Bioactive peptides and carbohydrates from seaweed for food applications: Natural occurrence, isolation, purification, and identification

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Abstract

Macroalgae or seaweed are relative un-explored and promising sources of novel molecules for the food industry including peptides and carbohydrates for their use as functional foods and nutraceuticals. Several algae-derived bioactive compounds have shown a wide range of biological activities both *in vitro* and *in vivo*, i.e. antihypertensive and antioxidant, that are strongly associated with the chemical structure of the peptides or carbohydrates. Multiple improvements in the purification and analytical tools to characterize these compounds have been reported in recent years, aiming to gain further insight into the complexity of different molecular structures of bioactive peptides and carbohydrates. This paper discusses the variable composition of algae and the opportunities of the use of this biomass to obtain novel functional bioactive peptides and carbohydrates for functional food applications. The main biological activities of the discovered bioactive peptides and carbohydrates together with the analytical procedures used to purify and characterize multiple compounds are also discussed.

Keywords: seaweed, bioactive peptides, functional food, peptide characterization, carbohydrate.

1. Introduction

Traditional food ingredients have gained increased scientific attention for their potential to generate functional foods and nutraceuticals. Functional foods are defined as foods that may impart a health benefit to the consumer that goes above and beyond basic human nutrition [1]. Several successful examples in the market include the use of fortified products with omega-3 polyunsaturated fatty acids of algal origin [1] and beverages containing bioactive peptides such as Evolus® or Calpis® [2].

Algae are a rich source of multiple valuable macro- and micro-nutrients, including proteins, carbohydrates, phenols, vitamins, and minerals [1, 3]. These compounds were traditionally exploited by the food and animal feed industries due to their nutritional value, i.e. as a source of proteins rich in essential amino acids [4] or the use of non-digestible carbohydrates from seaweed as a source of dietary fibre [5]. This rich and variable composition of algae is being explored for its potential to obtain functional ingredients including bioactive peptides and carbohydrates [1, 5-8]. Bioactive peptides are sequences of 2 to 30 amino acids in length that display hormone-like beneficial properties when released from their parent protein [9, 10]. Biological effects described to date include antihypertensive, antioxidant, antithrombotic, antimicrobial, and immunomodulatory properties [1,5]. Currently there are a number of products in the market containing bioactive peptides, including a peptide soup containing the peptides LKPNM obtained from dried bonito and a sardine-derived product rich in the di-peptide VY [1]. Moreover, algal carbohydrates such as alginates or carrageenan are industrially commercialized as thickening and gelling agents with multiple applications in the food, textile, biotechnological, and biomedical industries [11]. These marine carbohydrates have recently come under the spotlight as functional food ingredients [5]. Seaweed carbohydrates such as alginates,

carrageenan, and fucoidan showed a wide range of biological activities including antiinflammatory, anticoagulant, antioxidant, antiproliferative, and immunostimulatory activities both "*in vitro"* and/or "*in vivo"* [5]. Furthermore, algal polysaccharides such as laminarin are non-digested in the upper gastrointestinal tract and are considered as dietary fibre [5]. The consumption of dietary fibre has a positive influence on human health with beneficial impacts including a reduced risk of suffering from colon cancer and constipation, but also reduced hypercholesterolemia, obesity, and diabetes [12].

The biological effects displayed by these marine bioactive peptides and carbohydrates depend on the chemical structure of these molecules [1, 13]. These differences could be attributed to biological factors affecting the seaweed biomass (i.e. season and location of collection), but also to structural modifications of the molecules during the processes of extraction and purification [5, 14]. The increased interest in developing functional bioactive peptides and polysaccharides represents a challenge for analytical scientist trying to identify the relationship between the chemical structure of these compounds and their biological activities. Multiple analytical approaches have been used to date to purify and analyse the chemical structure of bioactive peptides and carbohydrates [5, 15].

This paper discusses the variable composition of algae and the opportunities of the use macroalgae to obtain novel functional bioactive peptides and carbohydrates for functional food applications. The main biological activities of the discovered bioactive peptides and carbohydrates together with the analytical procedures used to purify and characterize multiple compounds are also discussed.

2. Chemistry and biochemistry of macroalgae

Macroalgae or seaweed comprise a heterogeneous group of approximately 10,000 species [16], being only few of them used for food aplications, mainly as food additives or flavouring materials, especially in Asian countries. Indeed, seaweed is served in approximately 21% of meals in Japan [17] and some eastern varieties are increasingly consumed in western countries since oriental food became more popular.

Algae can be divided into three main groups or phyla: brown (Phaeophyceae), red (Rhodophyceae), and green (Chlorophyceae). Moisture content of fresh marine algae is very high and can account for up to 94% of the biomass [18]. In addition, algae have a highly variable composition, with large differences in their final content in minerals (including calcium, phosphorus, and potassium), vitamins, proteins, lipids, and fibre [8]. Seaweed composition, i.e. protein and carbohydrate contents, depends not only on the species, but also on the time of collection and habitat, and on external conditions such as temperature, light intensity, and nutrient concentration in water [16].

2.1 Protein and amino acid contents in macroalgae

2.1.1 Protein content

As mentioned previously, the protein content of seaweed differs depending on different factors. In addition, comparison of the protein content among algae is difficult because of methodological differences, especially during protein extraction, and the large number of species identified to date [19]. Although brown seaweed usually contains a low protein content when compared to that of green or red seaweed, Lourenço, Barbarino, De-Paula, Pereira and Marquez [19] reported a relatively high protein content ranging between 10 and 15% for the species *Chnoospora minima*, *Dictyota menstrualis*, *Padina gymnospora*, and *Sargassum vulgare*. Common brown seaweed includes *Laminaria digitata*, *Ascophyllum nodosum*, *Fucus vesiculosus*, and

Himanthalia elongata. The average protein content of *Laminaria digitata* was recently calculated as 6.8%, with highest and lowest protein levels in the first and third quarter of the year, respectively [20]. This value was similar to that obtained by Peinado, Girón, Koutsidis and Ames [21], who calculated the protein content of *Laminaria digitata* as 5.8%. Comparable results were observed in this study for other brown algae species such as *Laminaria hyperborea*, *Saccharina latissima*, and *Alaria esculenta* with average protein levels ranging between 6.8 and 11.0% of the total dry weight (DW) [20] and *Ascophyllum nodosum*, *Pelvetia canaliculata*, *Fucus vesiculosus*, and *Fucus spiralis* which presented an average protein content of 5.2, 7.3, 5.8, and 5.9%, respectively [21].

Although previous studies highlighted that in some green seaweed, such as those species belonging to the genus *Ulva*, the protein content can range between 10 and 26% of the total DW [22], a more recent study reported that the protein content of *Ulva lactuca*, harvested between May and June, was below 8.6% [23]. Mæhre, Malde, Eilertsen and Elvevoll [23] obtained similar results from other green algae such as *Cladophora rupestris* and *Enteromorpha intestinalis*, which were also harvested between May and June in Scotland, and had a total protein content of 3.4 and 11.3%, respectively. In addition, Lourenço, Barbarino, De-Paula, Pereira and Marquez [19] calculated the protein content of the green algae *Caulerpa fastigiata*, *Caulerpa racemosa*, *Codium decorticatum*, *Codiu, spongiosum*, *Codium taylorii*, and *Ulva fasciata,* collected in Brazil in autumn, and results suggested that the protein content varied between 11 and 20%, depending on the species.

Higher protein concentrations were reported in red seaweed, such as *Phorphyra tenera* and *Palmaria palmata*, which can be up to 47% [22]. However, in a more recent

study, Mæhre, Malde, Eilertsen and Elvevoll [23] calculated the protein content of *Palmaria palmata* and *Vertebrata lanosa*, harvested between May and June in Scotland, as 12.2 and 11.5%, respectively. Results correlate well with those obtained by Galland-Irmouli, Fleurence, Lamghari, Luçon, Rouxel, Barbaroux, Bronowicki, Villaume and Guéant [24], who showed that the protein content of *Palmaria palmata* significantly varied depending on the season, with the highest protein content (21%) at the end of the winter season and the lowest (11%) in early autumn. The total protein content of the species *Acanthophora spicifera*, *Aglaothamnion uruguayense*, *Cryptonemia seminervis*, *Gracilariopsis tenuifrons*, *Laurencia flagellifera*, Plocamium *brasiliense*, *Pterocladiella capillacea*, and *Porphyria acanthophora* was determined by Lourenço, Barbarino, De-Paula, Pereira and Marquez [19], who reported a variable protein content ranging between 12% (*Acanthophora spicifera*) and 27% (*Aglaothamnion uruguayense*) on a DW basis.

Numerous seaweed-derived proteins have been identified, and these can be used for the generation of biologically active peptides with health-promoting properties. However, among the different classes of proteins identified, it is important to emphasize lectins and phycobiliproteins. Lectins are glycoproteins with carbohydrate-binding properties which allows them to agglutinate microbes, yeasts, tumour cells, and erythrocytes [25]. Lectins are very interesting for a diversity of applications in immunological and histochemical studies [26] as well as in agricultural and medical applications due to their antimicrobial, antitumor, and antiviral activities [27]. In addition, phycobiliproteins are brilliantly coloured and highly fluorescent components which are currently used in numerous fluorescence-based techniques such as immunofluorescence, fluorescence-activated cell sorting, and fluorimetric microplate assays [28].

