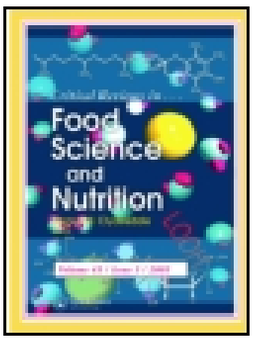

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Harshani Nadeeshani, Amira Hassouna & Jun Lu (2021): Proteins extracted from seaweed *Undaria pinnatifida* and their potential uses as foods and nutraceuticals, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2021.1898334

To link to this article: https://doi.org/10.1080/10408398.2021.1898334

Published online: 11 Mar 2021.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2021.1898334

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REVIEW

Proteins extracted from seaweed *Undaria pinnatifida* and their potential uses as foods and nutraceuticals

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ABSTRACT

Isolation and utilization of proteins from seaweeds have been a novel trend in the world at present due to the increasing demand for healthy non-animal proteins. The attention of scientific community has been paid on the protein derived from seaweed *Undaria pinnatifida* due to their high nutritional quality and bioactivity. This article aims to provide an integrated overview on methods of extraction, isolation and purification of *U. pinnatifida*-derived proteins and composition, nutritional value and potential nutraceutical and food applications with an interest to stimulate further research to optimize the utilization. Potential food applications of *U. pinnatifida* derived proteins are nutritional components in human diet, food ingredients and additives, alternative meat and meat analogues and animal and fish feed. Excellent antioxidant, antihypertension, anticoagulant, anti-diabetes, antimicrobial and anti-cancer activities possessed by proteins of *U. pinnatifida* enable the use of these proteins in various nutraceutical applications. A number of studies have been carried out on antioxidant and antihypertensive activities of *U. pinnatifida* proteins, whereas other bioactivites are yet to be further studied. Hence, more research works are crucial to be done in order to facilitate and promote the emerging novel foods and nutraceuticals, using proteins from seaweed *U. pinnatifida*.

KEYWORDS

Proteins; nutraceutical; *Undaria pinnatifida*; meat analogue; bioactivity

Introduction

There is a growing worldwide interest on natural compounds derived from seaweed, since they can be utilized in various food and nutraceutical applications, in order to promote human and animal health and wellbeing. Recently, the scientific community is paying more attention to all groups of macroalgae as they produce a variety of bioactive substances (Cotas, Leandro, Pacheco, Gonçalves, and Pereira 2020). Seaweeds are basically macroscopic marine algae which include various types of red (Rhodophyta), brown (Phaeophyta) and green (Chlorophyta) macroalgae.

Undaria pinnatifida (*U. pinnatifida*) named as Japanese kelp or wakame in Japanese is a brown macroalgae which belongs to order *Laminariales* and family *Alariaceae*. It was first found in China, Japan and Korea and has spread to more than twelve countries comprising Spain, Australia, France, Italy, North and South America, Argentina and New Zealand (Mak et al. 2014). Though *U. pinnatifida* favors cold water (5–20 °C), it shows greater tolerance to sunlight and temperature. It is capable of withstanding high wave exposure, salinity and other harsh environmental conditions (Hewitt et al. 2005). Generally, they are 60–120 cm long seaweeds which reach to 2–3 m length at maturity. Thallus of *U. pinnatifida* is fixed with fibrous holdfast which acts as the root of plant while the midrib of pant is attached to rolled wing-like blades at the end. Sporophyll is only present in mature plants and stipe extents sway from holdfast becoming the midrib (Figure 1).

According to current food applications, *U. pinnatifida* is added into soups, salads and side dishes in dried or salted form. Blades of *U. pinnatifida* preserve green color and delicate sweet flavor even after cooking. Products of *U. pinnatifida* can be categorized into three major types: cooked and salted blades, frozen sporophylls and midribs. These three varieties of products are processed further into numbers of different food products including instant, seasoned and dehydrated or dried products. The quantity of *U. pinnatifida* production in 2014 from China, the major producer in the world, was 203,099 tonnes dry weight from 7693 production sites. It corresponded to 2,030,990 tonnes of wet weight and the total global production was 2,359,000 tonnes of wet weight in the same year (FAO 2016). The total global productions of *U. pinnatifida* in past 50 years are illustrated in Figure 2. China, Japan and Republic of Korea mainly

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contribute to the global production of *U. pinnatifida* and the extensive commercial cultivations have been developed in these countries due to growing demand for *U. pinnatifida* as food and feed ingredient, especially after 2002.

Research activities have been progressively carried out to find more bioactive natural compounds from marine organisms in the last decade (Leandro, Pereira, and Gonçalves 2019). Seaweeds comprise exceptional quantities of functional nutrients and secondary metabolites that are evident by being the primary producer in the sea, and major nutrient and energy source for other marine organisms (Samarakoon and Jeon 2012). Among brown seaweeds, *U. pinnatifida* is a rich source of protein, fiber, calcium, sodium, potassium, iron, magnesium, vitamin B, vitamin A and antioxidants (Kolb et al. 2004; Prabhasankar et al. 2009). Taboada, Millán, and Miguez (2013) reported that *U. pinnatifida* contained 37% carbohydrate, 16.8% protein, 1% fat and 16.9% fiber.

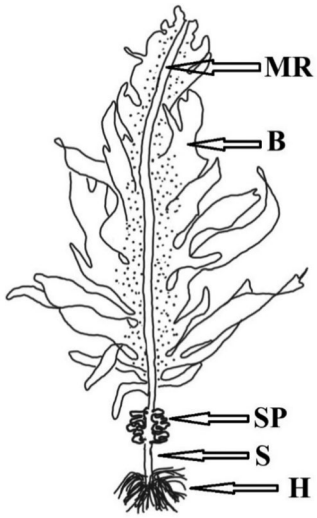

Figure 1. Parts of *U. pinnatifida* (MR - midrib, B - blade, SP - sporophyll, S - stipe, H - holdfast).

Seaweeds have been given a substantial role in human and animal diet due to inherent functional properties in polysaccharides, polyunsaturated fatty acids and mineral content. Furthermore, much attention has been given to the protein content of seaweeds, since protein contents of some red seaweed are higher than that of some pulses namely, soybean (Fleurence 1999a). In addition to that, seaweeds such as *Undaria*, *Ulva* and *Entormorpha* comprise protein contents which are equivalent to those found in ordinary vegetables, while *U. pinnatifida* contains the highest protein content among brown seaweeds (Amano and Noda 1990). The mostly consumed brown seaweed in Asian countries, such as China, Japan, Korea and the Philippines, is *U. pinnatifida*. Furthermore, sporophyll, blade and midrib of *U. pinnatifida* have been used as important dietary components and traditional Chinese medicine from the ancient time (Ye et al. 2005). The consumption of seaweeds is gaining an increasing interest not only as sea vegetables, but also as processed novel foods and dietetic products. Protein extracts and purified protein fractions are also being utilized as ingredients in food and nutraceuticals.

Extracts of *U. pinnatifida* possess exceptional bioactivities such as antioxidant, anticancer, anti-coagulant, anti-inflammatory, anti-diabetes and anti-microbial properties which are mainly exerted by polysaccharides, carotenoids, tocopherols, phycobilins, phycocyanins, vitamins, fatty acids and sterols (Wang et al. 2018), as well as amino acids, peptides and proteins (Zhang, Pang, and Han 2014). Thus, a great deal of attention has been paid on the utilization of biofunctional peptides and proteins in the development of functional foods and nutraceuticals. This review summarizes the methods of extraction, isolation and purification of bioactive

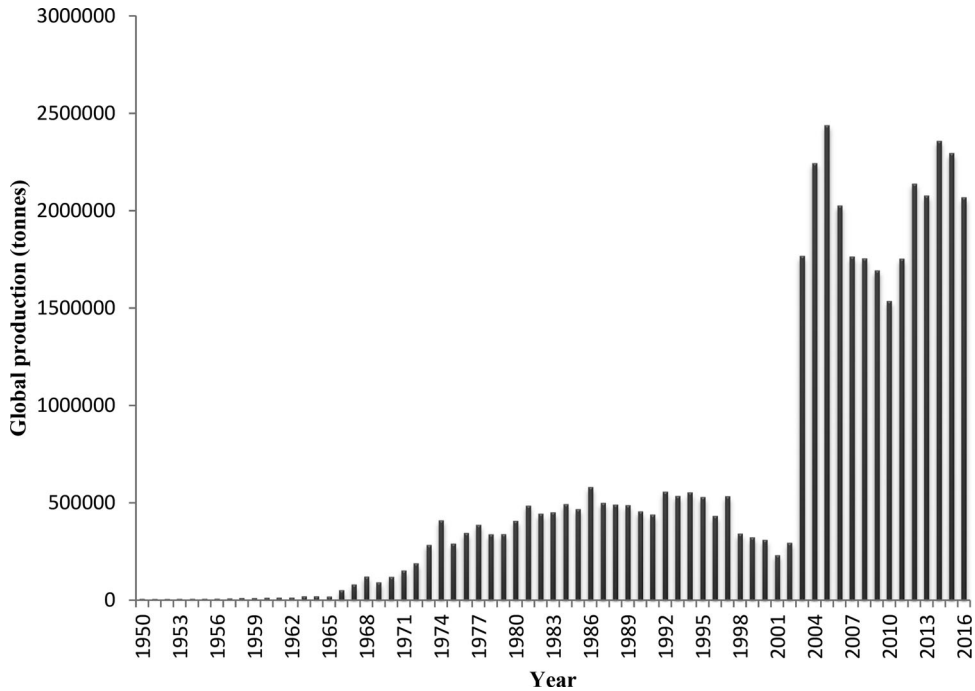

Figure 2. Global production (in tonnes, live weight) of *U. pinnatifida* (FAO, 2016).

and functional proteins from *U. pinnatifida*, and the proteins' nutritional value, composition, structure, nutraceutical properties and potential food and nutraceutical applications.

