Review

Open camera or QR reader and scan code to access this article and other resources online.

Biorefining of Brown Seaweeds Catalyzed through Innovative Enzyme Processes

Morten Schiøtt,[1] Madeleine Jönsson,[2] Leila Allahgholi,[2] Eva Nordberg Karlsson,[2] Lene Lange,[3] Guðmundur O. Hreggviðsson,[4,5] and Anne S. Meyer[1]

[1]*Department of Biotechnology and Biomedicine, DTU Bioengineering, Technical University of Denmark, Lyngby, Denmark*
[2]*Biotechnology, Department of Chemistry, Lund University, Lund, Sweden*
[3]*LLa-BioEconomy, Research & Advisory, Valby, Denmark*
[4]*Matís Ohf, Reykjavík, Iceland*
[5]*Faculty of Life and Environmental Sciences, University of Iceland, Reykjavík, Iceland*

Abstract

The current expansion of seaweed farming to North America and Europe can be a cornerstone in a new "blue bioeconomy" in the Northern Hemisphere. In this domain, the focus of R&D efforts is on creating value-added products through new biorefining processes for valorizing the unique polysaccharides of seaweeds. Apart from direct consumption of seaweeds as food—particularly in the Asian cuisine—commercial seaweed products are primarily natural hydrocolloids used to make viscous suspensions and gels, but new valuable products exerting bioactivity are coming into focus. This recent development rests on targeted, gentle extraction and modification of the seaweed polysaccharides using tailormade bioprocessing enzyme technologies. Since brown seaweed cultivation is rising in the Northern Hemisphere, this article provides an overview of recent advances and prospects in brown seaweed biorefining.

Keywords: brown seaweeds, biorefining, functional carbohydrate products, enzyme bioprocessing

Current and Future Products Based on Seaweeds

Cultivation of seaweeds has substantial environmental, climate, and socioeconomic benefits. A recent esti mate predicts that enhanced seaweed farming has significant growth potential and can contribute to at least 9 of the 17 U.N. Sustainable Development Goals helping to shape a sustainable, livable planet.[1] Consumption of seaweeds has increased globally because of new food trends, but it is the polysaccharides of seaweeds, i.e., agar, alginate, and carrageenans, that are the main commercial drivers of seaweed cultivation. These polysaccharides have for decades been extracted from certain seaweed species and used for their unique hydrocolloid properties as thickeners, stabilizers, and gelation agents in food, personal care, biotech, and pharmaceutical applications. Presently, commercial objectives target increased cultivation of seaweeds for production of the established seaweed-derived hydrocolloids. In the longer term, however, enhanced use of seaweeds as a resource is predicted to involve targeted biorefining with tailored production of poly- and oligosaccharides for a broader spectrum of applications including high-value functional, nutritional, and biomedical products as well as functional additives in novel materials as listed below:

- Gut health and immune modulation[2,3]
- Antibacterial[4]
- Antidiabetic[4]
- Anti-osteoporotic action[4]
- Cosmetics/cosmeceuticals[5]
- Construction materials[6]
- Food packaging and coating materials[7]
- Polysaccharides for three-dimensional printing[8]
- Plant stimulants[9]

New types of enzyme-based processing, extraction, and modification of the specific polysaccharides of seaweeds involve gentle biorefining for producing tailormade oligosaccharides with

specific functions and beneficial effects, which can be exploited for different purposes. Enzyme-based methods are thus being explored for both targeted extraction of specific polysaccharides from seaweeds and for modification of the polysaccharides after extraction. Application of enzymes to modify the polysaccharides gives more uniform products to help ensure consistent functionality and potentially higher value products.

Brown Macroalgae Production

Currently, Asian countries account for about 98% of the global production of seaweeds, but seaweed production has lately gained increased attention in Europe and North America.[1] The most common commercially used (mostly cultivated) brown macroalgae are kelps belonging to the genera *Saccharina*, *Laminaria*, *Macrocystis*, *Undaria*, and *Alaria* within the order Laminariales. In addition, brown algae species within the Fucales order (*Fucus*, *Sargassum*, *Durvillaea*) are also produced commercially.

The polysaccharide and biochemical compositions of brown kelp seaweeds vary among species and season, and several factors, including water temperature, affect the chemical composition.[10] The temperate waters of the Atlantic and Pacific oceans are well suited for growing kelp, as the long days and clear water provide optimal light and temperature conditions during the summer and the cold water reduces the risk of fouling of the kelp by epibionts.[11] Cultivation of seaweeds moreover provides ecosystem service due to the uptake of carbon dioxide, which mitigates climate change. Seaweeds may also provide shelter to marine organisms, reduce sediment resuspension, release oxygen, and absorb nutrients from the water, which contributes to mitigating algal blooms and the resulting oxygen depletion.[12] These factors provide the foundation for the current expansion of seaweed farming to the Northern Hemisphere, as well as the prospects for establishing a new "blue bioeconomy" in North America and Europe based on this new seaweed farming.

Extraction and Purification of Polysaccharides from Brown Seaweeds

Commercial extraction methods for seaweed hydrocolloids rely on a combination of heating and chemical treatment—the latter to change the pH and thus viscosity of the polysaccharide suspension. However, many different alternative extraction methods have been suggested to secure high yield, partial fractionation, and purity of polysaccharides extracted from seaweeds; these include ultrasound, microwaves, pressure, and supercritical conditions (*Table 1*). Techniques such as pressurized liquid extraction (also known as subcritical water extraction) and supercritical fluid extraction are better suited for extraction of smaller molecular substances than polysaccharides from seaweeds. Other advanced techniques such as pulsed electric fields and ultrasound-assisted extraction methods have also been suggested in the literature.[13,15] Several of these methods will both extract and partially fractionate the polysaccharides, because the chemical structure of the polysaccharides may be affected during the extraction treatment.[14] This aspect has recently come into focus with biorefining of the seaweeds, i.e., when utilization of different types of polysaccharides and substances from the seaweeds is being explored. In general, mainly due to their higher cost and more advanced equipment requirements, the industrial implementation of alternative physical methods is not widespread.