2.1.2 Amino acid composition

The amino acid composition of seaweed proteins has been repeatedly studied and compared to that of other foods. Most seaweed contain all the essential amino acids, and aspartic and glutamic acids were suggested to constitute a large part of their amino acid fraction [22]. Indeed, these two residues represent 22 and 44% of the total amino acids in certain brown seaweed [29]. Brown seaweed have been suggested as rich sources of threonine, valine, leucine, lysine, glycine, and alanine previously, and amino acids such as cysteine, methionine, histidine, tryptophan, and tyrosine were recorded at lower levels [18]. Lourenço, Barbarino, De-Paula, Pereira and Marquez [19] studied the amino acid profile of four brown seaweed species namely *Chnoospora minima*, *Dictyota menstrualis*, *Padina gymnospora*, and *Sargassum vulgare*. All samples were collected in Brazil in June and September, and showed a high content of aspartic and glutamic acid, and a relatively high (over 8%) content of leucine.

The green seaweed *Ulva pertusa* contains high levels of proline [30], and the amino acid profile of the green seaweed *Ulva lactuca* revealed that this specie contained all the essential amino acids, in levels which were comparable to the dietary recommendations proposed by the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) [31]. Lourenço, Barbarino, De-Paula, Pereira and Marquez [19] reported high concentrations of aspartic and glutamic acids in six species of green algae, namely *Caulerpa fastigiata*, *Caulerpa racemosa*, *Codium decorticatum*, *Codium spongiosum*, *Codium taylorii*, and *Ulva fasciata*. The authors also recorded a relatively high content of the residues leucine and alanine for all the studied species. In addition, leucine, threonine, isoleucine, valine, and methionine are well represented in the essential amino acid fraction of

Palmaria palmata, with similar leucine and valine concentrations to those generally reported for ovalbumin, and similar isoleucine and threonine contents to those recorded for leguminous proteins [22].

The amino acid profile of the red seaweed *Hypnea charoides* and *Hypnea japonica* revealed that both species contained all the essential amino acids (excluding tryptophan), which accounted for approximately 42-48% of the total amino acid content [31]. Red seaweed proteins also contain high quantities of glutamic and aspartic acid. However, previous studies suggested that the quantities of glutamic and aspartic acid in red seaweed were significantly lower than those observed in brown and green seaweed [32]. Lourenço, Barbarino, De-Paula, Pereira and Marquez [19] reported a concentration of aspartic and glutamic acid ranging between 10 and 15% in numerous red algae species including *Acanthophora spicifera*, *Aglaothamnion uruguayense*, *Cryptonemia seminervis*, *Gracilariopsis tenuifrons*, *Laurencia flagellifera*, and *Porphyria acanthophora*. Relatively high concentrations (over 7%) of leucine, valine, and glycine were recorded for the red seaweed *Porphyria tenra* [30], and concentrations of approximately 10% of alanine, threonine, proline, and histidine were reported for *Acanthophora delillii* [33]. Similar results were obtained for the red algae *Hypnea muciformis*, *Sebdania polydactlyla*, and *Scinia indica*, which had an approximate proline concentration of 10% [33].

Overall, glutamic and aspartic acid were the most abundant amino acids in most species. Methionine content was reported to be low in most of the species, and the mean values for individual amino acids were, in the majority of the reviewed studies, similar in brown, green, and red algae.

2.2 Carbohydrate contents in macroalgae

Seaweed also contain large amounts of polysaccharides with important functions for the macroalgal cells including structural and energy storage [5]. The total polysaccharide concentrations in macroalgae ranged from 4 to 76% of DW with the highest contents described in *Ascophyllum*, *Porphyra* and *Palmaria*; although other green species such as *Ulva* showed contents of up to 65% on a DW basis [18]. Seaweed polysaccharides include relevant bioactive compounds such as alginate, carrageenan, fucoidan, and laminarin. Furthermore other phycocolloids from macroalgae, i.e. agar, are commonly used in the food and animal feed industries as stabilisers, thickeners, and emulsifiers [13, 18].

Alginates are linear unbranched polysaccharides containing β-D-mannuronic acid (M) and α-L-guluronic acid (G) units linked by 1-4 glycosidic bonds [35]. These monomers are mainly arranged in sequences of homopolymeric blocks (MM and GG blocks) and heteropolymeric blocks (MG or GM blocks) [34]. Alginates are currently produced from brown seaweed of the genus *Laminaria*, *Saccharina*, *Lessonia*, *Macrocystis*, *Durvillaea*, *Ecklonia*, and *Ascophyllum* [34], being *Durvillaea potatorum* and *Macrocystis pyrifera* the species with the highest yields of alginates of up to 55 and 45% of DW, respectively [35, 36].

Carrageenans (CRGs) are sulphated linear galactans, consisting of alternating β-1,4- and α-1,3- linked d-galactosyl residues (D- and G-units). Carrageenans are characterized depending on the disaccharide repeating unit and the degree of sulphation of the molecule [37]. The three most industrially exploited types, namely Kappa, Iota, and Lambda are distinguished by the presence of one, two, and three ester-sulphate groups per repeating disaccharide unit respectively. However, the sulphate contents of the commercial carrageenans can vary depending on the

seaweed specie or batch [38]. *Chondrus crispus* and *Kappaphycus* sp. contain up to 71 and 88% of carrageenan, respectively [18].

Fucoidans are cell wall polysaccharides present in brown macroalgae which play a crucial role in the protection of macroalgae against environmental challenges [5]. Fucoidans are fucose containing sulphated polysaccharides formed of a backbone of (1 → 3)-linked α-l-fucopyranosyl or alternating (1 → 3)- and (1 → 4)-linked α-l-fucopyranosyl residues with variable degrees of sulphation [5]. These polysaccharides are a chemically diverse group with molecular weights ranging from 43 to 1600 kDa [39]. The content and composition of fucoidan varies depending on the season and seaweed specie [5]. *Fucus vesiculosus* had fucoidan levels ranging from 16 to 20%, while *Undaria pinnatifida* contained approximately 1.5% [18]. Furthermore, recent studies also showed differences in the fucoidan content depending on the stage of growth of the seaweed. Cultured *Laminaria japonica* showed the highest contents of fucoidan in October, when the seaweed blades were matured rather than at younger stages of development [40].

Laminarins are a group of energy storage polysaccharides composed by 1,3-linked β-d-glucose monosaccharides with variable branching at β-(1,6) [5]. The laminarin structure may vary in the degree of branching and degree of polymerization depending on the seaweed specie, season, and other parameters such as extraction and purification procedures [5]. Laminarin is present in high yields in *Laminaria* sp. (up to 32%, depending on the season) and *Saccharina* sp., but these molecules have been also described in *Ascophyllum*, *Fucus,* and *Undaria* sp. [18]. Seasonal studies on *Laminaria* sp. showed higher accumulation of storage carbohydrates during summer

and autumn that will be used during the winter as an energy source for new tissue growth [21].

3. Seaweed-derived bioactive compounds

3.1 Bioactive peptides derived from edible seaweed

As mentioned previously, algae are known to contain high concentrations of high quality polysaccharides, minerals, and vitamins, as well as bioactive compounds including proteins, lipids, and numerous polyphenols [18]. Bioactive peptides have been generated from a wide variety of natural sources including meat [10, 41, 42], milk [43, 44], cereals [45, 46], and fish [47, 48]. Although there has been a growing demand to isolate bioactive peptides from marine algae [9, 49], the number of biologically active peptides generated from seaweed is still limited. A number of functional foods containing seaweed-derived peptides are currently commercialized, mainly in Japan. The Japanese Ministry of Health and Welfare established a policy for approving some selected functional food products as Foods for Specified Health Uses (FOSHU) whose health claims are legally permitted [50]. Seaweed-derived peptide-containing products with FOSHU approved antihypertensive claims include Wakame peptide jelly (Riken Vitamin Co., Ltd., Tokyo, Japan) and Nori peptide S (Shirako Co., Ltd., Tokyo, Japan) [51].

3.1.1 Antioxidant peptides

A free radical can be defined as a chemical substance capable of independent existence with one or more unpaired orbital electrons, and can be produced either from normal cell metabolism *in situ* of from external sources such as radiation or pollution [52]. When free radicals are produces in excess, and cannot gradually be

destroyed, their accumulation in the body generates a phenomenon called oxidative stress [52]. The regulation of oxidative stress is an important factor in both, tumour development and responses to anticancer therapies [53]. Antioxidants can counteract oxidative stress, and these are either produced by the human body *in situ* or incorporate through diet.

Seaweed are a rich source of antioxidants. For example, the known antioxidant peptides carnosine and glutathione, which are generally present in animal muscle, were identified in seaweed species previously [54]. In addition, as mentioned previously, seaweed can contain relatively high protein content, and seaweed-derived proteins can be used for the generation of antioxidant hydrolysates and peptides. For example, Heo, Park, Lee and Jeon [55] generated a large amount of antioxidant hydrolysates of proteins isolated from *Ecklonia cava*, *Ishige okamurae*, *Sargassum fullvelum*, *Sargassum horneri*, *Sargassum coreanum*, *Sargassum thunbergii*, and *Scytosipon lomentaria* using the commercial enzymes Alcalase, Flavourzyme, Neutrase, Protamex, and Kojizyme. In this study, antioxidant activity was assessed using four different antioxidant scavenging assays and results demonstrated that the antioxidant potential of the Alcalase hydrolysates of *Sargassum horneri* were dose-dependent and thermally stable. In a more recent study, Cian, Martínez-Augustin and Drago [56] obtained different enzymatic hydrolysates from co-products of *Porphyra columbina* using alcalase, trypsin, and combinations of both. Bioavailability of peptides with antioxidant properties has been also evaluated. Indeed, Cian, Garzón, Ancona, Guerrero and Drago [57] observed that antioxidant activity of peptides derived from *Pyropia columbina* increased after a simulated gastrointestinal digestion.