Extraction of seaweed proteins

Extraction of protein from seaweeds has been paid great attention with the increased global demand for vegetable proteins (Harrysson et al. 2018). The protein extraction from most sea weeds is not an easy task, since the digestibility and the extractability of proteins are hindered by the profusion of polyphenols and strong cell walls rich in polysaccharides like carrageenans in Rhodophyta and alginates in Phaeophyta (Fleurence 1999b). There are conventional as well as more modern methods to extract proteins from seaweeds.

Conventional protein extraction methods

Conventional physical, chemical and enzymatic methods are still utilized to extrcat proteins from seaweeds. Polysaccharides like xylans, alginates, carrageenans, galactans, cellulose, fucoidan and laminarin are degraded enzymatically in order to extract proteins of seaweeds using enzymes such as cellulase, xylanase, *k*-carrageenase, *β*-agarase and *β*-glucanase (Harnedy and FitzGerald 2011). Mechanical grinding, aqueous treatment, homogenization, osmotic stress, high shear force and precipitation are some of the physical methods which are utilized to extract proteins from seaweeds. Two-phase acid, alkali and aqueous treatments are used to extract proteins as chemical methods (Barbarino and Lourenço 2005). Disadvantages of these conventional methods are being time consuming and laborious as well as reducing the quality of extracted proteins caused by releasing protease enzyme from vacuoles in the cytoplasm (Ganeva, Galutzov, and Teissié 2003).

Cazón et al. (2014) extracted proteins from *U. pinnatifida* using physical methods such as mechanical grinding and centrifugation followed by two-phase chemical extraction using NaOH and trichloroacetic acid. Park, Kim, and Jeong (2008) extracted protein from *U. pinnatifida* using mechanical grinding and high-pressure extraction. In addition, they described that high-pressure extraction yielded a protein content higher than that from normal extraction. Apart from that, they also performed enzymatic extraction of protein from *U. pinnatifida*, degrading polysaccharides by cellulase and pectinase. Types of amino acids in proteins of *U. pinnatifida* sporophyll were identified and quantified by Qi et al. (2017) after extracting proteins from sporophyll using enzymatic hydrolysis.

Improved modern protein extraction methods

The improved protein extraction methods are fundamentally based on rupturing tough cell walls of seaweeds by cell-disruption techniques which raise the accessibility to proteins, followed by protein extraction. These improved methods are pulsed electric field, ultrasound-assisted, microwave-assisted and sub- and super-critical fluid extractions. By pulsed electric field, cell membranes or cell walls of seaweeds are disrupted due to applied high electric currents, while the electroporation allows penetration of the target components of cells. The extractability of seaweed protein is increased by ultrasound-assisted extraction with the aid of formation, expansion and collapse of microbubbles which is produced by ultrasonic field rather than ultrasound waves (Ashokkumar et al. 2008). Microwave-assisted extraction increases the amount of protein extracted from seaweeds by heating, moisture evaporating and disrupting contents in the cells of seaweeds (Barba, Grimi, and Vorobiev 2015). Proteins of seaweeds can also be extracted using water with high temperature and pressure allowing water to maintain liquid and supercritical conditions, in sub- and super-critical fluid extraction techniques, respectively (Herrero, Cifuentes, and Ibañez 2006).

Though bioactive compounds such as fucoidan and fucoxanthin have been extracted from *U. pinnatifida* using these improved extraction techniques (Cheng, Huang, and Xiao 2014), the studies in which *U. pinnatifida* proteins are extracted using these novel extraction techniques are scarce. Nevertheless, a number of studies have proven that modern extraction methods not only improve protein yield, but also reduce processing time than conventional methods (Qu et al. 2013). Passos, Carretero, and Ferrer (2015) have illustrated that microwave-assisted protein extraction is more efficient giving higher content of extracted protein from marine algae than ultrasound-assisted extraction. Sub- and super-critical fluid extraction techniques have been used for the extraction of bioactive polysaccharides, polyunsaturated fatty acids, polyphenols and among others from seaweeds, they are rarely used for protein extraction (Gallego, Bueno, and Herrero 2019).

Isolation of bioactive peptides

Bioactive peptides are specific protein fragments with unique amino acid composition and sequence. They impact orpositively influence functions of the body to provide specific health benefits. There is an increasing demand for isolation of bioactive peptides from seaweeds. Extracted proteins from seaweeds are hydrolyzed to liberate bioactive peptide segments. Enzymatic hydrolysis has been used more in the health sector and in industries than chemical hydrolysis methods, because it gives higher purity and yield, as well as preserving the bioactive properties and nutritional value of peptides (Admassu et al. 2018b). In order to isolate peptides, proteins can be hydrolyzed using either proteolytic enzymes produced by microorganisms or plants, or digestive enzymes obtained from animals (Korhonen and Pihlanto 2006). Enzymes such as trypsin, chymotrypsin, pepsin, papain, fungal proteases, alcalase and flavourzyme are commonly used for the hydrolysis of protein. Suetsuna and Nakano (2000) and Sato, Hosokawa, et al. (2002) have isolated bioactive peptides from *U. pinnatifida* using pepsin and protease enzymes.

Optimum physical and chemical conditions, such as temperature and pH, are also important to the enzymatic hydrolysis of proteins. To facilitate complete isolation and purification, hydrolyzed protein fragments are fractionated further using ultra-filtration technique, while the boundaries of molecular masses are established with the aid of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Optimum isolation and purification can be achieved by chronological chromatographic procedures, like gel filtration, ion exchange and high-performance liquid chromatography (HPLC) (Harnedy and FitzGerald 2011). Furthermore, molecular structures and masses of bioactive peptides are characterized by spectrophotometric techniques such as mass-mass spectrophotometry (MS-MS) and liquid chromatography-mass spectrophotometry (LC-MS) (Samarakoon and Jeon 2012). Sato, Hosokawa, et al. (2002) isolated bioactive peptides from *U. pinnatifida* using three-step HPLC while these peptides classified by LC-MS, amino acid sequence and composition analysis. Suetsuna and Nakano (2000) isolated bioactive peptides from *U. pinnatifida* using ion-exchange chromatography, gel-filtration and column chromatography, followed by identification using sequence analysis and mass spectrophotometry.

Nutritional quality of protein extracted from *U. pinnatifida*

Seaweeds are regarded as a rich source of protein and red, green and brown seaweeds contain 20–47, 9–26 and 3–15% of protein, respectively in dry weight (Pliego-Cortés et al. 2019). Though most of the brown seaweeds comprise 15% (dw) of maximum protein content, *U. pinnatifida* has been reported to contain the highest protein content (11–24%, dw) among other brown seaweed species (Burtin, 2003). Different forms of proteins are contained by seaweeds, including peptides, glycoproteins, enzymes, cell wall-attached proteins, lectins, phycobiliproteins and amino acids. Nevertheless, nitrogen which is derived from non-protein sources in seaweeds such as nitrates, nucleic acids and pigments causes overestimation of the total protein content. Thus, specific seaweed nitrogen-to-protein conversion factors have been ascertained, to be exact 5.38, 5.13 and 4.59 for brown, green and red seaweeds, respectively, that are deviated from the standard nitrogen-to-protein conversion factor of 6.25 (Barbarino and Lourenço 2005).

The nutritional value of *U. pinnatifida* is influenced by not only the quantity but also the quality of proteins. The first two criteria to determine nutritional quality or value of dietary protein are amino acid composition and bioavailability. Moreover, the nutritional quality of structurally different proteins depends on amino acid composition, ratios of essential amino acid, purity, propensity to hydrolysis during digestion and processing effects (Han, Chee, and Cho 2015).

Amino acid composition

The total nitrogen content as well as amino acid composition and contents of seaweeds are affected by the season of harvesting (Fleurence 1999a). Moreover, Zhou et al. (2015) studied and reported that the total nitrogen content and amino acid profile of *U. pinnatifida* depends on geographical location, time of harvest and plant parts. Seaweed protein comprise almost all amino acids and the amino acid profile of seaweeds is found to be analogous to that of leguminous plants and ovalbumin. Moreover, Seaweeds contain around 40 mg/100 mg (dw) of total amino acid content, whereas 50% of total amino acid correspond to essential amino acids (Paiva et al. 2014). Vieira et al. (2018) found that *U. pinnatifida* consists of 44.2 mg/100 (dw) mg of total amino acid with the ratio of essential amino acids to non-essential amino acid (EAA/NEAA) is 0.87:1. Generally, glutamic and aspartic acid make up a huge fraction of amino acid content in seaweeds. For an example, glutamic and aspartic acid constitute 20–44% of total amino acids in brown seaweeds (Fleurence 1999a). The amino acid compositions of *U. pinnatifida* determined in several studies and a comparison of amino acid composition with red and green seaweed species are given in Table 1.