For separation, purification, and concentration of the seaweed polysaccharide products, classical downstream techniques such as centrifugation, membrane technology, and chromatography are used.[18,19] Extraction of alginate using classical pH manipulation for viscosity adjustment and Ca^{2+}-driven precipitation has so far dominated the process development. Fouling can be a severe issue in membrane technology with polysaccharides, but may gain traction in cascade valorization processes for concentration and fractionation of alginate and fucoidan present in liquid side streams after alginate separation.[20] Ion exchange chromatography is well suited for preparing homogeneous oligosaccharide populations of similar

Table 1. Physicochemical Methods for Extraction, Fractionation, and Purification of Seaweed Polysaccharides

METHOD PRINCIPLE	DESCRIPTION	REFERENCES
Chemical extraction (acidic or alkaline conditions)	Degrades polysaccharide linkages, leading to oligo/polysaccharides. Unspecific cleavage; desulfation. Environmentally hazardous	13
Physicochemical fractionation	Precipitation with organic solvents such as ethanol, or by specific cationic salts, e.g., $CaCl_2$ or quaternary ammonium salts	13,14
Ultrasound-assisted extraction	Implosion creates extremely high local temperatures (up to 4500°C) and pressure (about 50 MPa), causing disruption of cell walls and cell membranes, in turn releasing polysaccharides	13,15
Microwave-assisted extraction	Electromagnetic radiation causes heat-induced disruption of the cell walls and cell membranes to release polysaccharides. Risk of undesirable polysaccharide modification, e.g., deacetylation	13,16
Supercritical fluid extraction	At supercritical conditions (~31°C, 74 MPa) CO_2 forms a liquid that can be used to extract chemical compounds from a matrix. By changing the pressure, different compounds can be extracted	17
Pressurized liquid extraction	Liquid solvent used under high temperature (50–200°C) and pressure (3.5–20 MPa) to accelerate extraction of the target compounds	13

chain length; however, large-scale implementation may be hampered by the low throughput of this technology.[18]

While the chemical and physicochemical methods have been optimized for extracting specific polysaccharides, notably alginate, the classical protocols are essentially unspecific, cannot capture specific structural intricacies, and are moreover considered environmentally unsustainable. To obtain higher value products, enzymatic extraction has been shown to have potential.[15,21]

Through selective biocatalysis, enzymes can thus provide the specificity needed to produce specific native products with high purity in an environmentally friendly manner, and be used to upgrade and tailor-make the polysaccharides for higher value applications via specific modifications (*Table 2*).

Brown Macroalgae Polysaccharides

The main polysaccharide structures of brown seaweeds (*Phaeophyceae*) include the storage polysaccharide laminarin, as well as the cell wall polysaccharides alginate and fucoidan. In addition, brown seaweed cell walls may contain some cellulose. Genome annotation evidence has confirmed that pathways for sucrose, starch, and glycogen synthesis are absent in this type of seaweed.[26] Due to their different structures and properties, the three main, distinct types of polysaccharides from brown seaweeds, alginate, fucoidan, and laminarin, are used in very different applications (*Table 3*).

Alginate and Enzymatic Modification of Alginate

Alginate is a family of unbranched uronic acid polysaccharides consisting of β-D-mannuronic acid (M) and α-L-guluronic acid (G) linked by 1,4-glycosidic bonds. The ratio of M to G differs between algal species and during the life cycle and season within the same individual. Consecutive stretches of Ms are referred to as M-blocks, consecutive stretches of Gs are referred to as G-blocks, while strictly alternating M- and G-residues are referred to as MG-blocks. G-blocks and MG-blocks may be cross-linked by divalent cations, typically Ca^{2+}, which causes gel formation (also in the seaweed cell walls). Alginate has high industrial usage, primarily in the food industry, where its gelling, viscosity-enhancing, and stabilizing properties are exploited for improving food and drinks texture and for packaging films. Alginates are food additives and are generally recognized as safe by the U.S. Food and Drug Administration and the European Food Safety Authority (EFSA). They have the following E numbers in the European Union: E 400 (alginic acid), E 401 (sodium alginate), E 403 (ammonium alginate), E 404 (calcium alginate), and E 405 (propane-1,2-diol alginate). Alginate is also used in the pharmaceutical industry for drug encapsulation, wound dressing, and tissue engineering, as well as in the textile printing and paper industries.[27]

Depolymerization of alginate is mediated by alginate lyases. In the database of carbohydrate-active enzymes (www.cazy.org), alginate lyases have been categorized in 12 different polysaccharide lyase families, and they are found in both eucaryotic and procaryotic organisms.[28] The majority of categorized alginate lyases cleave internal bonds in the alginate sequence, i.e., work as endo-acting enzymes, but some have been shown to be exo-acting, cleaving single monomers from the end of the polymer.[29] The enzymes catalyze depolymerization of alginate by *β*-elimination, eventually resulting in monomeric 4-deoxy-l-erythro-5-hexoseulose uronic acid, which is converted to pyruvate and glyceraldehyde-1-phosphate. Alginate lyases either targets M-blocks (EC 4.2.2.11) and G-blocks (EC 4.2.2.3) or cleaves the bonds between M and G (and G and M) residues.[30]