3.1.2 Bioactive peptides and their role in diseases related to metabolic syndrome

The term metabolic syndrome describes a combination of medical disorders which increase the risk of developing cardiovascular disease, and these include obesity, diabetes, hypertension, lipid disorders, and alterations in the thrombotic potential [58]. Some of these disorders can be prevented or controlled by the inhibition of a number of enzymes and regulation systems by the use of chemically synthesised drugs or the ingestion of nutraceuticals or functional foods.

Venous thromboembolism (VTE) is a common and underestimated condition and one of the main causes of death and disability in high-income countries [59]. Blood coagulation is a complex mechanism with numerous coagulation factors and enzymes involved, and because of this, thrombus formation can be inhibited using different mechanisms [60]. The role of anticoagulants such as aspirin in the primary prevention of VTE has been evaluated in various clinical studies that associated this drug with a reduction of up to 50% in the risk to suffer VTE [61]. However, treatment with aspirin was also associated with an increased risk of bleeding. For this reason, investigations into the identification of novel antithrombotic compounds from different sources, including seaweed, are on-going. Antithrombotic peptides have been previously generated from milk proteins [60]. Up to the best of our knowledge, the peptide NMEKGSSSVVSSRMKQ, recently reported by Indumathi and Mehta [62], was the first antithrombotic peptide generated from seaweed. This peptide was generated by hydrolysis of *Porphyra yezoensis* proteins using pepsin. *In vitro* anticoagulation results showed a dose-dependent prolongation of activated partial thromboplastin time, a medical test that characterizes blood coagulation, and the authors suggested that the

peptide interacted with the clotting factors involved in the intrinsic pathway of coagulation. In addition, Indumathi and Mehta [62] recently isolated the anticoagulant peptide NMEKGSSSVVSSRM(+15.99)KQ from a Nori hydrolysate. Anticoagulation was measured by microtiter plate reader and was found to be non-cytotoxic and to have a similar order of activity as that of heparin.

Seaweed-derived bioactive peptides identified to date also include enzymatic inhibitors of the enzymes renin (EC 3.4.23.15) and angiotensin-I-converting enzyme (ACE-I; EC 3.4.15.1). Inhibition of renin, ACE-I, and other enzymes involved in the renin-angiotensin-aldosterone system (RAAS) plays a key role in the treatment of hypertension [9, 63]. These enzymes can be inhibited by the use of chemically synthesised drugs or the ingestion of functional foods. There are a number of bioactive peptide-containing functional foods which are commercially available worldwide. For example, the Japanese company Senmi Ekisu Co. Ltd. obtained generally recognized as safe (GRAS) status for its hydrolysed sardine muscle protein from the Food and Drug Administration (FDA) of the United States (US). This product, labelled as Valtyron®, is rich in the di-peptide VY with known ACE-I inhibitory and antihypertensive benefits [64]. In addition, the European Food Safety Authority (EFSA) issued a positive opinion regarding the safety of hydrolysed sardine muscle protein, for use as a novel food ingredient in 2010 [7]. Bioactive algae-derived hydrolysates and peptides with *in vitro* ACE-I inhibitory activity include enzymatic hydrolysates of *Spirulina platensis* (cyanobacteria) and *Polysiphonia urceolata*, which inhibited the activity of ACE-I by half at concentrations ranging between 0.1 and 1.0, depending on the enzymatic treatment [49], and the tetra-peptides AIYK, YKYY, KFYG, and YNKL, which were identified from a peptic digest of *Undaria pinnatifida* and showed ACE-I IC_{50} values of 213, 64, 90, and 21 μM, respectively [65].

3.2 Seaweed-derived bioactive carbohydrates

Macroalgal carbohydrates display a wide range of biological properties including antiinflammatory, immunostimulatory, antioxidant, anticoagulant, antiviral, antiproliferative, and antitumour properties [5, 13]. These biological activities could be attributed to the high content of sulphated polysaccharides of seaweed (i.e. carrageenans, and fucoidan), which cannot be found in terrestrial plants [13]. Furthermore, seaweed polysaccharides such as alginates, fucoidan, and laminarin, are non-digested in the upper digestive track of the animals, being macroalgae considered as a rich source of dietary fibre [5, 13]. Despite these beneficial properties, seaweed carbohydrates are being currently exploited industrially for their physicochemical properties, i.e. the ability of alginates and carrageenans to form gels, allowing their use as thickening, gelling, and protein-suspending agents [13, 38].

Alginates from brown macroalgae showed a wide potential for its application in the biomedical and bioengineering fields, due to its gelling capacity, biocompatibility, biodegradability and lack of toxicity [68]. Nutritionally, alginates are dietary fibres and contribute to the gut's health due to its water binding properties and thus its effects in faecal bulking, decreasing the colonic transit times which could be beneficial in preventing colonic cancer [69]. Furthermore, the Food and Drug Administration (FDA) named alginic acid and its salts as generally regarded as safe (GRAS) ingredients for oral administration [68].

Other macroalgal polysaccharides such as laminarin and fucoidan are also considered as dietary fibres [5]. Laminarin modulates the intestinal metabolism by affecting the biochemical and microbiology of the human gut microflora [70]. *In vivo* studies showed a down-regulation of pro- and antiinflammatory cytokines in post-weaning pigs

supplemented with laminarin [71]. Laminarin and fucoidan supplementation to sows during the periods of pregnancy and lactation showed an increase on immunoglobulin concentrations in the colostrum and a decrease of *E. coli* in suckling [72] and weaned piglets [73].

Further biological properties described for fucoidan with potential biomedical applications include the antioxidant and antiinflammatory activities, anticancer, anticoagulant and antithrombotic activities related to the structure and the degree of sulphation of the polysaccharide [74, 75]. Fucoidan from *Fucus evanescens* showed anticoagulant activities similar to other drugs such as heparin *in vitro* and *in vivo* [76].

Other sulphated polysaccharides such as carrageenans have also gathered scientific attention due to its biological properties including anti-inflammatory and immunomodulatory, but also antitumor, antihyperlipidemic, antiviral and anticoagulant properties using different *in vitro* and *in vivo* models [38]. Carrageenan gels from *Chondrus crispus* had antiviral properties against HIV virus and other sexual transmitted diseases such as herpes simplex virus [38]. As in the case of fucoidan, the main explanation for the anticoagulant activities of carrageenan appeared to be related with the degree of sulphation of the molecules with λ-carrageenan showing the highest antithrombotic activities followed by κ-carrageenan [18].

4. Analysis of bioactive peptides and carbohydrates

4.1 Purification of bioactive peptides and carbohydrates

For the determination of the sequence of a peptide and to perform individual activity assays, an effective purification of the target specie is necessary. Purification of peptides and obtaining them in sufficient quantities is a complex and expensive

process. Ultrafiltration membrane systems are fast and economic devices that are used to separate peptides with the desired MW by choosing the correct molecular weight cut-off (MWCO). Several studies enriched enzymatic hydrolysates and peptides derived from not only seaweed but other food sources using MWCO membranes. For example, Bondu, Bonnet, Gaubert, Deslandes, Turgeon and Beaulieu [77] utilized 1 and 10 kDa MWCO membranes for the purification of proteins and peptides derived from several macroalgae varieties including *Ulva lactuca*, *Solieria chordalis*, *Palmaria palmata*, and *Saccharina longicruris*. A similar strategy was followed by Beaulieu, Sirois and Tamigneaux [78] to purify peptides from *Palmaria palmata*. However, membranes have been reported to be poorly reproducible and could remove peptides below the desired MW [79]. Interactions between the peptide and the membrane can occur. The most hydrophobic peptides can adsorb on the membrane and be depleted from the sample [80]. Therefore, the separation and purification of not only peptides but also polysaccharides from seaweed for later identification is commonly achieved using one or multiple chromatographic techniques which have the advantage that can be used for qualitative and quantitative analysis as well as for preparative purposes.

Liquid chromatography (LC) has also been used extensively for the purification of bioactive peptides include mainly RP-HPLC, using columns packed with silica particles modified with C-18 chains [9, 54, 66, 67, 81]. Several lengths (50-400 mm), inner diameters (1.4-2.6 mm), and particle sizes (1.7-5.0 µm) were utilized in order to achieve a suitable resolution between the target compounds. In addition, in relation to mobile phases used, mixtures of formic acid in water and formic acid in acetonitrile are the most commonly used (table 1). UPLC applies the principles of separation of HPLC. UPLC uses low particle size columns (less than 2 µm in diameter) and UPLC

instrumentation (solvent and sample manager units) to operate at higher pressures, increasing the resolution, sensitivity, and speed of the analysis [82]. UPLC has been used recently not only for the purification of bioactive compounds but also to characterize the amino acid composition of 21 seaweed species from Norway [83]. LC is not regularly used for the purification of polar compounds such as carbohydrates. However, this separation technique could be used to analyse the monosaccharide composition of carbohydrates. Recently Liu, Wang, Song, He, Ren, Cong and Wu [84] analysed the monosaccharide composition of fucoidan extracts using a C-18 column (ZORBAX Eclipse XDB, 5 μm, 4.6 mm × 250 mm) following the acidic hydrolysis and derivatization of the compounds using 1-phenyl-3-methyl-5-pyrazolone (PMP) for its detection with UV at 250 nm. UPLC systems were also used to purify ad characterize multiple carbohydrates in products of plant [85, 86] and animal origin [87, 88]. Recently, Adrien, Dufour, Baudouin, Maugard and Bridiau [89] used UPLC coupled with MS to analyse the monosaccharide composition of seaweed extracts from *Himanthalia elongata*, *Laminaria digitata*, *Ascophyllum nodosum*, *Fucus vesiculosus*, *Ulva lactuca*, and *Chondrus crispus* with anticoagulant properties.