Similar to other seaweed species, the highest occurring amino acids in *U. pinnatifida* are glutamic and aspartic acids which play a foremost role in taste generation in seaweeds. Additionally, Glutamic acid is the major contributor for the "umami" flavor of seaweeds. Though cysteine, methionine, isoleucine, threonine, tryptophan, histidine and lysine are recognized as generally limiting amino acids in most seaweeds, these contents are higher than those existing in terrestrial plants (Dawczynski, Schubert, and Jahreis 2007). Amino acid score (AAS) is used for the intention of identifying a complete protein. The actual availability of individual essential amino acid is assessed by the AAS as it is compared and related to a reference protein or dietary requirements (FAO 1991). According to Cofrades et al. (2010), all essential amino acids of *U. pinnatifida* fulfilled the FAO/WHO requirements. Zhou et al. (2015) reported similar findings and added that the amount of valine, histidine and isoleucine in *U. pinnatifida* is higher than the FAO/WHO requirements (Table 2).

Protein digestibility

Bioavailability of a protein is dependent on the digestibility, as well asthe solubility in the digestive tract, the absorption to the circulatory system and the assimilation into the intended spot of exploitation. Protein digestibility and accessibility of seaweeds have been performed mostly in vitro using extracted proteins. It is a promising and preliminary screening tool to assess the nutritional quality of a food protein. Four categories of methods are used to evaluate the in vitro digestibility of a protein, such as solubility, dialyzability, gastrointestinal chambers and cell models. Small intestine can absorb merely undersized and soluble molecules. These small molecules can be estimated by high performance liquid chromatography (HPLC), atomic absorption spectrophotometry (AAS) or mass spectrophotometry (MS). The ability of a food component to penetrate a membrane is assessed by dialyzability assays (Hur et al. 2011).

Table 1. Comparison of amino acid composition of *U. pinnatifida* with red and green seaweed species.

	U. pinnatifida					
Amino acids	Japan (mg/g, dw)[a]	Spain (mg/g, dw)[b]	China, Japan and Korea (g/16 g N)[c]	New Zealand (g/100 g, dw)[d]	*Porphyra columbina* (Argentina, g/100 g, dw)[e]	*Ulva lactuca* (USA, mg/100 g, dw)[f]
Aspartic acid	10.18 ± 1.27	6.0 ± 0.7	8.7 ± 1.1	0.70 ± 0.11	12.22 ± 0.20	1487.0 ± 8.5
Glutamic acid	10.65 ± 0.57	11.5 ± 1.5	14.5 ± 3.2	0.82 ± 0.15	10.50 ± 0.56	1508.4 ± 9.5
Serine	5.76 ± 0.08	2.9 ± 0.4	4.0 ± 0.4	0.33 ± 0.04	6.16 ± 0.09	833.2 ± 5.9
Threonine*	7.33 ± 0.57	0.8 ± 0.2	4.4 ± 0.6	0.24 ± 0.04	5.91 ± 0.13	797.8 ± 7.5
Glycine	8.76 ± 0.87	3.9 ± 0.5	5.1 ± 0.7	0.41 ± 0.06	8.87 ± 0.14	815.6 ± 5.7
Alanine	27.20 ± 0.70	7.8 ± 1.1	4.7 ± 0.6	0.43 ± 0.06	12.54 ± 0.29	1096.4 ± 10.5
Arginine	8.41 ± 1.17	13.9 ± 3.0	5.2 ± 0.2	0.27 ± 0.04	6.19 ± 0.16	486.6 ± 3.5
Proline	5.52 ± 0.40	4.8 ± 0.5	3.6 ± 1.6	ND	3.96 ± 0.41	0.7 ± 0.1
Valine*	16.84 ± 0.32	3.5 ± 0.5	5.2 ± 0.5	0.53 ± 0.06	5.85 ± 0.11	339.2 ± 4.5
Methionine*	3.58 ± 0.42	1.9 ± 0.3	1.7 ± 0.5	0.25 ± 0.02	1.68 ± 0.07	671.7 ± 8.5
Isoleucine*	7.91 ± 0.18	3.0 ± 0.4	4.1 ± 0.3	0.34 ± 0.04	2.71 ± 0.05	550.0 ± 7.1
Leucine*	13.70 ± 1.36	4.8 ± 0.7	7.4 ± 0.6	0.56 ± 0.07	7.38 ± 0.11	1034.5 ± 8.9
Tryptophan*	0.43 ± 0.02	2.0 ± 0.3	2.9 ± 0.5	ND	0.63 ± 0.01	ND
Phenylalanine*	7.80 ± 0.17	16.6 ± 2.0	4.7 ± 0.3	0.33 ± 0.09	3.70 ± 0.06	1245.4 ± 12.5
Cystine	2.41 ± 0.17	ND	0.9 ± 0.2	ND	1.89 ± 0.03	55.0 ± 6.5
Lysine*	11.12 ± 1.07	5.7 ± 1.0	5.6 ± 0.4	0.53 ± 0.06	6.01 ± 0.10	723.3 ± 8.5
Histidine*	5.25 ± 0.37	5.3 ± 0.5	2.5 ± 0.3	0.49 ± 0.06	1.26 ± 0.08	133.9 ± 1.5
Tyrosine	4.31 ± 0.16	2.0 ± 0.3	2.9 ± 0.5	0.30 ± 0.04	2.55 ± 0.05	435.2 ± 1.5
Total	157.16 ± 9.87	94.4 ± 4.4	85.7 ± 7.9	6.53	100.01	12213.5
EAA/NEAA	0.89:1	0.83:1	0.7:1	1:1	0.54:1	0.82:1

*Essential amino acids (EAA).
ND - Not detected.
a- Kolb et al. (2004),
b- Sánchez-Machado et al. (2003),
c- Dawczynski, Schubert, and Jahreis (2007),
d- Zhou et al. (2015),
e- Cian et al. (2014) and
f- Ortiz et al. (2006)

Table 2. AAS of essential amino acid of *U. pinnatifida* and FAO/WHO requirements.

Essential amino acids	Spain[a]	New Zealand[b]	WHO/FAO requirement
Threonine	53.6	42.7	34
Histidine	21.6	68.2	19
Valine	31.1	75.2	35
Methionine	61	46.1	63
Isoleucine	7.3	30.9	25
Leucine	47.3	50.8	28
Phenylalanine	89	78.5	66
Lysine	58	69.6	58

a- Cofrades et al. (2010),
b- Zhou et al. (2015)

Several in vitro gastrointestinal models have been developed to investigate digestion, microbial colonization and nutrient absorption in the colon. The ability of a food component to be absorbed in the intestine has been reproduced and assessed using a range of in vitro cell culture models (Hur et al. 2011). In vitro studies of bioaccessibility are performed by either dynamic or static evaluating systems, whereas the dynamic system model is more accurate. However, it is costly with less efficiency and throughput. On the contrary, static system model is inexpensive, simple to perform, and highly efficient with high throughput. In the static systems, liberation of free amino acids from a food protein is measured after hydrolysis by gastrointestinal enzymes with distinct temperature and pH conditions. As a substitute, the dynamic systems are used by regulating temperature, pH, addition of enzymes, blending and holding time within compartments with the aim of setting up more accurate replica of gastrointestinal digestion (Alegría, Garcia-Llatas, and Cilla 2015).

Usually, enzymes such as trypsin, chymotrypsin, pancreatin, pepsin and pronase are used in a sequence of few or as a mixture of few for the protein digestion. Subsequently, the relative digestibility of seaweed proteins is compared with casein protein digestibility (100%). Machů et al. (2014) studied the digestibility of *U. pinnatifida* proteins after digesting for 6 hours while 81.7, 88.5 and 92.3% of digestibility were observed with pepsin, pancreatin and combined pepsin and pancreatin enzymes, respectively. According to MišurCoVá et al. (2010), *U. pinnatifida* proteins showed 69.1 and 87.5% digestibility in pepsin and pancreatin enzymes, though having the lowest protein digestibility compared to red and green seaweeds. Horie, Sugase, and Horie (1995) studied the influence of dietary fibers of *U. pinnatifida* on in vitro pepsin digestibility, and observed that pepsin activity and accessibility of dietary proteins are significantly reduced by soluble rather than insoluble dietary fibers. Additionally, Galland-Irmouli et al. (1999) declared the influence of abundance of polysaccharides in seaweeds on lowering the protein digestibility.