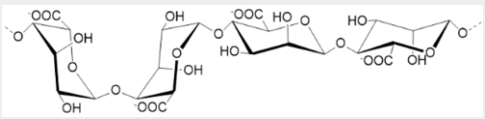
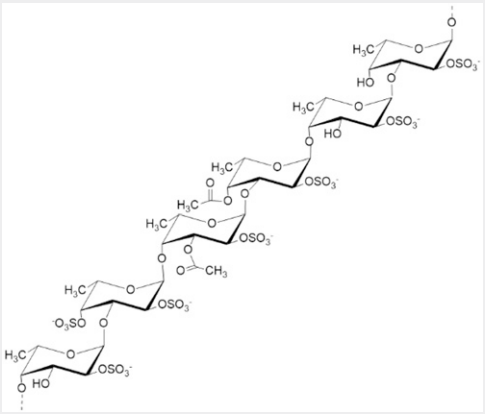
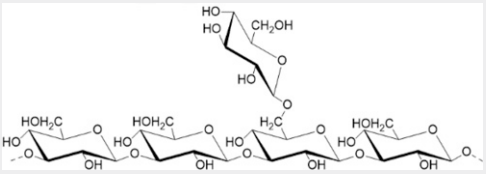
β-D-Mannuronic acid and α-L-guluronic acid are C-5 epimers, and these can be interconverted by epimerases, which will change the gel-forming properties of the alginate. Alginate is synthesized by the polymerization of D-mannuronic acid whereupon mannuronan C5-epimerase (EC 5.1.3.37) converts some residues to L-guluronic acid.[30] Most characterized alginate epimerases are of bacterial origin, but alginate epimerases from brown seaweeds have been heterologously expressed in *Escherichia coli* to confirm their ability to convert M to G in alginate.[31,32]

Acetylation of alginate appears to improve the ability of the alginate to work as a protective film.[29] Acetylation will also work as a steric hindrance for epimerases.[33] The mannuronan C-5-epimerase from *Pseudomonas syringae*, denoted PsmE, has been shown to harbor a unique *N*-region that has acetylhydrolase activity, enabling the enzyme to deacetylate M residues and convert them to G residues.[25,34] The ratio between M and

Table 2. Examples of the Use of Enzymes for Targeted Extraction, Modification, and Purification of Brown Seaweed Polysaccharides

PURPOSE	APPROACH	REFERENCES
Gentle fucoidan extraction: Enzymatic removal of unwanted polysaccharides	Enzymatic removal of alginate and laminarin from fucoidan extracts	21
Enzymatic production of oligosaccharides for increased physiological effects	Depolymerization of laminarin into bioactive oligosaccharides that can stimulate human monocytes to produce TNF-α cytokines	22
Extraction of bioactive fractions from seaweeds	Sequential use of cellulases and proteases for extraction of functional fractions from different types of seaweeds	23
Enzymatic de-sulfation of fucoidan	Targeted removal of sulfate groups from fucoidan	24
Enzymatic change of viscosity	Converting M alginate to G alginate using deacetylase and epimerase	25

Table 3. Signature Structures and Examples of Application Targets of the Main Polysaccharides of Brown Seaweeds (Main Seaweed Source Species Given for Each Type of Polysaccharide)

TYPICAL SEAWEED SOURCE	TYPE OF CARBOHYDRATE	MAIN STRUCTURAL CHARACTERISTICS	APPLICATION
Laminaria spp. *Saccharina* spp.	Alginate		Gelation Viscosity
Fucus spp. *Saccharina* spp.	Fucoidan		Various potential pharmaceutical uses
Laminaria spp. *Saccharina* spp.	Laminarin		Plant stimulant Potential immune-boosting uses

G residues can also be manipulated by selectively removing M residues with mannuronan-specific lyases. One such example is the exo-acting PL8 enzyme PsMan8A from the marine fungus *Paradendryphiella salina*, which specifically attacks polyM sequences without attacking polyG or mixed alginate sequences.[35]

LAMINARIN AND ENZYMATIC UPGRADING FOR ENHANCED FUNCTIONALITY

Laminarin is a mixed β-1,3(1,6)-linkage glucan with β-(1-3)-linked glucose units interspersed sporadically with β-1,6 branching points (*Table 3*) or β-1,6 kinks in the backbone chain. The laminarin glucan chain may be capped with mannitol at C1 of the glucose residue in the reducing end. The laminarin β-glucans form both soluble and insoluble fractions after water-based extractions of brown seaweeds. Detailed structures have only been resolved in few cases. The structure is species dependent and the ratio between β-1,6 and β-1,3 linkages varies: it is very low in *Laminaria hyperborea*, as the laminarin mainly contains β-1,3-linkages[36]; moderate in laminarin from *Laminaria digitata*, with a ratio of $\sim$1:7; and high in laminarin from *Eisenia bicyclis*, with a ratio of β-1,6 to β-1,3 linkages of $\sim$1:3–2:3.[37,38] Laminarin typically has an average molecular weight of 2000–5000 Da (up to 7000 Da), i.e., a degree of polymerization of $\sim$20–40, depending on the brown seaweed species.[39]

Laminarin is degraded by the endo-acting 1,3-β-glucanases (laminarinases) (EC 3.2.1.39 and EC 3.2.1.6) that cleave the laminarin into oligosaccharides and the exo-acting 1,3-β-glucosidases (EC 3.2.1.58) that cleave off glucose residues form the nonreducing end. Laminarinases are categorized in 14 different glycoside hydrolase (GH) CAZy families in the open CAZy database (www.cazy.org), whereas 1,3-β-glucosidases are found in eight CAZy families.[28] Side chains linked by β-1,6 linkages can be removed by β-1,6-glucanases (EC 3.2.1.75) that belong to CAZy families GH5 and GH30.[28]