Nevertheless, the direct application of one LC method does not allow the detection of the investigated compounds, especially if they are present in the mixture in low quantities. Therefore, multidimensional approaches are generally used. Ion exchange chromatography (IEC) is one of the classic methods among multidimensional approaches. IEC achieves the separation of peptides and carbohydrates based on the adsorption of the charged analytes on to immobilized ion exchange groups of an opposite charge in the stationary phase of the column and its elution by changing the concentration or pH of the mobile phase [5]. Carbohydrates from seaweed are normally separated using IEC containing positively charged resins as stationary phase

with affinity for negatively charged analytes (anion-exchange chromatography or AEC). AEC is one of the most commonly used purification techniques used to separate alginates and sulphated polysaccharides such as fucoidan [5] and carrageenan [38]. The sulphate ester groups linked to the backbone of these seaweed polysaccharides exhibit high anionic charges, which facilitate the use of AEC following different experimental conditions described in detail in table 2. The elution of the adsorbed carbohydrates from different resins was performed mainly by stepwise [90] or linear gradient [91-93] of NaCl or NaOH to achieved multiple polysaccharide fractions. In addition, IEC purification of fucoidan extracts followed by ESI-MS analysis was used to determine the monosaccharide composition of the different fucoidan fractions obtained from *Sargassum fusiforme* [90], while IEC followed by ESI-MS/MS was used to characterize fucoidan carbohydrates with anticancer activity from *Padina boryana* [94]. ESI-MS/MS was used to characterize the monosaccharide units of immunomodulatory and anti-HIV fucoidan fractions from *Sargassum mcclurei*, *Sargassum polycystum* and *Turbinara ornate* [95] and from *Sargassum crassifolium* and *Padina australis* [96], respectively.

SEC is also a common stem in multidimensional fractionation of bioactive peptides. Today, SEC is the most widely accepted and used analytical method for the measurement of MW distributions of biopolymers [97]. Briefly, SEC is a liquid chromatographic technique in which a sample solution is introduced into a column filled with porous packing, often silica-based, carried through the column by a solvent, and separated by differential pore permeation [97]. Unlike IEC, polymers do not bind to the inert material of the columns and thus the composition of the buffer has no effect on the separation of the compounds, enabling to use the type of solvent that better suits the preservation of the compounds of interest for further use or purification and

chemical characterization [5]. SEC can provide a rapid estimate of the MW of a peptide, obtained under denaturing conditions, when all polypeptides have the same random coil structure [98]. Small peptides with just a few amino acid residues in length do not usually adopt stable structures in solution. However, in rare occasions small peptides were shown to form stable secondary structures. For example, the fragment Aβ (25-35) of the Alzheimer β-amyloid peptide was demonstrated to undergo from a random coil to a β-sheet structure previously [99]. Peptide bonds absorb UV light with a maximum around 187 nm. However, because of interference from buffer components, wavelengths ranging between 210 and 220 nm are the most frequently used [98]. SEC has been used for the determination of the MW distributions of multiple seaweed carbohydrates as seen in table 2. The separation of different MW alginates and fucoidans was achieved by the use of a single column or various columns connected successively, being the elution of the different compounds monitored by refractive index detector (RI) [90, 93, 94, 100] or evaporative light-scattering detector (ELSD) [101]. Both IR and ELSD are commonly used to detect compounds that cannot be efficiently detected with UV spectra. Recent innovations in the purification of carbohydrates include the separation of polysaccharides such as dextran, fucoidan, alginic acid, and laminarin using ionic liquid-modified silica rather than the conventional SEC silica [102].

4.2 Sequence validation

The identification of the sequence responsible of the observed bioactivities has been largely neglected or was performed to identify the most probable sequence. Edman degradation was the first approach used for peptide identification [80]. However, due to their accuracy and sensitivity, chromatographic techniques, especially LC-MS/MS

methods have become more popular over the last decade and are considered as a reference for bioactive compound analysis (Table 1). Other technologies such as nuclear magnetic resonance (NMR) have been also studied. MS is an analytical technique that can provide useful data on the structure and concentration of the analytes of interest in a sample after their conversion to ions [96]. In MS the analytes are ionised using high-energy electrons, causing a molecular fragmentation of the analytes that pass through a mass-to-charge analyser and are detected as a function of mass-to-time or mass-to-charge ratio (m/z) [103]. In the last decade, MS has become increasingly important for analysis of peptides and carbohydrates due to its high sensitivity, accuracy, and fast processing times compared to NMR and other detectors [38]. MS techniques commonly used to analyse macroalgal peptides and carbohydrates include electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) which differ on the strategy followed to ionize the analytes. ESI uses electrical energy to transfer ions from an initial solution into a gas stage prior to its detection with MS. This ionization into gas requires the dispersion of charged droplets, followed by the evaporation of the solvent and ejection of ions from the highly charged droplets [104]. ESI-MS is a sensitive, robust and reliable method capable to detect femtomole quantities of multiple compounds, including non-volatile and thermolabile analytes [104]. ESI-MS coupled with LC for molecular fractionation prior to MS analysis (LC/ESI-MS) or to MS in tandem (LC/ESI-MS/MS) are powerful methods recently used to analyse peptides and carbohydrates in a complex biological samples, including seaweed extracts [90, 94]. The conversion of the analytes to a gaseous phase performed during ESI could be difficult to achieve when analysing complex proteins and carbohydrates due to their high polarity and mass [99]. In MALDI, the analytes are normally mixed with a matrix to form a solid-state mixture that

will be later irradiated with UV laser pulses (337-355 nm), generating ions analysed based on its mass-to-charge ratio by MS. Choosing the appropriate matrix for an analyte is crucial for successful MALDI analysis [105, 106].

MALDI continues to be a major technique for the analysis of carbohydrates [105]. MALDI-TOF mass spectra was used to analyse the structure of antiinflammatory κ/β-carrageenans from red algae *Tichocarpus crinitus* [107]. Furthermore, MALDI was recently used to analyse fucoidan fractions with multiple biological properties from seaweed, including *Saccharina cichorioides* [108], *Saccharina gurjanovae* [109], *Sargassum muticum* [110] and *Alaria angusta* [111].

NMR spectroscopy is one of the most powerful analytical tools available to date to determine the structure of bioactive compounds [38, 112, 113]. NMR is based on the principle of nuclear spin or the fact that when a molecule is placed in a magnetic field, this momentum will be aligned either in the same or opposite direction to the field, resulting in 2 states separated by an energy gap and thus, in resonance frequency [112]. This difference in resonance frequency will depend on the chemical environment of the nucleus in a molecule and on the on the magnetic field strength in an effect known as chemical shift [112]. Most elements have at least one naturally occurring NMR active isotope with variable frequency of occurrence i.e. the natural abundance of ^{1}H is almost 100 % being easier to detect by NMR compared to ^{13}C and ^{15}N isotopes that appeared naturally in 1.1 and 0.4 % respectively [119]. For characterization of carbohydrates, the resonances of ^{1}H NMR in the anomeric region (4.4-5.5 ppm) and the ^{13}C NMR spectra (95-110 ppm) provide useful information on the number of monosaccharide units of the carbohydrates [112]. ^{1}H NMR resonances bellow 1 ppm indicate the presence of CH_3-groups and above 2 ppm reveal N-acetyl and/or O-acetyl

groups [112]. NMR have been used extensively to characterize the structure of seaweed' carbohydrates. Carrageenan can be qualitatively and quantitatively characterized by NMR techniques, allowing the determination of molar rations and the presence monosaccharides present in the extracts. Both ^{1}H and ^{13}C NMR spectroscopy are applicable for these purposes, but ^{1}H NMR has the advantage of a relatively high sensitivity and lower time of analysis [38, 112]. ^{1}H NMR was also used to identify and characterize the structural units of alginates (MM, GG, MG or GM blocks) from multiple seaweed species [34, 93]. ^{1}H NMR and ^{13}C NMR spectra together with 2D NMR was also used to characterize a fucoidan fraction with antiangiogenic activity from macroalgae *Sargassum fusiforme* [90]. ^{13}C NMR analysis was also performed to characterize fucoidan fractions with hepatoprotective activities from *Kjellmaniella crassifolia* [84]. NMR spectra using ^{1}H and ^{13}C amongst other techniques were also used by Youssouf, Lallemand, Giraud, Soulé, Bhaw-Luximon, Meilhac, D'Hellencourt, Jhurry and Couprie [114] alginates extracted from *Sargassum binderi* and *Turbinaria ornata* and carrageenans from the red macroalgae *Kappaphycus alvarezii* and *Euchema denticulatum*. NMR was recently used for the characterization of a glycoprotein with 36.24% carbohydrate, composed of rhamnose, galactose, glucose, and mannose with a mole ratio of 38:30:26:6 [115]. NMR spectra proved that the above mentioned glycoprotein, isolated from the green seaweed *Codium decorticum*, contained protein and carbohydrate portions with (1 → 4)-linked β-galactose residues and β-linked glucose residues. Senthilkumar and Jayanthi [115] demonstrated the anticancer properties of this compound by different cell lines.