Potential food and feed applications of protein extracted from *U. pinnatifida*

The continuously growing world population together with the increased food demand, urges the necessity of exploring new nutrients and food sources. Moreover, declining world agricultural lands and diminishing freshwater reserves make mariculture a better substitute of farm lands produce and other sources of nutrients. There is an increasing movement within the food industry to find consumer friendly and healthy plant protein sources to replace those from animal sources. Among these, plant proteins and seaweed proteins are receiving a growing attention due to their nutritional

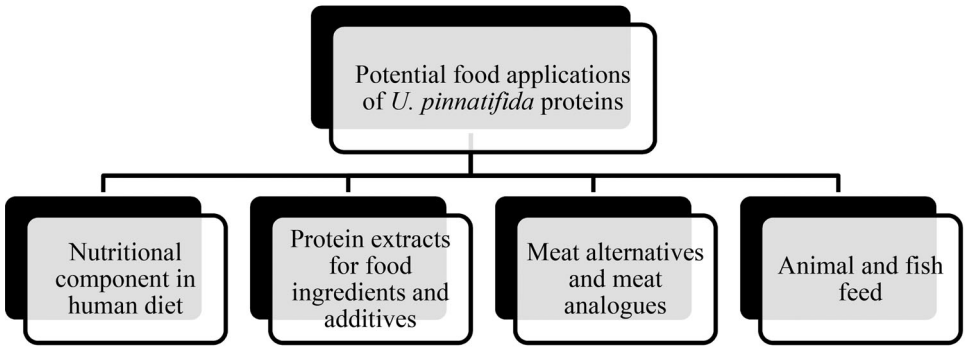

Figure 3. Potential food applications of protein extracted from *U. pinnatifida*.

and functional properties. Examples of potential food applications of the brown seaweed species *U. pinnatifida*-derived proteins are shown in Figure 3.

Some seaweeds consist of nearly 50% of protein on a dry weight basis, equivalent to conventional sources of proteins such as egg, meat and soybean in addition to high abundance of essential amino acids. Functional properties of seaweed proteins are hydration capacity, protein solubility, foaming ability, emulsification ability, water and fat binding capacity and gelation (Chronakis and Madsen 2011). Although seaweeds, including *U. pinnatifida* have been consumed by people of Asian countries for centuries, and became very common ingredients in diet of some Western countries, knowledge of functional properties and digestibility of seaweed proteins are still limited (Kazir et al. 2019).

Although, seaweed proteins were often extracted as a by-product of polysaccharide extraction, specific and robust methods have been developed to extract proteins from seaweeds, separately as valuable functional ingredients due to increasing demand for animal protein alternatives. The seaweed cell wall acts as an obstacle to extract proteins. The cell wall is comprised of crystalline cellulose microfibrils which exist parallel to the cell surface. Generally, brown algae contain mannitol, laminarin and cellulose in the cell wall, whereas other seaweeds contain various other sulfonated hydrocolloids. Hence, various fractionation protocols have been developed for separation of seaweed proteins from polysaccharides such as high-pressure processing, ultrasound-assissted extraction, sonication and autoclave pretreatments, among others (O'Connor et al. 2020).

Nutritional component in human diet

Amino acids which resulted from breaking down proteins, are utilized for cellular repair and immune response and as hormones, co-enzymes and other molecules vital for life (Rostom and Shine 2018). From the ancient time, seaweeds have been an important source of nutrients in human diet. They are designated as sea vegetables, owing to their impressive content and composition of proteins, which is equivalent to or sometimes more than that of some other plant protein sources. Nowadays, attention of vegans and athletes have been gained by this excellent protein source, for fulfilling their daily protein requirement without using animal proteins. As described by Pati et al. (2016), *U. pinnatifida* is very popular among vegetarians in countries like China, Korea, Japan, Indonesia, Thailand and the Philippines due to its high protein content. Additionally, Minato et al. (2006) studied the nutritional status of Japanese athletes provided with Miso soup which included *U. pinnatifida* and tofu as a good protein source. Berning (2000) highlighted the importance of consuming a protein dense plant food sources to cater increasing protein requirements of strict vegetarian athletes without taking excessive protein supplements. Thus, *U. pinnatifida* has a good potential to fulfill the high demand for protein-energy requirements of strict vegetarian athletes, serving as a high-quality source of protein.

U. pinnatifida is available, not only as fresh seaweeds, but also as cooked and salted blades, frozen sporophylls and midribs. Seasoned and dried *U. pinnatifida* products can be found commercially. They are incorporated into the diet as soups, salads and side dishes in dried or salted form. Beak (2007) stated that Korean *U. pinnatifida* market comprises 55% of dried, 25% of instant noodle base, 13% instant soup base and 7% fresh or salted seaweed and sliced stalk products. Taboada, Millán, and Miguez (2013) studied the nutritional value of *U. pinnatifida* and the potential of use as a food supplement, by including this seaweed into the diet of rodents. They found that *U. pinnatifida* was an excellent source of nutrients and was well-accepted by experimental animals. Dried plant parts of *U. pinnatifida* has been added into numbers of functional foods as an ingredient, such as pasta, bread, noodles, biscuits, confectionaries, beverages and beer.

Kim et al. (2006) developed a jam product from sporophyll of *U. pinnatifida,* masking sea mustard flavor with strawberry and cinnamon flavors. Prabhasankar et al. (2009) developed a pasta product incorporating 10% of wakame which is sensorially acceptable with improved nutritional value, especially with protein content, in addition to improving the interaction between protein matrix and starch granules by 20%. Furthermore, Cofrades et al. (2008) added dried and powdered *U. pinnatifida* to gel/emulsion meat systems prepared with pork meat and back fat. Interestingly, it has been found that the addition of *U. pinnatifida* improved fat and water binding properties and enhanced chewiness and hardness. It reduced cohesiveness and springiness in the gel/emulsion meat system. Kim et al. (2015) were able to produce a reduced-fat/low-salt meat emulsion from pork

ham and back fat by reducing fat and salt with the incorporation of dried powder of *U. pinnatifida*.

Protein extracts for food ingredients and additives

Currently, there is a high demand for inexpensive, sustainable and natural protein substitutes to meat and dairy sources, which comprise comparable nutritional quality and functional properties. Although, seaweeds have been added to diet from ancient time, purified protein of seaweeds are still underutilized in the food industry. Nevertheless, there is a huge potential to utilize seaweed proteinswith functional properties, such ashydration capacity, protein solubility, foaming ability, emulsification ability, gelation, and water and fat binding capacity, in producing a wide range of food products such as cake, bread, sausages, salad dressing, and soup. Roohinejad et al. (2017) have reviewed the application of seaweed extracts on formulating new food products. They described that these seaweeds products have better functional properties and are a rich source of essential amino acids. However, studies on functional properties of seaweed proteins are limited, unlike the abundant studies carried out on seaweed polysaccharides.

The industrial extraction protocols of seaweed proteins are not well-established unlike seaweed polysaccharides. Proteins are mainly removed from polysaccharide extractions as by-products. Ge and Yang (2010) investigated about the best method to remove proteins from polysaccharide extracts of *U. pinnatifida*. They found that enzymatic method was more efficient than trichloroacetic acid and Sevag methods. Additionally, Park, Kim, and Jeong (2008) studied different methods to obtain protein extracts from *U. pinnatifida* in order to raise the yield. It was found that pressure extraction for 20 minutes followed by enzymatic hydrolysis with 0.1% of pectinase and 2 h additional extraction was the best method to yield the highest quantity of protein.

One of the industrial applications of seaweed proteins is a food colorant which has been developed in Japan from a blue chromoprotein, namely phycocyanin, which belongs to phycobiliprotein group. This green color pigment is mainly extracted from *Spirulina* species and used in commercial food products such as dairy products, soft drinks, chewing gums and green colored pasta. Red and purple color pigments namely R-phycoerythrin and R-phycocyanin have been extracted from Rhodophyta. On the other hand, Freitas et al. (2012) illustrated the potential of using protein extracted from marine sources as thickening agents, stabilizing agents, gelling agents and protein replacements in the food industry. Furthermore, Fitzgerald et al. (2014) formulated a bread product incorporating red seaweed *Palmaria palmata* protein hydrolysate. They indicated that the color, texture profile, volume, crumb structure, moisture and sensory quality attributes of that product were not affected by the addition of seaweed proteins.

Lipids and proteins in food are subjected to oxidation-during processing steps and storage, reducing consumer acceptability and producing reactive oxygen species (ROS). ROS as hydroxyl radical, singlet oxygen, hydrogen peroxide, superoxide anionradical and peroxiradical are injurious to human health. Therefore, in order to prevent oxidative deterioration of food components, antioxidant food additives are added to food products. Although synthetic antioxidants have better antioxidant activity, natural antioxidants have been given much more priority in food products as minimally processed food and fresh food items, due to the new trend toward eating healthy and avoiding toxicity and health risks associated with synthetic antioxidants (Admassu et al. 2018b). Hence, antioxidant potential of bioactive peptides extracted from seaweeds, and potential of food applications as antioxidant food additives are being progressively investigated by many researchers in the world (Kazir et al. 2019).