Enzymatic transglycosylation, which results in the formation of new glycoside or oligosaccharide products, is catalyzed by certain retaining GHs, which can use acceptors other than water (i.e., carbohydrates). Transglycosylation reactions with laminarin take place in two steps: first, the enzyme cleaves a donor β-glucan; second, the part containing the nonreducing end is added to an acceptor β-glucan with a β-1,6 linkage.[40] Bacterial GH17 glucanosyltransferases have been found in a relatively narrow spectrum of bacteria, mostly Proteobacteria

and Bacteroidetes. The particular enzyme Glt20 from *Bradyrhizobium diazoefficiens* cleaves off two terminal glucose residues from the reducing end of laminarin oligosaccharides and then transfers the remaining part of the molecule to an acceptor laminarin oligosaccharide with a β-1,6-linkage. The newly formed branch may then be used as the acceptor for a new transfer event, and this can be repeated a third time to produce highly branched end products.[41] Other characterized bacterial GH17 glucanosyltransferases active on β-1,3-glucans include the enzymes Glt1, Glt3, and Glt7, of which Glt7 shows the highest capability of making β-1,6 branches on laminarin acceptors, whereas notably Glt1 mainly elongates laminarin acceptors via β-1,3 linkages of transferred oligosaccharides.[42] The AfBgt2p glucanosyltransferase from *Aspergillus fumigatus* always transfers the cleaved-off β-glucan to an internal glucose residue of the acceptor molecule, resulting in a branched β-glucan. In contrast, AfBgt1p from *A. fumigatus* and the ScBgl2p from yeast transfer the liberated β-glucan (laminaribiose in case of laminarin) to the terminal glucose residue of the nonreducing end of the acceptor molecule, producing an elongated, kinked β-(1,3;1,6)-glucan.[40]

β-Glucans from cereals, seaweed, fungi, and cyanobacteria are marketed as food supplements in the form of crude extracts or powders. In general, β-1,3(1,6)-glucans are presumed to have a range of biological activities for applications in nutraceuticals, pharmaceuticals, and cosmeceuticals.[43] Laminarin seems to act as a modulator of gut intestinal metabolism through short-chain fatty acid production, especially butyrate, accomplished by the gut microbiota.[44] Yeast-derived β-glucans consist of complex, high-molecular-mass polysaccharides derived from the cell wall of baker's yeast *Saccharomyces cerevisiae*. Like laminarin, yeast β-glucans comprise a β-1,3-linked glucan backbone with β-1,3 glucan branches linked to the backbone via β-(1,6) glycosidic linkages. Yeast β-glucans are considered safe as food supplements at dose levels of up to 375 mg per day and in foods for particular nutritional uses even at dose levels of up to 600 mg per day.[45] Purified yeast β-glucans have been shown to activate innate immune effector cells in human blood samples, triggering a coordinated anticancer immune response via complex formation with anti-β-glucan antibodies.[46,47] The precise determinants of potency are not completely resolved, but the activities seem to relate to molecular size and the content of 1,6 linkages, and appear to be confined to β-1,3-glucans including β-1,3(1,6) glucans and not β-1,3-1,4 mixed linkage glucans.[48] Indeed, about 20 years ago, enzymatically depolymerized low-molecular-mass laminarin (from *L. digitata*) was reported to inhibit the proliferation of human leukemia cells *in vitro*, whereas the original laminarin had only little activity.[22] More preclinical mechanistic understanding of the putative structure–function benefits of laminarin is clearly required, but the available bioactivity findings on laminarin and yeast β-glucans provide interesting options for new, potent high-value uses of laminarin.

FUCOIDAN POLYSACCHARIDES AND THEIR ENZYMATIC MODIFICATION

Fucoidan polysaccharides are a part of the cell walls of brown seaweeds and designate a family of sulfated polysaccharides having a backbone rich in fucose residues, specifically α-L-fucosyls. Recently, fucoidans have been shown to resist degradation and aggregate into particulate organic matter that will eventually sink to the bottom of the sea thus potentially working as a carbon sink.[49]

The fucoidan polysaccharides are highly diverse molecules and may be classified into the following five groups based on their monosaccharide composition and backbone linkage types: group 1, polymers of α-1,3-L-fucose; group 2, polymers of repeating α-1,3- and α-1,4-L-fucose residues; group 3, galactofucans and fucogalactans; group 4, fucoidans with backbones that in addition to fucose and galactose also contain mannose and uronic acids; and group 5, fucoidans with fucose, galactose, mannose, xylose, glucose, arabinose, and uronic acids.[50] Fucoidans are moreover often branched and acetylated, and sulfations are found on C2, C3, or C4, or combinations thereof. The diversity of fucoidan structures is reflected in the large variation of activity that different fucoidans exert in bioactivity assays. The available data do not provide firm correlations between activity and molecular weight of the fucoidan, level of sulfation, or the content of specific monosaccharides. Rather, it appears that different properties of the fucoidans are relevant for mediating their biological effects in the different physiological processes investigated in the assays.

Fucoidanases catalyze the depolymerization of fucoidan. Endo-acting fucoidanases (EC 3.2.1.211 endo-α-1,3-L-fucanase; EC 3.2.1.212 endo-α-1,4-L-fucanase) cleave within the fucoidan molecule thus producing fucoidan oligosaccharides of varying lengths. So far, only about 18 endo-fucoidanases have been characterized, i.e., 17 belonging to family GH107 and 1 to family GH168 (www.cazy.org). In the past few years, several new endo-acting fucoidanases, notably of family GH107, have been discovered, and a few have even been structurally characterized (*Table 4*). A recent finding of a new type of endo-1,3-fucanase has laid the foundation of family GH174, a new GH family in the CAZy database[28,59] (*Table 4*).