5. Conclusions

Seaweed are relative un-explored and promising of novel molecules for its use as functional foods and nutraceuticals including bioactive peptides and carbohydrates. Several seaweed derived bioactive compounds have shown a wide range of biological activities both *in vitro* and *in vivo*. Amongst the biological activities reported by bioactive peptides and polysaccharides the most promising include antihypertensive, antioxidant, anticoagulant, antitumour, anti-inflammatory, and immunostimulatory activities. These biological activities have increased the scientific interest in developing functional ingredients and to study the relationship between the chemical structure of the compounds and the biological activities. Multiple analytical approaches have been used to date to purify and analyse the chemical structure of bioactive peptides and carbohydrates. Recent improvements in the purification and characterization methodologies gradually increased the use of novel chromatographic techniques i.e. ultra-performance liquid chromatography (UPLC) and analytical techniques such as NMR spectroscopy (^{1}H and ^{13}C NMR) for qualitative and quantitative analysis and mass spectrometry (ESI and MALDI) to gain further insight into the complexity of different molecular structures of bioactive peptides and carbohydrates.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

Conflict of Interest Statement

The authors report no commercial or proprietary interest in any product or concept discussed in this article.

References

[1] M. Garcia-Vaquero, M. Hayes, Red and green macroalgae for fish and animal feed and human functional food development, Food Reviews International, 32 (2016) 15-45.

[2] M.L. Ragavan, N. Patnaik, R. Muniyasamy, A. Roy, L. Deo, N. Das, Biochemical Characterization and Enzymatic profiling of Potential Probiotic Yeast Strains, Research Journal of Pharmacy and Technology, 12 (2019) 3941-3944.

[3] G. Rajauria, Optimization and validation of reverse phase HPLC method for qualitative and quantitative assessment of polyphenols in seaweed, Journal of pharmaceutical and biomedical analysis, 148 (2018) 230-237.

[4] M. Garcia-Vaquero, M. Lopez-Alonso, M. Hayes, Assessment of the functional properties of protein extracted from the brown seaweed Himanthalia elongata (Linnaeus) SF Gray, Food Research International, 99 (2017) 971-978.

[5] M. Garcia-Vaquero, G. Rajauria, J.V. O'Doherty, T. Sweeney, Polysaccharides from macroalgae: Recent advances, innovative technologies and challenges in extraction and purification, Food Research International, 99 (2017) 1011-1020.

[6] S. Bleakley, M. Hayes, Algal proteins: extraction, application, and challenges concerning production, Foods, 6 (2017) 33.

[7] C. Fitzgerald, R.E. Aluko, M. Hossain, D.K. Rai, M. Hayes, Potential of a renin inhibitory peptide from the red seaweed Palmaria palmata as a functional food ingredient following confirmation and characterization of a hypotensive effect in spontaneously hypertensive rats, Journal of agricultural and food chemistry, 62 (2014) 8352-8356.

[8] C. Fitzgerald, E. Gallagher, D. Tasdemir, M. Hayes, Heart health peptides from macroalgae and their potential use in functional foods, Journal of agricultural and food chemistry, 59 (2011) 6829-6836.

[9] M. Garcia-Vaquero, L. Mora, M. Hayes, In Vitro and In Silico Approaches to Generating and Identifying Angiotensin-Converting Enzyme I Inhibitory Peptides from Green Macroalga Ulva lactuca, Marine Drugs, 17 (2019) 204.

[10] T. Lafarga, M. Hayes, Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients, Meat science, 98 (2014) 227-239.

[11] S. Kraan, Algal polysaccharides, novel applications and outlook, Carbohydrates-comprehensive studies on glycobiology and glycotechnology, InTech2012.

[12] S. Kadam, P. Prabhasankar, Marine foods as functional ingredients in bakery and pasta products, Food Research International, 43 (2010) 1975-1980.

[13] M. Hayes, B. Tiwari, Bioactive Carbohydrates and Peptides in Foods: An Overview of Sources, Downstream Processing Steps and Associated Bioactivities, International Journal of Molecular Sciences, 16 (2015) 22485-22508.

[14] A.H. Pripp, T. Isaksson, L. Stepaniak, T. Sørhaug, Quantitative structure-activity relationship modelling of ACE-inhibitory peptides derived from milk proteins, European Food Research and Technology, 219 (2004) 579-583.

[15] T. Lafarga, R.E. Aluko, D.K. Rai, P. O'Connor, M. Hayes, Identification of bioactive peptides from a papain hydrolysate of bovine serum albumin and assessment of an antihypertensive effect in spontaneously hypertensive rats, Food research international, 81 (2016) 91-99.

[16] H.P. Makkar, G. Tran, V. Heuzé, S. Giger-Reverdin, M. Lessire, F. Lebas, P. Ankers, Seaweeds for livestock diets: a review, Animal Feed Science and Technology, 212 (2016) 1-17.

[17] J. Yoshinaga, M. Morita, M. Yukawa, K. Shiraishi, H. Kawamura, Certified reference material for analytical quality assurance of minor and trace elements in food and related matrixes based on a typical Japanese diet: interlaboratory study, Journal of AOAC International, 84 (2001) 1202-1208.

[18] S.L. Holdt, S. Kraan, Bioactive compounds in seaweed: functional food applications and legislation, J Appl Phycol, 23 (2011) 543-597.

[19] S.O. Lourenço, E. Barbarino, J.C. De-Paula, L.O.d.S. Pereira, U.M.L. Marquez, Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds, Phycological Research, 50 (2002) 233-241.

[20] P. Schiener, K.D. Black, M.S. Stanley, D.H. Green, The seasonal variation in the chemical composition of the kelp species Laminaria digitata, Laminaria hyperborea, Saccharina latissima and Alaria esculenta, J Appl Phycol, 27 (2015) 363-373.

[21] I. Peinado, J. Girón, G. Koutsidis, J. Ames, Chemical composition, antioxidant activity and sensory evaluation of five different species of brown edible seaweeds, Food research international, 66 (2014) 36-44.

[22] J. Fleurence, Seaweed proteins: biochemical, nutritional aspects and potential uses, Trends in Food Science & Technology, 10 (1999) 25-28.

[23] H.K. Mæhre, M.K. Malde, K.E. Eilertsen, E.O. Elvevoll, Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and

evaluation of their potential as food and feed, Journal of the Science of Food and Agriculture, 94 (2014) 3281-3290.

[24] A.-V. Galland-Irmouli, J. Fleurence, R. Lamghari, M. Luçon, C. Rouxel, O. Barbaroux, J.-P. Bronowicki, C. Villaume, J.-L. Guéant, Nutritional value of proteins from edible seaweed Palmaria palmata (Dulse), The Journal of nutritional biochemistry, 10 (1999) 353-359.

[25] A.J. Smit, Medicinal and pharmaceutical uses of seaweed natural products: a review, J Appl Phycol, 16 (2004) 245-262.

[26] K.H. Cardozo, T. Guaratini, M.P. Barros, V.R. Falcão, A.P. Tonon, N.P. Lopes, S. Campos, M.A. Torres, A.O. Souza, P. Colepicolo, Metabolites from algae with economical impact, Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 146 (2007) 60-78.

[27] R. Hamid, A. Masood, I.H. Wani, S. Rafiq, Lectins: proteins with diverse applications, (2013).

[28] C. Juin, J.-R. Chérouvrier, V. Thiéry, A.-L. Gagez, J.-B. Bérard, N. Joguet, R. Kaas, J.-P. Cadoret, L. Picot, Microwave-assisted extraction of phycobiliproteins from Porphyridium purpureum, Applied biochemistry and biotechnology, 175 (2015) 1-15.

[29] I.-M. Munda, Differences in amino acid composition of estuarine and marine fucoids, Aquatic Botany, 3 (1977) 273-280.

[30] T. Fujiwara-Arasaki, N. Mino, M. Kuroda, The protein value in human nutrition of edible marine algae in Japan, Hydrobiologia, 116 (1984) 513-516.

[31] K. Wong, P.C. Cheung, Nutritional evaluation of some subtropical red and green seaweeds: Part I—proximate composition, amino acid profiles and some physico-chemical properties, Food chemistry, 71 (2000) 475-482.

[32] J. Fleurence, Seaweed proteins, Proteins in food processing. Woodhead publishing, Cambridge, (2004) 197-213.

[33] R. Qasim, Amino acid composition of some common seaweeds, Pakistan journal of pharmaceutical sciences, 4 (1991) 49-54.

[34] C. Peteiro, Alginate Production from Marine Macroalgae, with Emphasis on Kelp Farming, in: B.H.A. Rehm, M.F. Moradali (Eds.) Alginates and Their Biomedical Applications, Springer Singapore, Singapore, 2018, pp. 27-66.

[35] A.J. Lorbeer, S. Charoensiddhi, J. Lahnstein, C. Lars, C.M. Franco, V. Bulone, W. Zhang, Sequential extraction and characterization of fucoidans and alginates from Ecklonia radiata, Macrocystis pyrifera, Durvillaea potatorum, and Seirococcus axillaris, J Appl Phycol, 29 (2017) 1515-1526.

[36] R. Westermeier, P. Murúa, D.J. Patiño, L. Muñoz, A. Ruiz, D.G. Müller, Variations of chemical composition and energy content in natural and genetically defined cultivars of Macrocystis from Chile, J Appl Phycol, 24 (2012) 1191-1201.

[37] S. Knutsen, D. Myslabodski, B. Larsen, A. Usov, A modified system of nomenclature for red algal galactans, Botanica marina, 37 (1994) 163-170.

[38] V.L. Campo, D.F. Kawano, D.B.d. Silva, I. Carvalho, Carrageenans: Biological properties, chemical modifications and structural analysis – A review, Carbohydrate Polymers, 77 (2009) 167-180.