As indicated by recently published studies, utilization of antioxidant peptides as potential food additives has already been studied, particularly at laboratory scale. However, further research is essential to establish the mechanism to obtain and release the specific active peptide sequences from the protein sources, because the optimum reaction between proteolytic agent and protein is a very crucial for the final composition. Predominately, animal-derived bioactive proteins, especiallly from dairy products and wastes of meat and seafood processing are used as sources for bioactive peptides than plant-derived bioactive proteins (Lorenzo et al. 2018). However, a recent trend for investigating bioactive peptides from seaweeds, including *U. pinnatifida* has opened up potential opportunities for developing antioxidant food additives from them (Admassu et al. 2018a).

Meat alternatives and meat analogues

Meat analogues are termed as food products which simulate and reproduce the chemical, organoleptic, visual and esthetic attribute of conventional meat products. Modern meat alternatives and meat analogues have been able to meet consumer expectations due to texture, mouth feel, flavor, and appearance which highly bear a resemblance to meat products. It is a global trend for those who are more concerned about animal welfare and environmental issues associated with meat processing industries and slaughterhouses, to substitute traditional meat products with meat analogues (Kyriakopoulou, Dekkers, and van der Goot 2019). Increasing health consciousness and the emphasis given on healthy aging among modern consumers, have instigated the replacement of animal proteins by plant-based proteins including seaweed proteins (Ismail et al. 2020). In general, meat analogues are formulated from soy protein, wheat gluten, mycoproteins and proteins from other legumes, cereals, nuts, mushrooms and some vegetables, among others. There is a huge potential to produce meat analogues from seaweed proteins due to its high availability, protein content and protein quality. A number of studies have been performed to elaborate this potentiality.

Dagevos, Tolonen, and Quist (2019) have discussed about the consumer demand and market trend, as well as creating a new market for novel meat alternatives formulated with seaweed proteins. It has been predicted that, although

vegetable and soy proteins are used predominately for the production of meat analogues, in the recent future, proteins extracted from seaweeds would be more popular and heavily exploited for formulating meat analogues. This is due to the fact that they can match and fulfill the consumer taste requirement better, and they provide maximum yield and fast growth rate even withoutuse of a land. Joshi and Kumar (2015) have mentioned algae protein as a highly potential meat alternative, while presenting an example of such meat alternative product which was produced in Germany, namely, Remis Algen. Trottet et al. (2018) developed a process to prepare meat analogues, which contains desired protein content using different protein sources including seaweed protein.

Animal and fish feed

Seaweed proteins are increasingly being used in feed of ruminants, poultry, pigs, rabbits and fish, due to ever growing global demand for animal and fish products. Substitutes for animal-originated proteins and soy proteins are necessitated by the feed industry in order to overcome predicted demand in the future. Apart from that, utilizing animal meal for animal nutrition has been prohibited in countries like Europe, which has created a tendency to exploit plant proteins as alternatives. Due to this arisen opportunity, there would be a high propensity for using seaweed proteins into animal feed formations.

Evans and Critchley (2014) stated the advantageous effects of brown seaweed incorporated into ruminant feed, which include improving growth and immune functions, lowering occurrence of pathogenic microorganisms in the final product, showing resistance to stress conditions, rising product quality and enhancing meat and milk productivity. Enhancement of growth, weight and colonization of beneficial bacteria in guts were reported in pigs which were fed with feed formula incorporated with brown seaweeds (Dierick, Ovyn, and De Smet 2009). Furthermore, several studies have been done by incorporating seaweeds into poultry feed, and they have observed benefits such as improved nutritional composition, productivity, quality, healthy lipid profile and mass of breast muscle of the final meat products (El-Deek and Brikaa 2009).

Studies on using seaweeds for fish feed were initiated two decades ago. It was found that fish can absorb 56-67% of existing proteins in seaweeds, which illustrate the aptness of using seaweeds as a protein source for fish nutrition. Fish feed incorporated with algal protein positively influences the growth rate, stress resistance, feed efficiency, disease resistance, survival rate, and nutritional quality and composition of final fish products (Fleurence 1999a). Fleurence et al. (2012) discussed the potentiality of using *U. pinnatifida* in aquaculture for feed as a source of protein, while Niu et al. (2015) observed advantageous effects of the diet incorporated with *U. pinnatifida* on growth, construction of intestine, and immunity of tiger prawn (*Penaeus monodon*). Furthermore, *U. pinnatifida* is used in abalone, shrimp and prawn feed in many countries.

Uchida, Numaguchi, and Murata (2004) studied dietary effect on growth and shell growth of young pearl oysters by feeding them marine silage added with *U. pinnatifida,* observing affirmative dietary effects. Although there are numbers of studies done on the incorporation of dried seaweed into animal and fish feed formulas, studies on adding seaweed proteins to animal or fish feed are extremely scarce. Fish and animal feed are produced from less-expensive raw materials, whereas extraction of protein from seaweed is costly and laborious. However, there is a potential to extract proteins from seaweeds using inexpensive and conventional extraction methods, and to incorporate them to animal and fish feed in order to improve their nutritional value.

Potential nutraceutical value and applications of protein extracted from *U. pinnatifida*

The term "nutraceutical" was introduced in 1989 by Stephen DeFelice combining the words; nutrition and pharmaceutical. It can be defined as a food or a part of a food which offers health or medical benefits, together with effects of treatment and/or prevention of a disease (Kalra 2003). With the inclination of consumers' attention toward healthy consumption, as well as relationship between diet and health and healthy ageing, people look for foods that are not simply aimed to indulging hunger and supplying vital nutrients, but also intending to lower health risks, prevent nutrition-linked diseases and improve health and wellbeing of humans. Seaweeds have gained immense attention lately due to discovering range of bioactive compounds including bioactive proteins, and peptides which can be used as nutraceuticals in food and supplement industries. *U. pinnatifida* exerted bioactivity through proteins, glycoproteins, cyclic peptides, linear peptides, peptide derivatives, depsipeptides, amino acids and amino acid-like components by showing drug like actions, imitating hormones' activity, modifying physiological functions, increasing positive influences by interacting on target cells and attaching to particular receptors (FitzGerald and Murray 2006).

Though some bioactive peptides derived from several macroalgae have been distinguished and characterized, most of the bioactive peptides in diverse species of seaweeds have remained as unexplored and untapped sources. Generally, bioactive peptides comprise 2–20 amino acids, and they are not active when entrenched in their main protein structure. Based on compound structure and amino acid composition and sequence, these bioactive peptides wield wide range bioactivities, including antioxidant, anticancer, antihypertensive, anticoagulant, immune modulation, antimicrobial, antidiabetic activities (Samarakoon and Jeon 2012) which contribute to various nutraceutical applications (Figure 4).

Antioxidant activity

There are complex and effective defence structures of antioxidants in seaweeds, in order to shield themselves from oxidation. Free radicals and other reactive oxygen species are frequently generated in sea water due to various harsh

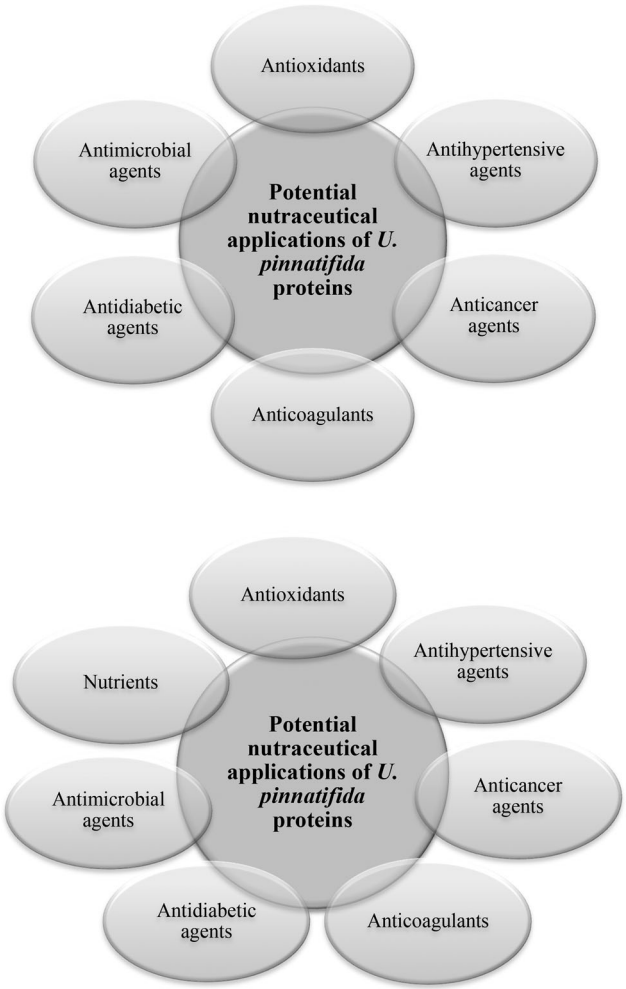

Figure 4. Potential nutraceutical applications of proteins extracted from *U. pinnatifida*.

conditions in this natural oxygenated environment such as varying temperature, light intensity and salinity. One of the antioxidant defence systems is bioactive proteins (Guedes, Amaro, and Malcata 2011). Seaweed polypeptides exert antioxidant activity by scavenging free radicals through two mechanisms. They are single electron transfer and hemolytic or hydrogen atom transfer mechanisms. These two mechanisms occur in parallel, but one can dominate the other depending on the partition coefficient, solubility and structure of the antioxidant peptide (Esfandi, Walters, and Tsopmo 2019).