Endo-fucoidan-lyases. Besides fucoidanases, fucoidan can also be degraded by endo-acting, bacterial fucoidan-lyases. Two such enzymes have been described so far, FdlA and FdlB. Both enzymes can degrade fucoglucuronomannan from *Kjellmaniella crassifolia* (Kj-fucoidan)[62] and have also been found to have activity on galactofucan from *Sargassum mcclurei*.[63] Currently, among these two, only FdlA is recognized as a characterized fucoidan lyase of family PL43. FdlA appears to cleave the α-1,4-linkage between D-mannose and D-glucuronic acid in Kj-fucoidan; this type of fucoidan has a branched sulfated fucose linked on the C-3 hydroxyl group of D-mannose.[61,62]

Exo-acting α-fucosidases are found in CAZy families GH29, GH95, GH139, GH141, and GH151, while β-fucosidases are found in families GH1 and GH30. The natural substrates of most of these enzymes are fucosyl groups on glycoproteins or oligosaccharides such as human milk oligosaccharides and Lewis antigens. Fucosidases isolated from *Wenyingzhuangia fucanilytica* have, however, shown activity toward fucooligosaccharides and

Table 4. Cleavage Points in Fucoidan (in Bold) and CAZy Family Affiliation of Endo-Acting, Microbial Fucoidanases, and the Sole Characterized Fucoidan Lyase, FdlA

CLEAVAGE SITE (BOLD)	ENZYME ACRONYM	CAZY FAMILY	REFERENCES
→3)-α-L-Fuc*p*2S-**(1→4)**-α-L-Fuc*p*2S,3S-(1→	MfFcnA	GH107	51,52
	FWf1	GH107	
→3)-α-L-Fuc*p*2S,4S-**(1→4)**-α-L-Fuc*p*2S,3S-(1→	FWf1	GH107	52
→3)-α-L-Fuc*p*2S-**(1→4)**-α-L-Fuc*p*2S-(1→	FFA2	GH107	52–54
	FWf1	GH107	
	Fhf1	GH107	
	Fhf2	GH107	
→?)-α-L-Fuc*p*?S-**(1→4)**-α-L-Fuc*p*?S-(?→	P5AFcnA	GH107	55,56
	P19DFcnA	GH107	
	Fp273	GH107	
	Fp277	GH107	
	Fp279	GH107	
→3)-α-L-Fuc*p*2S-**(1→4)**-α-L-Fuc*p*2S-(1→	Mef1	GH107	57
→4)-α-L-Fuc*p*?S-**(1→3)**-α-L-Fuc*p*?S-(1→	Mef2	GH107	58
→4)-α-L-Fuc*p*2S-**(1→3)**-α-L-Fuc*p*-(1→	FunA	GH168	59,60
	Fun174A	GH174	
→2)-(α-L-Fuc*p*3S-[1→3])-α-D-Man*p*-**(1→4)**-β-D-Glc*p*UA-(1→	FdlA (lyase)	PL43	61

fucoidans, and with different fucosidases showing differences in selectivity regarding linkage types.[64]

Sulfatases. Exo-acting sulfatases catalyze the removal of sulfate from the ends of the carbohydrate (reducing or non-reducing end), while endo-acting sulfatases catalyze the cleavage of sulfate from internal residues of the carbohydrate. The *W. fucanilytica* genome harbors six sulfatase genes in their fucoidan degrading gene cluster, but only three have been functionally characterized. SWF1 and SFW4 have no action on native fucoidan, only on oligosaccharides, but have different substrate specificity, with SWF4 removing C3 sulfates from the nonreducing end of oligosaccharides with 2,3S sulfation, and SWF1 removing C2 sulfates from the nonreducing end of oligosaccharides with 2S sulfation.[65] SWF5 is another fucoidan endo-acting sulfatase, which selectively eliminates 4*O*-sulfation in sulfated fucans and fucooligosaccharides composed of alternating α-(1,3)- and α-(1,4)-linked residues of sulfated L-fucopyranose, but the enzyme is not active on fucoidans composed of only α-(1,3)-linked sulfated L-fucopyranose residues.[24] A surprisingly thermostable exo-acting sulfatase, PsFucS1, from a *Pseudoalteromonas sp*. was found in the gut of a sea cucumber; the enzyme acts on fucoidan oligosaccharides with a thermal optimum of 68°C.[66] Thermostable enzymes are interesting for biorefining applications as processing at elevated temperatures decreases contamination risk and allows higher conversion rates.

RISK OF TOXIC ELEMENTS IN BROWN SEAWEEDS

The growing interest in the application and refining of brown seaweeds into products for human consumption and use has raised concern about the presence of potentially toxic elements (PTEs).[67] There is lack of knowledge of whether certain elements are preferentially accumulated, but PTEs include heavy metals arsenic, cadmium, lead, and mercury.[68]

In Europe, recommendations and guidelines are being developed concerning the content in products for the market. The European Commission has set maximum levels for certain contaminants in foodstuffs, and the EFSA has implemented tolerable daily/weekly intake levels (*Table 5*). In some cases, previous set guidelines are *considered no longer appropriate*, as indicated in *Table 5*, but not yet replaced by other recommendations. Uptake of elemental compounds, including PTEs, in seaweed is postulated to derive from the cell wall polysaccharide components, where the functional groups (e.g., carboxyl, hydroxyl, and sulfate) are important for binding. In brown seaweeds, the content of alginate and fucoidan has been shown to correlate positively to mercury and cadmium levels,[67] whereas arsenic might interact through weak bonds. In alginate, the M-blocks appear more selective for Cd^{2+} while G-blocks have a higher affinity for Ca^{2+}.[69] In addition, high iodine content in some species, e.g., *Saccharina latissima*, may pose a potential risk for human health and a maximum tolerable intake has been established (*Table 5*).

Table 5. Overview of Recommendations and Maximum Levels for the Potentially Toxic Elements, Arsenic, Cadmium, Lead, Mercury, as Well as Iodine, from Different Sources

POTENTIALLY TOXIC ELEMENTS	FEED[a]	ALGAE BROUGHT TO MARKET	MAXIMUM TOLERABLE DAILY/ WEEKLY INTAKE LEVELS ACCORDING TO EFSA[b]
Arsenic (total)	40 mg kg^{-1}	No data	No data
Arsenic (inorganic)	2 mg kg^{-1}	3 mg kg^{-1} (dry weight)	25 μg kg^{-1} body weight (b.w.) *considered no longer appropriate*
Cadmium	1 mg kg^{-1}	3 mg kg^{-1} (dry weight), [supplements, 0.5 mg kg^{-1} (dry weight)]	2.5 μg kg^{-1} b.w.
Lead	10 mg kg^{-1}	0.5 mg kg^{-1} (dry weight)	25 μg kg^{-1} b.w. *considered no longer appropriate*
Mercury	0.1 mg kg^{-1}	0.2 mg kg^{-1} (dry weight)	4 μg kg^{-1} b.w. for inorganic mercury
Iodine	n.a.	2000 mg kg^{-1} (dry weight)	60 μg kg^{-1} b.w.[c]