[39] L.E. Rioux, S.L. Turgeon, M. Beaulieu, Characterization of polysaccharides extracted from brown seaweeds, Carbohydrate Polymers, 69 (2007) 530-537.

[40] M. Honya, H. Mori, M. Anzai, Y. Araki, K. Nisizawa, Monthly changes in the content of fucans, their constituent sugars and sulphate in cultured Laminaria japonica, Sixteenth International Seaweed Symposium, Springer, 1999, pp. 411-416.

[41] L. Mora, M. Reig, F. Toldrá, Bioactive peptides generated from meat industry by-products, Food Research International, 65 (2014) 344-349.

[42] J. Wu, F. Jahandideh, W. Yu, K. Majumder, Bioactive Peptides from Meat Proteins as Functional Food Components, Functional Polymers in Food Science: From Technology to Biology, (2015) 181-208.

[43] V. Raikos, T. Dassios, Health-promoting properties of bioactive peptides derived from milk proteins in infant food: a review, Dairy science & technology, 94 (2014) 91-101.

[44] I. Ricci-Cabello, M. Olalla Herrera, R. Artacho, Possible role of milk-derived bioactive peptides in the treatment and prevention of metabolic syndrome, Nutrition reviews, 70 (2012) 241-255.

[45] L. López-Barrios, J.A. Gutiérrez-Uribe, S.O. Serna-Saldívar, Bioactive peptides and hydrolysates from pulses and their potential use as functional ingredients, Journal of food science, 79 (2014).

[46] M. Malaguti, G. Dinelli, E. Leoncini, V. Bregola, S. Bosi, A.F. Cicero, S. Hrelia, Bioactive peptides in cereals and legumes: agronomical, biochemical and clinical aspects, International journal of molecular sciences, 15 (2014) 21120-21135.

[47] S.S. Khora, Marine fish-derived bioactive peptides and proteins for human therapeutics, Int. J. Pharm. Pharm. Sci, 5 (2013) 31-37.

[48] L. Mora, M. Hayes, Cardioprotective cryptides derived from fish and other food sources: Generation, application, and future markets, Journal of agricultural and food chemistry, 63 (2015) 1319-1331.

[49] H.L. He, X.L. Chen, H. Wu, C.Y. Sun, Y.Z. Zhang, B.C. Zhou, High throughput and rapid screening of marine protein hydrolysates enriched in

peptides with angiotensin-I-converting enzyme inhibitory activity by capillary electrophoresis, Bioresour Technol, 98 (2007) 3499-3505.

[50] S. Arai, Functional food science in Japan: state of the art, Biofactors, 12 (2000) 13-16.

[51] H. Fukami, Functional foods and biotechnology in Japan, Biotechnology in functional foods and nutraceuticals, (2010) 29.

[52] L.A. Pham-Huy, H. He, C. Pham-Huy, Free radicals, antioxidants in disease and health, International journal of biomedical science: IJBS, 4 (2008) 89.

[53] C. Gorrini, I.S. Harris, T.W. Mak, Modulation of oxidative stress as an anticancer strategy, Nature reviews Drug discovery, 12 (2013) 931.

[54] P.A. Harnedy, R.J. FitzGerald, Bioactive proteins, peptides, and amino acids from macroalgae, Journal of Phycology, 47 (2011) 218-232.

[55] S.-J. Heo, E.-J. Park, K.-W. Lee, Y.-J. Jeon, Antioxidant activities of enzymatic extracts from brown seaweeds, Bioresource Technology, 96 (2005) 1613-1623.

[56] R.E. Cian, O. Martínez-Augustin, S.R. Drago, Bioactive properties of peptides obtained by enzymatic hydrolysis from protein byproducts of Porphyra columbina, Food Research International, 49 (2012) 364-372.

[57] R.E. Cian, A.G. Garzón, D.B. Ancona, L.C. Guerrero, S.R. Drago, Hydrolyzates from Pyropia columbina seaweed have antiplatelet aggregation, antioxidant and ACE I inhibitory peptides which maintain bioactivity after simulated gastrointestinal digestion, LWT-Food Science and Technology, 64 (2015) 881-888.

[58] T. Fulop, D. Tessier, A. Carpentier, The metabolic syndrome, Pathologie Biologie, 54 (2006) 375-386.

[59] M. Franchini, P.M. Mannucci, A new era for anticoagulants, European journal of internal medicine, 20 (2009) 562-568.

[60] R. Rojas-Ronquillo, A. Cruz-Guerrero, A. Flores-Nájera, G. Rodríguez-Serrano, L. Gómez-Ruiz, J.P. Reyes-Grajeda, J. Jiménez-Guzmán, M. García-Garibay, Antithrombotic and angiotensin-converting enzyme inhibitory properties of peptides released from bovine casein by Lactobacillus casei Shirota, International Dairy Journal, 26 (2012) 147-154.

[61] C. Becattini, G. Agnelli, A. Schenone, S. Eichinger, E. Bucherini, M. Silingardi, M. Bianchi, M. Moia, W. Ageno, M.R. Vandelli, Aspirin for preventing the recurrence of venous thromboembolism, New England Journal of Medicine, 366 (2012) 1959-1967.

[62] P. Indumathi, A. Mehta, A novel anticoagulant peptide from the Nori hydrolysate, Journal of Functional Foods, 20 (2016) 606-617.

[63] K. Mercier, H. Smith, J. Biederman, Renin-angiotensin-aldosterone system inhibition: overview of the therapeutic use of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, mineralocorticoid receptor antagonists, and direct renin inhibitors, Primary Care: Clinics in Office Practice, 41 (2014) 765-778.

[64] T. Kawasaki, E. Seki, K. Osajima, M. Yoshida, K. Asada, T. Matsui, Y. Osajima, Antihypertensive effect of valyl-tyrosine, a short chain peptide derived from sardine muscle hydrolyzate, on mild hypertensive subjects, Journal of human hypertension, 14 (2000) 519.

[65] K. Suetsuna, T. Nakano, Identification of an antihypertensive peptide from peptic digest of wakame (Undaria pinnatifida), The Journal of nutritional biochemistry, 11 (2000) 450-454.

[66] P.A. Harnedy, M.B. O'Keeffe, R.J. FitzGerald, Purification and identification of dipeptidyl peptidase (DPP) IV inhibitory peptides from the macroalga Palmaria palmata, Food chemistry, 172 (2015) 400-406.

[67] C. Fitzgerald, E. Gallagher, P. O'Connor, J. Prieto, L. Mora-Soler, M. Grealy, M. Hayes, Development of a seaweed derived platelet activating factor acetylhydrolase (PAF-AH) inhibitory hydrolysate, synthesis of inhibitory peptides and assessment of their toxicity using the zebrafish larvae assay, Peptides, 50 (2013) 119-124.

[68] M. Szekalska, A. Puciłowska, E. Szymańska, P. Ciosek, K. Winnicka, Alginate: Current use and future perspectives in pharmaceutical and biomedical applications, International Journal of Polymer Science, 2016 (2016).

[69] P. MacArtain, C.I. Gill, M. Brooks, R. Campbell, I.R. Rowland, Nutritional value of edible seaweeds, Nutrition reviews, 65 (2007) 535-543.

[70] C. Devillé, M. Gharbi, G. Dandrifosse, O. Peulen, Study on the effects of laminarin, a polysaccharide from seaweed, on gut characteristics, Journal of the Science of Food and Agriculture, 87 (2007) 1717-1725.

[71] A. Walsh, T. Sweeney, C. O'Shea, D. Doyle, J. O'Doherty, Effect of dietary laminarin and fucoidan on selected microbiota, intestinal morphology and immune status of the newly weaned pig, The British journal of nutrition, 110 (2013) 1630-1638.

[72] S. Leonard, T. Sweeney, B. Bahar, J. O'Doherty, Effect of maternal seaweed extract supplementation on suckling piglet growth, humoral immunity, selected microflora, and immune response after an ex vivo lipopolysaccharide challenge, Journal of animal science, 90 (2012) 505-514.

[73] S. Leonard, T. Sweeney, B. Bahar, B. Lynch, J. O'Doherty, Effect of dietary seaweed extracts and fish oil supplementation in sows on performance, intestinal microflora, intestinal morphology, volatile fatty acid concentrations and immune status of weaned pigs, British journal of nutrition, 105 (2011) 549-560.

[74] E. Deniaud-Bouët, K. Hardouin, P. Potin, B. Kloareg, C. Hervé, A review about brown algal cell walls and fucose-containing sulfated polysaccharides: Cell wall context, biomedical properties and key research challenges, Carbohydrate polymers, 175 (2017) 395-408.

[75] W. Wijesinghe, Y. Jeon, Biological activities and potential industrial applications of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: A review, Carbohydrate Polymers, 88 (2012) 13-20.

[76] T. Kuznetsova, N. Besednova, A. Mamaev, A. Momot, N. Shevchenko, T. Zvyagintseva, Anticoagulant Activity of Fucoidan from Brown Algae Fucus evanescens of the Okhotsk Sea, Bulletin of Experimental Biology and Medicine, 136 (2003) 471-473.

[77] S. Bondu, C. Bonnet, J. Gaubert, É. Deslandes, S.L. Turgeon, L. Beaulieu, Bioassay-guided fractionation approach for determination of protein precursors of proteolytic bioactive metabolites from macroalgae, J Appl Phycol, 27 (2015) 2059-2074.

[78] L. Beaulieu, M. Sirois, É. Tamigneaux, Evaluation of the in vitro biological activity of protein hydrolysates of the edible red alga, Palmaria palmata (dulse) harvested from the Gaspe coast and cultivated in tanks, J Appl Phycol, 28 (2016) 3101-3115.