Antioxidant system, including enzymatic and non-enzymatic antioxidant compounds in human body, defends all tissues and internal organs from oxidative damages caused by diverse noxious reactive oxygen species. Examples of these endogenous antioxidant enzymes are catalase, superoxide dismutase and glutathione peroxidase. Non-enzymatic antioxidants are ascorbic acid, tocopherols, carotenoids, selenium and ferritin. The disparity between reactive oxygen species and endogenous antioxidants leads to triggering severe health issues and chronic diseases, such as cardiovascular disease, cancer, diabetes mellitus, neurodegenerative diseases, inflammation, hypertension and early ageing.

Recently, these disease conditions are aggravated among populations due to new food habits, excessive artificial, instant and high-fat food consumption, smoking, alcohol intake as well as environmental pollution (Valko et al. 2007). Therefore, in order to prevent humans from exposing to these injurious reactive free radical substances, studies are being continuously carried out to find highly potential antioxidants which can be taken as nutraceuticals and food supplements. Currently, higher attention has been paid on extraction of seaweed protein hydrolysate and peptides with antioxidant activity, than other antioxidant organic solvents from seaweeds. Nevertheless, some studies have focused on extraction and utilization of antioxidant peptides as nutraceuticals, while, most of the highly potential seaweed proteins and peptides remain undiscovered. Heo et al. (2005) observed higher hydrogen peroxide scavenging activity (nearly 90%) in protease enzymatic extracts of seven species of brown seaweeds than that of commercial antioxidants. Although, antioxidant activity of organic extracts of *U. pinnatifida* has been studied by many researchers, antioxidant activity of proteins and peptides of *U. pinnatifida* has not been studied extensively.

Rafiquzzaman et al. (2013) investigated antioxidant activity of glycoprotein extracted from *U. pinnatifida.* They suggested that glycoprotein of *U. pinnatifida* is a good natural and bioaccessible antioxidant as well as DNA-protective agent. Furthermore, they stated that these bioactivities are assay-specific and pH-dependent, in addition to being fairly stable during digestion process. However, the needs of further studies have been highlighted to examine the mechanism of biofunction and determine the structures of glycoproteins. In another study, the anti-inflammatory and anti-Alzheimer's activities of glycoproteins isolated from *U. pinnatifida* were examined by Rafiquzzaman et al. (2015). The findings highlighted the high potential of using *U. pinnatifida* derived glycoprotein as nutraceuticals for prevention of oxidative-stress related diseases including Alzheimer's disease and inflammatory diseases. Je et al. (2009) studied the antioxidant activity of protease enzyme assisted extracts of *U. pinnatifida,* and strong radical scavenging activity of the extracts against hydroxyl and DPPH radicals were observed. Wang et al. (2018) summarized the antioxidant potential of peptides of *U. pinnatifida* in the review. It has been found that, peptides and protein hydrolysate with low molecular weight are more likely to own free radicals scavenging activity than those with high molecular weight. This is due to the fact that the molecular weight and structural characteristics of peptides affect mainly the movement and penetration of peptides within the body (Chang, Wu, and Chiang 2007).

Among identified antioxidant peptides from various protein sources, glutathione and carnosine have been found in seaweeds as antioxidant peptides, which are abundant in meat proteins as well (Fleurence 2004). According to all findings of these researches, protein hydrolysates, peptides and amino acids extracted from *U. pinnatifida* appear to have good antioxidant potential.

Antihypertensive activity

Hypertension is an ever-increasing health risk factor among world population which causes the development of coronary

heart disease, stroke, heart failure and end-stage kidney disease (Lawes, Vander Hoorn, and Rodgers 2008). The rennin-angiotensin system (RAS) and kallikrein-kinin system (KKS) play vital roles in controlling and maintaining blood pressure in human body. Hypertension is managed by means of lifestyle changes, drugs, food-derived drugs and nutraceuticals. The recommended lifestyle changes are: reducing alcohol and sodium intake, giving up smoking, reducing body weight, doing physical activities frequently, and dietary changes such as, consumption of vegetables, fruits and non- or low-fat milk products, regularly. As far as drug treatments are considered, mostly used methods are inhibiting the renin-angiotensin system either by blocking angiotensin receptors (AT1) or inhibiting angiotensin-I converting enzyme (ACE). ACE is a peptidase which belongs to the zinc metalloenzyme family and it takes part into key functions in KKS and RAS by converting angiotensin-I to the potent vasoconstrictor angiotensin-II and raising blood pressure by inactivating the vasodilator bradykinin (Fitzgerald et al. 2011).

However, drugs which are used as ACE inhibitors comprise wide range of side effects, such as angioedema, hypotension, enhanced potassium levels in body, inability to sense taste, and chronic cough (FitzGerald, Murray, and Walsh 2004). Therefore, much attention has been paid on intake of food-derived antihypertensive peptides with ACE and renin inhibitory activity, as function foods, nutraceuticals and food supplements. With this new trend, there has been a growing interest among scientific community in order to extract, isolate and utilize novel antihypertensive peptides from seaweeds, due to high protein content and reported exceptional bioactivity in seaweed protein (Kim and Wijesekara, 2010).

Suetsuna and Nakano (2000) investigated four tetra-peptides with ACE inhibiting activity from peptic digest of *U. pinnatifida.* These peptides were extracted, isolated and identified by ion-exchange chromatography, gel filtration, column chromatography and mass spectrometry. These peptides were found to be able to lower blood pressure significantly in spontaneously hypertensive rats after oral administration. Sato, Hosokawa, et al. (2002) studied antihypertensive effects of ACE inhibitory peptides of *U. pinnatifida* by oral administration to spontaneously hypertensive rats. Peptides were isolated from hydrolysates of *U. pinnatifida* by protease hydrolysis, followed by purification with high performance liquid chromatography. It was found that, the isolated peptides possessed considerable antihypertensive properties, while peptides such as Ile-Try, Val-Try, Phe-Try and Ile-TRp significantly reduced blood pressurein rats. In addition to that, 10 dipeptides were isolated from hot water extract of *U. pinnatifida* using different stages of high-performance liquid chromatography, AEC inhibitory activity and antihypertensive effects were examined by Suetsuna, Maekawa, and Chen (2004). Blood pressure of spontaneously hypertensive rats was observed to be declined significantly by ingestion of these dipeptides. Identified peptide sequences and their IC_{50} values of anti-ACE activity are given in Table 3.

Table 3. Amino acid sequences and IC_{50} values of antihypertensive peptides derived from proteins of *U. pinnatifida*.

Amino acid sequence of peptides	IC_{50} values	Reference
Ala-Ile-Tyr-Lys	213 μM	Suetsuna and Nakano (2000)
Tyr-Lys-Tyr-Tyr	64.2 μM	
Lys-Phe-Tyr-Gly	90.5 μM	
Tyr-Asn-Lys-Leu	21 μM	
Val-Tyr	35.2 μM	Sato, Hosokawa, et al. (2002)
Ile-Tyr	6.1 μM	
Ala-Trp	18.8 μM	
Phe-Tyr	42.3 μM	
Val-Trp	3.3 μM	
Ile-Trp	1.5 μM	
Leu-Trp	23.6 μM	
Tyr-His	5.1 μM	Suetsuna, Maekawa, and Chen (2004)
Lys-Trp	10.8 μM	
Lys-Tyr	7.7 μM	
Lys-Phe	28.3 μM	
Phe-Tyr	3.7 μM	
Val-Trp	10.8 μM	
Val-Phe	43.7 μM	
Ile-Tyr	2.7 μM	
Ile-Trp	12.4 μM	
Val-Tyr	11.3 μM	

Another study was done by Sato, Oba, et al. (2002), looking into antihypertensive and ACE inhibitory activity of hydrolysates of *U. pinnatifida,* hydrolyzed using 17 proteases. Hydrolysates of *U. pinnatifida* were identified as effective physiological functional food, regardless of inherited slight bitterness with observed significant reduction of systolic blood pressure of spontaneously hypertensive rats. Moreover, a clinical study was conducted by Hata et al. (2001), using 37 elderly patients with hypertension, to investigate the effect after ingestion of dried *U. pinnatifida* powder on serum biochemical parameters and blood pressure. According to the results of this study, *U. pinnatifida* was recommended as a dietary regimen for hypertension treatments due to observed significant decrease of systolic and diastolic blood pressure, and it was stated that *U. pinnatifida* would be promoted as supplemental remedy once the mechanism of action is clarified.

Considering all the published literature regarding antihypertensive activity of proteins of *U. pinnatifida,* a huge potential of using peptides extracted from *U. pinnatifida* as nutraceuticals and dietary supplements is indicated. This should be followed by more human clinical trials and studies to examine the mechanism of antihypertensive activity.