[a]Directive 2002/32/EC specifies undesired substances in animal feed; levels concern feed with 12% moisture.
[b]Maximum tolerable weekly intake (TWI), as established from the European Food Safety Authority (EFSA).
[c]Iodine is based on an average body weight of 70 kg.
Table adapted from Jönsson and Nordberg Karlsson, 2023.[67]

Arsenic content can be effectively reduced with hot aqueous treatment. Iodine content has also been significantly reduced by hot water and pH shift treatments,[70,71] showing that reduction can be achieved by simple scalable methods. Cadmium is of concern, while lead and mercury are of lesser concern and usually below the tolerable levels. Many methods that reduce arsenic and iodine cause an undesirable concentrating effect of cadmium, probably because of complexation with alginate (especially M-block).[67] Use of chelating agents such as EDTA, especially in combination with ultrasound, has shown significant cadmium reduction in *L. hyperborea*.[72] Enzymatic conversion has received limited attention in this context but could play a role in PTE reduction. Taking alginate as an example, epimerases may reduce M-block in favor of G-block alginate; likewise, selective alginate lyase catalysis can remove mannuronic acid. Clearly, analyses of PTEs and iodine must be included in seaweed biorefineries and protocols must be introduced for their removal, where necessary.

ENZYME DISCOVERY

The great potential of seaweed polysaccharides will require the discovery of new enzymes that target these polysaccharides for purification, fragmentation, and modification purposes. While such enzymes are available for polysaccharides from terrestrial systems, much less emphasis has been put into enzyme discovery from marine systems. Traditionally, such discovery endeavors have started from isolating microorganisms from the substrate in question, followed by enzyme purification or PCR amplification of relevant enzyme genes. The advent of mass DNA sequencing technology has more recently favored strategies by which metagenomes from relevant (seaweed biomass degrading) environments have been sequenced and clustered into separate metagenome assembled genomes. Another hotspot environment for the discovery of seaweed bioprocessing is the gut microbiome of seaweed-eating animals, like sea cucumber, snails, etc. Relevant enzyme sequences can subsequently be identified in the genomic sequences using search algorithms based on sequence similarity or structural similarity to already known proteins, or by close genomic proximity to genes involved in the same pathways, which, in bacteria typically are in so-called polysaccharide utilization loci that are coregulated to be transcribed simultaneously in the presence of the targeted substrate. A third option is to make direct use of the vast amounts of sequence data available in public repositories such as GenBank (https://www.ncbi.nlm.nih.gov/) using targeted sequence search strategies. Heterologous expression of the identified enzyme gene sequences in *E. coli*, yeast, or filamentous fungi, followed by purification of the expressed proteins, allows for a detailed characterization of the enzymes to determine their substrate specificity, optimal reaction conditions, and specific product formation.

Outlook and Impact

To understand the functional and very promising bioactivity effects of seaweed polysaccharides, and exploit these in new products, it is important to retain the specific structural traits of the polysaccharides and obtain pure, homogeneous, and well-characterized products. Enzyme-assisted processing enables such production, thus allowing distinct functional properties to be made from mass reared seaweed. Endo-acting enzymes that catalyze depolymerization of alginate, laminarin, and fucoidan will generally produce a population of oligomeric products having different backbone length, and hence different properties. A new wave of enzyme research is expected to target the details of enzyme action and function to achieve more homogeneous products directly by enzymatic processes. More well-defined products obtained in this way are foreseen to foster new high-value applications of seaweed carbohydrates with

verified beneficial bioactivity effects in a range of different products. Such processing requires discovery of new enzymes with distinct activities on brown seaweed polysaccharides but will ultimately lead to sustainable and environmentally friendly production methods.

The substantiation of the beneficial effects of products obtained by enzyme-assisted processing is still at the research stage, but several of the bioactivity effects reported, e.g., enzymatically modified fucoidan[73] or laminarin[38] molecules, may pave the way for the manufacture of unique, beneficial, and high-value products.

Biorefining is principally defined as the manufacturing of multiple products from one feedstock. Biorefining via cascade valorization involving fractionation of brown seaweed side streams after a primary alginate extraction has shown resource management gains and potential business benefits.[20] However, it is presently uncertain whether such full biorefining of whole seaweeds or whether smaller scale, targeted reactions on portions of the seaweed, designated for upgrading of specific polysaccharides, will drive the industrial development. Regardless, the ability of enzymes to catalyze robust, precise modifications that provide superior products for novel, high-value applications is expected to play a role in brown seaweeds refining to support a new global "blue bioeconomy" based on seaweed production from new seaweed farming in the Northern Hemisphere. Apart from offering new products for medicine, food and personal products, it is an important asset that large-scale seaweed cultivation offers substantial ecosystem services that contribute to climate change mitigation.

Authors' Contributions

M.S., E.N.K., G.O.H., L.L., A.S.M.: Conceptualization. M.S., M.J., L.A., E.N.K., L.L., G.O.H., A.S.M.: Writing, Original draft preparation. M.S., E.N.K., L.L., G.O.H., A.S.M.: Reviewing, Editing.

Author Disclosure Statement

No competing financial interests exist.

Funding Statement

This study received funding from the Nordic Council of Ministers Fisheries Cooperation via the MacroCat Seaweed Contract, Nanoq no. 22184560 "Novel high value products from seaweed polysaccharides/MacroCat Norrænt Netverk um nýtingu þörunga."