[79] A.L. Capriotti, C. Cavaliere, S. Piovesana, R. Samperi, A. Laganà, Recent trends in the analysis of bioactive peptides in milk and dairy products, Analytical and bioanalytical chemistry, 408 (2016) 2677-2685.

[80] S. Piovesana, A.L. Capriotti, C. Cavaliere, G. La Barbera, C.M. Montone, R.Z. Chiozzi, A. Laganà, Recent trends and analytical challenges in plant bioactive peptide separation, identification and validation, Analytical and bioanalytical chemistry, 410 (2018) 3425-3444.

[81] C.n. Fitzgerald, L. Mora-Soler, E. Gallagher, P. O'Connor, J. Prieto, A. Soler-Vila, M. Hayes, Isolation and characterization of bioactive pro-peptides with in vitro renin inhibitory activities from the macroalga Palmaria palmata, Journal of agricultural and food chemistry, 60 (2012) 7421-7427.

[82] M.E. Swartz, UPLC™: an introduction and review, Journal of Liquid Chromatography & Related Technologies, 28 (2005) 1253-1263.

[83] I. Biancarosa, M. Espe, C. Bruckner, S. Heesch, N. Liland, R. Waagbø, B. Torstensen, E. Lock, Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters, J Appl Phycol, 29 (2017) 1001-1009.

[84] S. Liu, Q. Wang, Y. Song, Y. He, D. Ren, H. Cong, L. Wu, Studies on the hepatoprotective effect of fucoidans from brown algae Kjellmaniella crassifolia, Carbohydrate Polymers, (2018).

[85] T. Cai, H. Ting, Z. Jin-lan, Novel identification strategy for ground coffee adulteration based on UPLC–HRMS oligosaccharide profiling, Food chemistry, 190 (2016) 1046-1049.

[86] N. Dartora, L.M. De Souza, A.P. Santana-Filho, M. Iacomini, A.T. Valduga, P.A. Gorin, G.L. Sassaki, UPLC-PDA–MS evaluation of bioactive compounds

from leaves of Ilex paraguariensis with different growth conditions, treatments and ageing, Food Chemistry, 129 (2011) 1453-1461.

[87] G. Fusch, A. Choi, N. Rochow, C. Fusch, Quantification of lactose content in human and cow's milk using UPLC–tandem mass spectrometry, Journal of Chromatography B, 879 (2011) 3759-3762.

[88] H.-H. Ri, Y.-A. Choe, J.-H. Yun, K.-R. Sin, Determination of carbohydrates in infant milk powders by ultra-performance liquid chromatography with evaporative light scattering detector and BEH HILIC column, arXiv preprint arXiv:1711.07473, (2017).

[89] A. Adrien, D. Dufour, S. Baudouin, T. Maugard, N. Bridiau, Evaluation of the anticoagulant potential of polysaccharide-rich fractions extracted from macroalgae, Natural product research, 31 (2017) 2126-2136.

[90] Q. Cong, H. Chen, W. Liao, F. Xiao, P. Wang, Y. Qin, Q. Dong, K. Ding, Structural characterization and effect on anti-angiogenic activity of a fucoidan from Sargassum fusiforme, Carbohydrate Polymers, 136 (2016) 899-907.

[91] T. Imbs, S. Ermakova, O. Malyarenko, V. Isakov, T. Zvyagintseva, Structural elucidation of polysaccharide fractions from the brown alga Coccophora langsdorfii and in vitro investigation of their anticancer activity, Carbohydrate Polymers, 135 (2016) 162-168.

[92] A. Ramu Ganesan, M. Shanmugam, R. Bhat, Producing novel edible films from semi refined carrageenan (SRC) and ulvan polysaccharides for potential food applications, International Journal of Biological Macromolecules, 112 (2018) 1164-1170.

[93] M. Sterner, M.S. Ribeiro, F. Gröndahl, U. Edlund, Cyclic fractionation process for Saccharina latissima using aqueous chelator and ion exchange resin, J Appl Phycol, 29 (2017) 3175-3189.

[94] R.V. Usoltseva, S.D. Anastyuk, I.A. Ishina, V.V. Isakov, T.N. Zvyagintseva, P.D. Thinh, P.A. Zadorozhny, P.S. Dmitrenok, S.P. Ermakova, Structural characteristics and anticancer activity in vitro of fucoidan from brown alga Padina boryana, Carbohydrate Polymers, 184 (2018) 260-268.

[95] T.T.T. Thuy, B.M. Ly, T.T.T. Van, N. Van Quang, H.C. Tu, Y. Zheng, C. Seguin-Devaux, B. Mi, U. Ai, Anti-HIV activity of fucoidans from three brown seaweed species, Carbohydrate Polymers, 115 (2015) 122-128.

[96] Y. Yuguchi, V.T.T. Tran, L.M. Bui, S. Takebe, S. Suzuki, N. Nakajima, S. Kitamura, T.T.T. Thanh, Primary structure, conformation in aqueous solution, and intestinal immunomodulating activity of fucoidan from two brown seaweed species Sargassum crassifolium and Padina australis, Carbohydrate Polymers, 147 (2016) 69-78.

[97] L.K. Kostanski, D.M. Keller, A.E. Hamielec, Size-exclusion chromatography—a review of calibration methodologies, Journal of biochemical and biophysical methods, 58 (2004) 159-186.

[98] G.B. Irvine, High-performance size-exclusion chromatography of peptides, Journal of biochemical and biophysical methods, 56 (2003) 233-242.

[99] E. Terzi, G. Hoelzemann, J. Seelig, Alzheimer. beta.-Amyloid Peptide 25-35: Electrostatic Interactions with Phospholipid Membranes, Biochemistry, 33 (1994) 7434-7441.

[100] N. Rhein-Knudsen, M.T. Ale, F. Ajalloueian, A.S. Meyer, Characterization of alginates from Ghanaian brown seaweeds: Sargassum spp. and Padina spp, Food Hydrocolloids, 71 (2017) 236-244.

[101] P.S. Saravana, Y.-J. Cho, Y.-B. Park, H.-C. Woo, B.-S. Chun, Structural, antioxidant, and emulsifying activities of fucoidan from Saccharina japonica using pressurized liquid extraction, Carbohydrate Polymers, 153 (2016) 518-525.

[102] Y.R. Lee, K.H. Row, Ionic liquid-modified mesoporous silica stationary phase for separation of polysaccharides with size exclusion chromatography, Separation and Purification Technology, 196 (2018) 183-190.

[103] M. Vitha, Chromatography: Principles and Instrumentation, Wiley2016.

[104] C.S. Ho, C. Lam, M. Chan, R. Cheung, L. Law, L. Lit, K. Ng, M. Suen, H. Tai, Electrospray ionisation mass spectrometry: principles and clinical applications, The Clinical Biochemist Reviews, 24 (2003) 3.

[105] D.J. Harvey, Analysis of carbohydrates and glycoconjugates by matrix-assisted laser desorption/ionization mass spectrometry: An update for 2011–2012, Mass spectrometry reviews, 36 (2017) 255-422.

[106] I.-C. Lu, C. Lee, Y.-T. Lee, C.-K. Ni, Ionization mechanism of matrix-assisted laser desorption/ionization, Annual Review of Analytical Chemistry, 8 (2015) 21-39.

[107] A.A. Kalitnik, S.D. Anastyuk, E.V. Sokolova, A.O. Kravchenko, E.I. Khasina, I.M. Yermak, Oligosaccharides of κ/β-carrageenan from the red alga Tichocarpus crinitus and their ability to induce interleukin 10, J Appl Phycol, 28 (2016) 545-553.

[108] S.D. Anastyuk, N.M. Shevchenko, R.V. Usoltseva, A.S. Silchenko, P.A. Zadorozhny, P.S. Dmitrenok, S.P. Ermakova, Structural features and anticancer

activity in vitro of fucoidan derivatives from brown alga Saccharina cichorioides, Carbohydrate Polymers, 157 (2017) 1503-1510.

[109] N.M. Shevchenko, S.D. Anastyuk, R.V. Menshova, O.S. Vishchuk, V.I. Isakov, P.A. Zadorozhny, T.V. Sikorskaya, T.N. Zvyagintseva, Further studies on structure of fucoidan from brown alga Saccharina gurjanovae, Carbohydrate Polymers, 121 (2015) 207-216.

[110] N. Flórez-Fernández, M. López-García, M.J. González-Muñoz, J.M.L. Vilariño, H. Domínguez, Ultrasound-assisted extraction of fucoidan from Sargassum muticum, J Appl Phycol, 29 (2017) 1553-1561.

[111] R.V. Menshova, S.D. Anastyuk, S.P. Ermakova, N.M. Shevchenko, V.I. Isakov, T.N. Zvyagintseva, Structure and anticancer activity in vitro of sulfated galactofucan from brown alga Alaria angusta, Carbohydrate Polymers, 132 (2015) 118-125.

[112] H. Jonsson, Exploring the structure of oligo-and polysaccharides: Synthesis and NMR spectroscopy studies, Department of Organic Chemistry, Stockholm University, 2010.

[113] E.N.G. Marsh, Y. Suzuki, Using 19F NMR to probe biological interactions of proteins and peptides, ACS chemical biology, 9 (2014) 1242-1250.

[114] L. Youssouf, L. Lallemand, P. Giraud, F. Soulé, A. Bhaw-Luximon, O. Meilhac, C.L. D'Hellencourt, D. Jhurry, J. Couprie, Ultrasound-assisted extraction and structural characterization by NMR of alginates and carrageenans from seaweeds, Carbohydrate Polymers, 166 (2017) 55-63.