Anticancer activity

Cancer is one of the major chronic degenerative diseases and cause of death in the world. The onset of a cancer is caused by transformation of normal cells in the body into cancer cells due to some mutations in DNA, which may disrupt the regulating process and balance between death of programmed cells (apoptosis) and cell proliferation. It can be progressed into cellular, as well as genetic level forming cells with uncontrolled division which subsequently develops into a tumor and spread to distant organs of the body (Suarez-Jimenez, Burgos-Hernandez, and Ezquerra-Brauer 2012). Occurrence of cancers is reported due to endogenous

genetic factors, as well as exogenous factors such as diet, lifestyle and environment aspects which generate mutations in the body. Chemotherapy is used as the main treatment technique for cancer therapies, nevertheless, frequent inception of resistance in cancer cells for chemotherapeutic treatments, as well as negative effects associated with other anticancer treatments have been overriding issues (Sheih et al. 2010). Therefore, dietary components and bioactive functional secondary metabolites have been isolated and used with the purpose of prevention, management and treatment of cancers.

Among those bioactive compounds, cationic low-molecular-weight peptides with anti-tumor activity have been discovered and identified as anticancer peptides. They are able to inhibit tumor angiogenesis and tumor cell proliferation and migration, efficiently thus, have many advantages over conventional chemotherapy attributable to their unique mechanism of action (Xie, Liu, and Yang 2020). Seaweeds have been used to extract valuable functional and bioactive metabolites, which are used for treatments of cancers. Anticancer properties of proteins and peptides derived from seaweeds have been given much attention recently. Pepsin- and papain-digested hydrolysates of red seaweed *Pyropia haitanensis* showed anti-proliferation activity against human lung cancer cells (A549), human liver cancer cells (HepG-2) and human breast cancer cells (MCF-7), with IC_{50} values ranging from 59.09 to 272.67 μg mL^{-1} (Mao et al. 2017). Moreover, Fan et al. (2017) have isolated five peptides from red seaweed *Porphyra haitanesis,* which showed anti-proliferation activity against cancer cell lines, such as HT-29, SGC-7901, HepG-2, A549 and MCF-7 (IC_{50}-191.61-316.95 μg mL^{-1}). There are many studies which have been done on anticancer activity of proteins extracted from seaweeds, nevertheless, report of anticancer activity of proteins extracted from *U. pinnatifida* are yet again scarce. There is a huge potential to discover and isolate proteins and peptides with anticancer properties from *U. pinnatifida* as it contains very high percentage of proteins rich in exceptional bioactive properties (Wang et al. 2018).

Considering the above findings regarding anticancer proteins and peptides of other seaweeds, as well as theirpotential occurrence in *U. pinnatifida*, there is a prospective utilization of proteins and peptides extracted from *U. pinnatifida* for the development of nutraceuticals, dietary supplements and pharmaceuticals. Therefore, more studies should be conducted in order to discover these proteins and peptides.

Anticoagulant activity

Blood coagulation is a vital component of haemostasis, in which the blood flow is ceased when there is an injury in a blood vessel wall, without unsettling the normal blood circulation. There are two stages of this haemostasis process, known as primary and secondary. During the primary constituent, platelets are aggregated, and platelet plug is formed, while insoluble fibrin, which is the end product of clotting cascade, deposits during the secondary part repairing the damaged blood vessel wall (Gale 2011). This coagulation cascade comprises series of proteolytic reactions that are involved in blood clotting by two dissimilar mechanisms, namely, intrinsic and extrinsic pathways. These pathways ultimately merge and convert soluble fibrinogen into insoluble fibrin. Anticoagulants obstruct the coagulation features prolonging or stopping blood coagulation process. Anticoagulants are used for treating thrombotic disorders, investigating the mechanism of blood coagulation as a cure for hemophilia. They are also used in equipments used for medical laboratory applications, such as renal analysis, blood transfusion and some other blood analysis in test tubes (Kim and Wijesekara 2010).

Commercial anticoagulants, used for therapeutic purposes, are heparin, warfarin and coumarine. These commercial anticoagulants have been associated with the development of several side effects, such as thrombocytopenia, ineptness in inborn or acquired antithrombin deficiencies, hemorrhagic effects and failure to inhibit thrombin bound to fibrin (Pereira, Melo, and Mourão 2002). Therefore, growing interest has been developed for discovering natural anticoagulants which are safe for long term human health. Occasionally, anticoagulant bioactive peptides are extracted from seaweeds and analyzed. These anticoagulant peptides act as inhibitors of single or multiple clotting factors such as thrombin, prothrombin, FX, FXI, FXII and VIIa/TF complex, while some prolong the prothrombin time and plasma recalcification time (Syed and Mehta 2018). On the other hand, protein related metabolites of seaweeds, like proteoglycan, have been recognized with anticoagulant activity.

Athukorala et al. (2007) evaluated hot water extracts from 22 species of brown and green seaweeds found in South Korea for anticoagulant activity, using activated partial thromboplastin time (APTT) and prothrombin time (PT) assays. It was observed that these extracts possessed significant anticoagulant activity. The anticoagulant activity was exerted by high molecular weight polysaccharides or complex compounds formed by binding proteins to polysaccharides, namely proteoglycans. Matsubara et al. (2000) isolated and analyzed anticoagulant activity of proteoglycans from green seaweeds *Codium pugniformi s*using APTT, PT and thrombin time (TT) assays. The results revealed that, proteoglycans held comparable but slightly weaker anticoagulant activity to heparin. Furthermore, another anticoagulant peptide has been isolated and identified from hydrolysate of red seaweed *Porphyra yezoensis*with a sequence of NMEKGSSSVVSSRM(+15.99)KQ using size exclusion, anion exchange, Sephadex and HPLC and MS methods (Indumathi and Mehta 2016). This purified peptide showed a considerable in vitro anticoagulant activity in APPT assay. Considering all these published literatures, there is a potential to use *U. pinnatifida*-derived proteoglycans and peptides to developanticoagulant nutraceuticals. Further studies are necessary to discover bioactive proteoglycans and peptides, identify their mechanism of action, and verify the safety and efficiency of their use as nutraceuticals.

Antidiabetic activity

Diabetes mellitus is a growing health issue in the world. Type 1 diabetes mellitus occurs due to inability of the pancreas to secrete insulin as a result of destruction of beta cells. Type 2 diabetes mellitus occurs due to either insufficient cellular response to insulin, or insufficient production of insulin (Anguizola et al. 2013). Bioactive peptides derived from proteins play a significant role in regulating glucose homeostasis. One type of therapy for the assistance of glycaemic control is the inhibition of enzymes such as α-glucosidase and α-amylase, which are responsible for hydrolyzing dietary carbohydrates and aiding subsequent absorption by small intestine. Another antidiabetic treatment is incretin analogues, like glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). These insulinotropic incretin hormones are utilized to regulate postprandial blood glucose level and increase secretion of glucose-dependent insulin. Dipeptidyl peptidase-4 (DPP-4) inhibitors are also used for antidiabetic therapies, as they are capable of inactivating these incretin peptide hormones by cleaving the N-terminal of X-alanine and X-proline sequences (Admassu et al. 2018a). Moreover, it has been found that antidiabetic peptides are able to increase cholecystokinin levels in the body, a gut hormone that regulate food intake. Hence, these bioactive peptides have established effects on glycemia reduction, insulin secretion and body fat loss (Rivero-Pino, Espejo-Carpio, and Guadix 2020).

Harnedy, O'Keeffe, and FitzGerald (2015) identified and purified three DPP-4 inhibitory peptides from red seaweed *Palmaria palmate* with amino acid sequence of Met-Ala-Gly-Val-Asp-His-Ile, Leu-Leu-Ala-Pro and Ile-Leu-Ala-Pro. Admassu et al. (2018b) identified two peptides with antidiabetic activity, which function by inhibiting α-amylase enzyme at IC_{50} value of 2.58 ± 0.08 mM. Amino acid sequences of these two peptides were Glu-Leu-Ser and GlyGly-Ser-Lys. They were stated as potential food, nutraceutical and pharmaceutical ingredients for the management of type 2 diabetes. Pimentel et al. (2019) have highlighted the potential of utilizing macroalgae derived protein hydrolysates and peptides as ingredients in functional food and nutraceuticals for the prevention and management of type 2 diabetes. As for the antidiabetic properties of proteins derived from *U. pinnatifida*, Wang et al. (2018) has stated that *U. pinnatifida* is rich in peptides which hold considerable antidiabetic activities. Although studies regarding antidiabetic activity of proteins extracted from *U. pinnatifida* are limited, there is still a high potential for extraction, isolation and utilization of antidiabetic peptides for nutraceuticals from it.

Antimicrobial activity

Antibiotic and multidrug resistant microorganisms have become a global challenge in current medical treatments and public health. Therefore, discovering new antibiotic drugs and potential antimicrobial nutraceuticals are important tasks for the betterment of future medicine. When viral infections are considered, it is very complicated and difficult to attack a target virus directly by a treatment without causing adverse effects on infected cells. However, if viral proteinase enzymes, which are vital for the onset of particular viral disease, inhibited using specific inhibitors, the virus would not be replicated, because maturation of the virus is entirely dependent on proteolytic operations on viral polyprotein precursors (Wagner et al. 1999). There is an ever-expanding preference among people in the world for using natural products for treating, managing and preventing various disease conditions. More researchers are working on discovery of novel antimicrobial nutraceuticals, functional foods and drugs, based on natural plant sources with high bioavailability and less side effects and toxicity.