REFERENCES

1. World Bank. Global Seaweed New and Emerging Markets Report 2023. Washington, D.C. Available from: http://documents.worldbank.org/curated/en/099081423104548226/P175786073c14c01609fe409c202ddf12d0
2. You L, Gong Y, Li L, et al. Beneficial effects of three brown seaweed polysaccharides on gut microbiota and their structural characteristics: An overview. Int J of Food Sci Tech 2020;55(3):1199–1206; doi: 10.1111/ijfs.14408
3. Murphy EJ, Fehrenbach GW, Abidin IZ, et al. Polysaccharides—Naturally occurring immune modulators. Polymers (Basel) 2023;15(10); doi: 10.3390/polym15102373
4. Perumal PK, Dong CD, Chauhan AS, et al. Advances in oligosaccharides production from algal sources and potential applications. Biotechnol Adv 2023; 67:108195; doi: 10.1016/j.biotechadv.2023.108195
5. López-Hortas L, Flórez-Fernández N, Torres MD, et al. Applying seaweed compounds in cosmetics, cosmeceuticals and nutricosmetics. Mar Drugs 2021; 19(10); doi: 10.3390/md19100552
6. Rossignolo JA, Duran AJFP, Bueno C, et al. Algae application in civil construction: A review with focus on the potential uses of the pelagic *Sargassum* spp. biomass. J Environ Manage 2022;303:114258; doi: 10.1016/j.jenvman.2021.114258
7. Carpintero M, Marcet I, Rendueles M, et al. Algae as an additive to improve the functional and mechanical properties of protein and polysaccharide-based films and coatings. A review of recent studies. Food Packaging Shelf 2023;38: 101128; doi: 10.1016/j.fpsl.2023.101128
8. Mandal S, Nagi GK, Corcoran AA, et al. Algal polysaccharides for 3D printing: A review. Carbohydr Polym 2023;300:120267; doi: 10.1016/j.carbpol.2022.120267
9. Shukla PS, Borza T, Critchley AT, et al. Seaweed-based compounds and products for sustainable protection against plant pathogens. Mar Drugs 2021;19(2); doi: 10.3390/md19020059
10. Manns D, Nielsen MM, Bruhn A, et al. Compositional variations of brown seaweeds *Laminaria digitata* and *Saccharina latissima* in Danish waters. J Appl Phycol 2017;29(3):1493–1506; doi: 10.1007/s10811-017-1056-z
11. Bak UG, Mols-Mortensen A, Gregersen O. Production method and cost of commercial-scale offshore cultivation of kelp in the faroe islands using multiple partial harvesting. Algal Res 2018;33:36–47; doi: 10.1016/j.algal.2018.05.001
12. Cotas J, Gomes L, Pacheco D, et al. Ecosystem services provided by seaweeds. Hydrobiology 2023;2(1):75–96; doi: 10.3390/hydrobiology2010006
13. Dobrinčić A, Balbino S, Zorić Z, et al. Advanced technologies for the extraction of marine brown algal polysaccharides. Mar Drugs 2020;18(3):168; doi: 10.3390/md18030168
14. Ale MT, Mikkelsen JD, Meyer AS. Designed optimization of a single-step extraction of fucose-containing sulfated polysaccharides from *Sargassum* sp. J Appl Phycol 2012;24(4):715–723; doi: 10.1007/s10811-011-9690-3
15. Terme N, Hardouin K, Cortès HP, et al. Emerging seaweed extraction techniques: Enzyme-assisted extraction a key step of seaweed biorefinery? In: Sustainable Seaweed Technologies, Chapter 10, Elsevier; 2020, pp. 225–56. 10.1016/B978-0-12-817943-7.00009-3
16. Gomez L, Tiwari B, Garcia-Vaquero M. Emerging extraction techniques: Microwave-assisted extraction. In: Sustainable Seaweed Technologies, Chapter 9, Elsevier; 2020, pp. 207–24. 10.1016/B978-0-12-817943-7.00008-1
17. Machmudah S, WahyudionoKanda H, et al. Emerging seaweed extraction techniques: Supercritical fluid extraction. In: Sustainable Seaweed Technologies, Chapter 11, Elsevier; 2020, pp. 257–86. 10.1016/B978-0-12-817943-7.00010-X
18. Batool A, Menaa F. Concentration and purification of seaweed components by chromatography methods. In: Sustainable Seaweed Technologies, Chapter 13, Elsevier; 2020, pp. 315–70. 10.1016/B978-0-12-817943-7.00013-5
19. Díaz-Reinoso B. Concentration and purification of seaweed extracts using membrane technologies. In: Sustainable Seaweed Technologies, Chapter 14, Elsevier; 2020, pp. 371–90. 10.1016/B978-0-12-817943-7.00014-7
20. Zhang X, Border A, Goosen N, et al. Environmental life cycle assessment of cascade valorisation strategies of South African Macroalga *Ecklonia maxima* using green extraction technologies. Algal Res 2021;58:102348; doi: 10.1016/j.algal.2021.102348
21. Nguyen TT, Mikkelsen MD, Tran VHN, et al. Enzyme-assisted fucoidan extraction from brown macroalgae *Fucus distichus* subsp. *evanescens* and *Saccharina latissima*. Mar Drugs 2020;18(6); doi: 10.3390/md18060296
22. Miyanishi N, Iwamoto Y, Watanabe E, et al. Induction of TNF-α production from human peripheral blood monocytes with β-1,3-glucan oligomer prepared from laminarin with β-1,3-glucanase from *Bacillus clausii* NM-1. J Biosci Bioeng 2003;95(2):192–195; doi: 10.1263/jbb.95.192

