now being evaluated on seaweed protein extraction, their food applications have to be consequently developed. However, seaweed proteins are now emerging as a future trend in pharmaceutical, cosmeceutical and food industries around the world.

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Abbreviations

Arabinogalactan proteins (AGPs), Hydroxyproline rich glycoproteins (HRGP), Oscillatoria agardhii agglutinin homolog (OAAH), Galanthus nivalis agglutinin (GNA), Adenosine diphosphate (ADP), Ultra Violet (UV), Mycosporine-like amino acids (MAA), Glycoprotein (GP), Ammonium sulfate ($(NH_4)_2SO_4$), Sodium hydroxide (NaOH), TCA (Trichloroacetic acid), DEAE cellulose (Diethylaminoethyl cellulose), Adenosine triphosphate (ATP), SfL (*Solieria Filiformis), Capsosiphon Fulvescens* (Cf-hGP), *Undaria Pinnatifida* (UPGP), mM (Millimoles), Pressurized Liquid Extraction (PLE), Accelerated Solvent Extraction (ASE), Intestinal Epithelial Cell (IEC), Human immunodeficiency virus (HIV),United Kingdom (UK), Sodium dodecyl sulphate- Polyacrylamide gel electrophoresis (SDS)-PAGE, Potential of Hydrogen (pH), (High Performance Liquid Chromatograph (HPLC), Fourier Transform Infrared (FTIR) spectroscopy, Ultra performance liquid chromatography (UPLC), Reversed phase high performance liquid chromatography(RP-HPLC), European Union (EU).

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