Among various natural sources of antimicrobials, proteins extracted from seaweeds are given increasing interest. Natural derived antimicrobial peptides can be categorized into peptides with cyclic peptides, α-helix, β-sheet and peptides with long lengths. Mechanism of action of antimicrobial peptides depends on structure, amphipathic property and amino acid sequence, among others. These peptides comprise diverse mechanisms to disrupt the membrane of microbes. Models such as detergent, carpet, toroidal pore worm hole, barrel-stave like modes are used as mechanisms of binding antimicrobial peptides to membranes of bacteria. Furthermore, other insertion models such as molecular electroporation and sinking raft models are used by the host defense antimicrobial peptides (Seyfi et al. 2020).

Several studies have been carried out to isolate functional metabolites and investigate their prospective usages. For an example, Beaulieu et al. (2015) discovered and characterized antibacterial peptides from protein hydrolysate of brown seaweed *Saccharina longicruris* for the purpose of restraining the food spoilage. The peptides showed significant antibacterial effect against *Staphylococcus aureus*. Cordeiro et al. (2006) observed significant antifungal activity from the protein extracted from red seaweed *Hypnea musciformis* against pathogenic yeasts *Candida albicans* and *C. Guilliermondii*. Lectin fraction was found to be responsible for growth inhibition of these pathogenic yeasts. Furthermore, strong antibacterial activity was detected from lectins of two red seaweed species by Liao et al. (2003) against *Vibrio Pelagius* and *V. Vulnificus,* showing high potential of economic value as microbial disease preventers.

As far as antiviral activity is considered, Hirayama et al. (2016) studied and reported significant antiviral activity of high-mannose specific lectins extracted from red algae *Kappaphycus alvarezii* against HIV virus. Muet al. (2017) found anti-influenza viral activity from high-mannose specific lectins extracted from green alga *Halimeda renschii*. Furthermore, a novel anti-HIV lectin protein namely, Griffithsin (GRFT), that has a potential microbicide activity for preventing the sexual transmission of AIDS, was discovered by Mori et al. (2005).

De Corato et al. (2017) studied in vitro and in vivo antifungal activities of crude extracts of *U. pinnatifida* against three postharvest pathogens, *Botrytis cinerea*, *Penicillium digitatum* and *Monilinia laxa*. Complete inhibition of mycelia growth and conidial germination were observed. Apart from that, few literatures can be found with regards to the

antimicrobial, antifungal and antiviral ability of proteins and protein-derived components extracted from *U. pinnatifida*, even though several authors have stated about fine antimicrobial activities in extracts of *U. pinnatifida* (Wang et al. 2018). Based on all the above, there is a high potential to use proteins and protein-derived components extracted from *U. pinnatifida* as ingredients in antimicrobial nutraceuticals and functional foods, as well as for developing prospective pharmaceuticals.

Protein as nutraceutical ingredients for nutrition

Protein is a vital nutrient in the human diet throughout the entire life span which is required for growth, supporting bone and muscle metabolism and ensuring the development and maintenance of nervous system as well as supporting to sustain physical performance and muscle mass in older ages (Kårlund et al. 2019). Physically active people have raised physiological protein requirements in order to sustain adequate protein synthesis, energy production, good gut integrity and immune functions throughout multi-stress conditions of prolonged, intensive, frequent and goal-directed workout routines.

Elderly people are often suffering from progressive decline in skeletal mass, causing the development of sarcopenia. It results poor quality of life, physical disability, mortality and great health care cost. Stimulation of muscle protein synthesis and preservation of muscle mass through taking protein and amino acid nutraceuticals is a potential remedy for sarcopenia. Therefore, other than protein rich foods, protein and amino acid supplements, as nutraceuticals are widely recommended for athletes, habitually active consumers and elderly (Cruz-Jentoft et al. 2010). It has been found that essential amino acids are main regulators for muscle protein synthesis, thus essential amino acids and high protein oral nutritional supplements are recommended.

Seaweed proteins are well-suited for this requirement due to its high quantity and quality comprising all essential amino acids. Protein content of seaweeds ranges from 5% to 47% in dry weight and the essential amino acid content represents nearly a half of the total amino acid contents, comprising compatible protein profile to that of egg protein (Černá 2011). High protein content in seaweeds can be a solution for finding a cheap and new alternative source of protein for addressing the global issue of protein malnutrition Therefore, including edible seaweeds into the daily diet is regarded as a nutraceutical food since its protein values superior or similar to soybean and legumes. Higher protein content is recovered from seaweeds using novel and improved protein extraction technologies along with alkaline and polysaccharide treatments (Cotas et al. 2020).

Consuming microalgae as dietary protein supplements is well-established in the world in the form of tablets, pills and powders, while it has spread into large scale commercial productions. Usage of protein extracted from seaweeds as nutraceutical ingredients for nutrition is challenging due to high processing cost and high technological requirements (Pangestuti and Kim 2015). However, some commercial protein powder products are commercially available. Moreover, seaweeds have been incorporated into a wide range of functional foods while many studies have been conducted on nutritional benefits of those functional foods (Bleakley and Hayes 2017). Likewise, *U. pinnatifida* seaweed powder has been incorporated into a number of functional foods as described under section "Nutritional component in human diet". Several studies have been carried out to evaluate the suitability of *U. pinnatifida* as a food supplement and their nutritional contribution to the dietary requirements (Kolb et al. 2004; Taboada et al. 2013). Further research is still necessary to develop novel methods to enable *U. pinnatifida* protein production, extraction and processing as affordable nutraceutical protein and amino acid supplements since studies on using *U. pinnatifida*-derived protein as nutraceuticals for nutrition are scanty.

Conclusions and futures perspectives

There is a worldwide inclination and interest to utilize functional nutrients and secondary metabolites from seaweeds, owing to the recent attention toward healthy eating and aging. Proteins extracted from seaweeds have been given an increasing attention as a healthy and alternative source to replace animal proteins. Among mostly harvested and used seaweeds, *U. pinnatifida* has been given a high focus due to its high protein content. Diverse forms of *U. pinnatifida* proteins are used for food and nutraceutical, including proteins, peptides, glycolproteins, enzymes, cell wall-attached proteins, lectins and amino acids. Nutritional quality of *U. pinnatifida* protein is considered as exceptional, due to its rare amino acid composition, high ratio of essential to non-essential amino acids, and high propensity to hydrolysis during digestion.

These nutritionally valuable proteins of *U. pinnatifida* can be extracted by conventional and more modern methods. Bioactive peptides can be isolated and purified using gel electrophoresis, chromatographic and spectrophotometric techniques. High nutritional quality and functional properties such as hydration capacity, protein solubility, foaming ability, emulsification ability, water and fat binding capacity and gelation uphold potential food applications of *U. pinnatifida*-derived proteins, including nutritional component in human diet, protein extracts for food ingredients and additives, meat alternatives and meat analogues, and animal and fish feed.

Developing functional foods from seaweed proteins and peptide benefits consumers since they confer substantial amounts of high-quality non-animal protein with complete amino acid profile. Moreover, the exceptional bioactivity imparted from the seaweed peptides reinforce the functional properties targeting prevention of a number of chronic diseases. Major limiting factors of using seaweed proteins and peptides in functional foods are lack of seaweed availability, cost-effective seaweed cultivation technology, cost-effective and precise isolation and purification technology and rigorous scientific studies on efficacy, safety, toxicity and functionality. Moreover, low shelf life of seaweeds, lack of

consumer awareness of health benefits, environmental threats associated with cultivation and competition with green vegetables are challenges for developing functional foods from protein derived from *U. pinnatifida.*

According to the findings of a number of studies, excellent bioactivities possessed in proteins of *U. pinnatifida* may lead to the utilisationof these proteins in various nutraceutical applications, as antioxidant, antihypertensive, anticancer, anticoagulant, antidiabetic and antimicrobial agents. Similar studies done on nutraceutical applications of proteins of other seaweed species warrant these potential applications. Although several nutraceutically important protein of *U. pinnatifida* identified and characterized, most of them remain as unexplored and unexploited sources. Furthermore, plenty of in vitro, in vivo and clinical research are needed to examine the mechanism, safety and efficacy of these nutraceutical proteins or protein fractions/conjugates. Overall, more research is essential to progress the novel food and nutraceutical applications of *U. pinnatifida* proteins. The research areas should also include novel food industrial applications as food additives and food ingredients, factors limiting digestibility and nutritional value, processing steps to avoid possible undesirable antinutritional factors, and sensory quality attributes associated with *U. pinnatifida* proteins.

Author contributions

Harshani Nadeeshani – Wrote the original draft and performed literature search.

Amira Hassouna – Edited the original draft.

Jun Lu – Conceived the idea, edited the final draft and revisions.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research is financially supported by New Zealand National Science Challenge, High Value Nutrition grant HVN 3719491 1905-13024.

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