[115] D. Senthilkumar, S. Jayanthi, Partial characterization and anticancer activities of purified glycoprotein extracted from green seaweed Codium decorticatum, Journal of Functional Foods, 25 (2016) 323-332.

[116] X. Fan, L. Bai, X. Mao, X. Zhang, Novel peptides with anti-proliferation activity from the Porphyra haitanesis hydrolysate, Process Biochemistry, 60 (2017) 98-107.

[117] P.A. Harnedy, M.B. O'Keeffe, R.J. FitzGerald, Fractionation and identification of antioxidant peptides from an enzymatically hydrolysed Palmaria palmata protein isolate, Food Research International, 100 (2017) 416-422.

[118] X. Mao, L. Bai, X. Fan, X. Zhang, Anti-proliferation peptides from protein hydrolysates of Pyropia haitanensis, J Appl Phycol, 29 (2017) 1623-1633.

[119] H. Admassu, M.A. Gasmalla, R. Yang, W. Zhao, Identification of bioactive peptides with α-amylase inhibitory potential from enzymatic protein hydrolysates of red seaweed (Porphyra spp), Journal of agricultural and food chemistry, 66 (2018) 4872-4882.

[120] T. Furuta, Y. Miyabe, H. Yasui, Y. Kinoshita, H. Kishimura, Angiotensin I converting enzyme inhibitory peptides derived from phycobiliproteins of dulse Palmaria palmata, Marine drugs, 14 (2016) 32.

Table 1. Seaweed-derived peptides identified using LC-MS/MS during the period 2013-2018.

Matrix	Peptide sequence	Activity	Sample purification	Column	Mobile phase	Reference
Palmaria palmata	IRLIIVLMPILMA	Renin inhibitory, antihypertensive in SHRs	Removal of polyethylene glycols using titanium dioxide.	ACQUITY BEH C18 column (100 µm × 100 mm, 1.7 µm) and Symmetry C18 (180 µm × 200 mm, 5.0 µm)	Solvent A: 0.1% (w/v) formic acid in water Solvent B: 0.1% (w/v) formic acid in acetonitrile Flow rate: 5 µL/min	Fitzgerald, Aluko, Hossain, Rai and Hayes [7]
Palmaria palmata	ILAP, LLAP, and MAGVDHI	DPP-IV inhibitory	SPE using a Phenomenex Strata-X 5 g/60 mL C18 followed by RP-HPLC with 0.1% formic acid in water (Solvent A) and 80:20 acetonitrile:water containing 0.1% formic acid at 5 mL/min.	ACQUITY BEH C18 (2.1 × 50 mm, 1.7 µm)	Solvent A: 0.1% (w/v) formic acid in water Solvent B: 0.1% (w/v) formic acid in 80:20 (v/v) acetonitile:water Flow rate: 0.2 mL/min	Harnedy, O'Keeffe and FitzGerald [66]
Pyropia (Nori)	NMEKGSSSVVSSRM(+15.99)KQ	Anticoagulant	(i) SEC using a Sephadex G-100 column (1.4 × 200 mm) in 0.05 M Tris buffer at 0.5 mL/min. (ii) IEC using a DEAE Sephadex column (1.4 × 50 mm) in 0.05 M	The purified peptide was desalted using a C-18 zip tip column, reconstituted in 0.1% formic acid, and injected.	-	Indumathi and Mehta [62]

			Tris buffer at 0.5 mL/min (iii) RP-HPLC using a Sephadex G-25 column (1.4 × 200 mm) in 0.05 M Tris buffer at 0.5 mL/min.			
Palmaria palmata	NIGK	PAF-AH inhibitory	RP-HPLC using a Phenomenex C10 column (100µm × 212 mm, 5 µm) eluted with 0.1% TFA in water at a flow rate of 1 mL/min	ACQUITY BEH C18 column (100 µm × 100 mm, 1.7 µm) and Symmetry C18 (180 µm × 200 mm, 5.0 µm)	Solvent A: 0.1% (w/v) formic acid in water Solvent B: 0.1% (w/v) formic acid in acetonitrile Flow rate: 5 µL/min	Fitzgerald, Gallagher, O'Connor, Prieto, Mora-Soler, Grealy and Hayes [67]
Porphyria haitanensis	VPGTPKNLDSPR and MPAPSCALPRSVVPPR	Antiproliferative	MWCO filtration using 3, 5, and 10 kDa membranes followed by RP-HPLC using a Sephadex G-15 column (2.6 × 400 mm) eluted with water at 0.35 mL/min	MALDI-TOF-MS. 5 mg/mL Alpha-cyano-4-hydroxycinnamic acid dissolved in 60:40 acetonitrile:water containing 0.1% trifluoroacetic acid was used as a matrix.	-	Fan, Bai, Mao and Zhang [116]

Palmaria palmata	SDITRPGGNM	Antioxidant	SPE using a Phenomenex Strata-X 5 g/60 mL C18 followed by RP-HPLC with 0.1% formic acid in water (Solvent A) and 80:20 acetonitrile:water containing 0.1% formic acid at 5 mL/min.	ACQUITY BEH C18 (2.1 × 50 mm, 1.7 µm)	Solvent A: 0.1% (w/v) formic acid in water Solvent B: 0.1% (w/v) formic acid in 80:20 (v/v) acetonitile:water Flow rate: 0.2 mL/min	Harnedy, O'Keeffe and FitzGerald [117]
Pyropia haitanensis	QTDDNHSNVLWAGFSR	Antiproliferative	MWCO filtration using 3, 5, and 10 kDa membranes followed by SEC using a Sephadex G-15 column (2.6 × 400 mm) eluted with water at 0.35 mL/min	MALDI-TOF-MS. 5 mg/mL Alpha-cyano-4-hydroxycinnamic acid dissolved in 60:40 acetonitrile:water containing 0.1% trifluoroacetic acid was used as a matrix.	-	Mao, Bai, Fan and Zhang [118]
Porphyra spp	GGSK and ELS	α-Amylase inhibitory				Admassu, Gasmalla, Yang and Zhao [119]
Palmaria palmate	VYRT, LDY, LRY, FEQDWAS	ACE-I inhibitory	Sequential filtration by Miller-GV (0.22 µm)	Peptides analysed by Edman degradation method using a	-	Furuta, Miyabe, Yasui,

			and Millex LG (0.20 µm). RP-HPLC using a Mightysil RP-18GP column (4.6 × 150 mm) using a linear gradient of ACN (1-20%) containing 0.1% TFA at a flow rate of 1 mL/min	Procise 492HT protein sequencer and MALDI-TOF/MS/MS		Kinoshita and Kishimura [120]

Table 2. Experimental conditions used for the purification of macroalgal carbohydrates.

Compounds of interest	Analytical method *	Experimental conditions	Detector	References
Alginates	SEC	Shodex OHpak SB-806 HQ (8 × 300 mm) column. Eluted with 0.1 M NaOAc (pH 6). Flow rate 0.5 mL/min.	Refractive index detector (RI)	Rhein-Knudsen, Ale, Ajalloueian and Meyer [100]
Alginates	SEC	PSS Suprema (300 × 8 mm) column. Eluted with 0.01 M NaOH. Flow rate: 1 mL/min.	Refractive index detector (RI)	Sterner, Ribeiro, Gröndahl and Edlund [93]
Fucoidan	SEC	TSKgel GMPWXL (7.8 mm × 300 mm) with guard column TSK PWXL (6.0 mm × 4.0 mm). Eluted with HPLC-grade water. Flow rate: 1 mL/min.	Evaporative light-scattering detector (ELSD)	Saravana, Cho, Park, Woo and Chun [101]
Fucoidan	SEC	Sephacryl S-300 column (100 × 2.6 cm). Equilibrated and eluted with 0.2 M NaCl.	Refractive index detector (RI)	Cong, Chen, Liao, Xiao, Wang, Qin, Dong and Ding [90]
Fucoidan	SEC	Shodex Asahipak GS-520 HQ and GS-620 HQ (7.5 mm × 300 mm) at 50 °C with elution by 0.5 M ammonium bicarbonate (0.8 mL/min).	Refractive index detector (RI)	Usoltseva, Anastyuk, Ishina, Isakov, Zvyagintseva, Thinh, Zadorozhny,

				Dmitrenok and Ermakova [94]
Alginate	IEC	CarboPac PA1 (4 × 250 mm) column. Eluted using 0.1 M NaOH increasing up to 0.16 M NaOH with 0.19 M sodium acetate. Flow rate: 1.50 mL/min.	Pulsed amperometric detector (PDA)	Sterner, Ribeiro, Gröndahl and Edlund [93]
Carrageenan	IEC	Q-SepharoseTM (EX 150 mm × 50 cm). Eluted with linear gradient 3 M NaCl. Flow rate: 1.50 mL/min.	-	Ramu Ganesan, Shanmugam and Bhat [92]
Fucoidan	IEC	DEAE-cellulose (50 × 5 cm, Cl^- form). Eluted stepwise with 0, 0.2, 0.4, 0.8 and 1.6 M NaCl, followed with 0.3 M NaOH	-	Cong, Chen, Liao, Xiao, Wang, Qin, Dong and Ding [90]
Laminarin and fucoidan	IEC	DEAE-cellulose (Fluca) column (3.0 × 14 cm). Eluted first with H_2O and the fractions obtained further eluted with a linear gradient 0–2 M·NaCl	-	Imbs, Ermakova, Malyarenko, Isakov and Zvyagintseva [91]

* Abbreviations are as follow: size exclusion chromatography (SEC) and ion-exchange chromatography (IEC).