23. Teixeira-Guedes C, Gomes-Dias JS, Cunha SA, et al. Enzymatic approach for the extraction of bioactive fractions from red, green and brown seaweeds. Food Bioprod Process 2023;138:25–39; doi: 10.1016/j.fbp.2022.12.005
24. Silchenko AS, Rasin AB, Zueva AO, et al. Discovery of a fucoidan endo-4O-sulfatase: Regioselective 4O-desulfation of fucoidans and its effect on anticancer activity in vitro. Carbohydr Polym 2021;271:118449; doi: 10.1016/j.carbpol.2021.118449
25. Bjerkan TM, Bender CL, Ertesvåg H, et al. The *Pseudomonas syringae* genome encodes a combined mannuronan C-5-epimerase and O-acetyl hydrolase, which strongly enhances the predicted gel-forming properties of alginates. J Biol Chem 2004;279(28):28920–28929; doi: 10.1074/jbc.M313293200
26. Michel G, Tonon T, Scornet D, et al. Central and storage carbon metabolism of the brown alga *Ectocarpus siliculosus*: Insights into the origin and evolution of storage carbohydrates in eucaryotes. New Phytol 2010;188(1):67–81; doi: 10.1111/j.1469-8137.2010.03345.x
27. Usman A, Khalid S, Usman A, et al. Algal polysaccharides, novel application, and outlook. In: Algae Based Polymers, Blends, and Composites: Chemistry, Biotechnology and Materials Science, 2017, Elsevier, pp. 115–53. 10.1016/B978-0-12-812360-7.00005-7
28. Drula E, Garron ML, Dogan S, et al. The carbohydrate-active enzyme database: Functions and literature. Nucleic Acids Res 2022;50(D1):D571–D577; doi: 10.1093/nar/gkab1045
29. Ertesvåg H. Alginate-modifying enzymes: Biological roles and biotechnological uses. Front Microbiol 2015;6(523); doi: 10.3389/fmicb.2015.00523
30. Rhein-Knudsen N, Meyer AS. Chemistry, gelation, and enzymatic modification of seaweed food hydrocolloids. Trends Food Sci Technol 2021;109:608–621; doi: 10.1016/j.tifs.2021.01.052
31. Fischl R, Bertelsen K, Gaillard F, et al. The cell-wall active mannuronan C5-epimerases in the model brown alga *Ectocarpus*;: From gene context to recombinant protein. Glycobiol 2016;26(9):973–983; doi: 10.1093/glycob/cww040
32. Inoue A, Satoh A, Morishita M, et al. Functional heterologous expression and characterization of mannuronan C5-epimerase from the brown alga *Saccharina japonica*. Algal Res 2016;16:282–291; doi: 10.1016/j.algal.2016.03.030
33. Rozeboom HJ, Bjerkan TM, Kalk KH, et al. Structural and mutational characterization of the catalytic A-module of the mannuronan C-5-epimerase AlgE4 from *Azotobacter vinelandii*. J Biol Chem 2008;283(35):23819–23828; doi: 10.1074/jbc.M804119200
34. Petersen AB, Tøndervik A, Gaardløs M, et al. Mannuronate C-5 epimerases and their use in alginate modification. Essays Biochem 2023;67(3):615–627; doi: 10.1042/EBC20220151
35. Pilgaard B, Vuillemin M, Holck J, et al. Specificities and synergistic actions of novel PL8 and PL7 alginate lyases from the marine fungus *Paradendryphiella salina*. J Fungi (Basel) 2021;7(2):80; doi: 10.3390/jof7020080
36. Nelson TE, Lewis BA. Separation and characterization of the soluble and insoluble components of insoluble laminaran. Carbohydr Res 1974;33(1):63–74; doi: 10.1016/S0008-6215(00)82940-7
37. Read SM, Currie G, Bacic A. Analysis of the structural heterogeneity of laminarin by electrospray-ionisation-mass spectrometry. Carbohydr Res 1996;281(2):187–201; doi: 10.1016/0008-6215(95)00350-9
38. Pang Z, Otaka K, Maoka T, et al. Structure of β-glucan oligomer from laminarin and its effect on human monocytes to inhibit the proliferation of U937 cells. Biosci Biotechnol Biochem 2005;69(3):553–558; doi: 10.1271/bbb.69.553
39. Spicer SE, Adams J, Thomas DS, et al. Novel rapid method for the characterisation of polymeric sugars from macroalgae. J Appl Phycol 2017;29(3):1507–1513; doi: 10.1007/s10811-016-0995-0
40. Gastebois A, Mouyna I, Simenel C, et al. Characterization of a new β(1–3)-glucan branching activity of *Aspergillus fumigatus*. J Biol Chem 2010;285(4):2386–2396; doi: 10.1074/jbc.M109.077545
41. Dobruchowska JM, Jonsson JO, Fridjonsson OH, et al. Modification of linear $\beta(1\rightarrow3)$-linked gluco-oligosaccharides with a novel recombinant β-glucosyltransferase (trans-β-glucosidase) enzyme from *Bradyrhizobium diazoefficiens*. Glycobiology 2016;26:1157–1170; doi: 10.1093/glycob/cww074
42. Hreggvidsson GO, Dobruchowska JM, Fridjonsson OH, et al. Exploring novel non-leloir β-glucosyltransferases from proteobacteria for modifying linear (B1→3)-linked gluco-oligosaccharide chains. Glycobiology 2011;21(3):304–328; doi: 10.1093/glycob/cwq165
43. Mantovani MS, Bellini MF, Angeli JPF, et al. β-Glucans in promoting health: Prevention against mutation and cancer. Mutat Res 2008;658(3):154–161; doi: 10.1016/j.mrrev.2007.07.002
44. Månberger A, Verbrugghe P, Guðmundsdóttir EE, et al. Taxogenomic assessment and genomic characterisation of *Weissella cibaria* strain 92 able to metabolise oligosaccharides derived from dietary fibres. Sci Rep 2020;10(1):5853; doi: 10.1038/s41598-020-62610-x
45. EFSA/European Food Safety Authority Panel. Scientific opinion on the safety of 'Yeast beta-glucans' as a novel food ingredient. EFSA J 2011;9:21–37; doi: 10.2903/j.efsa.2011.2137
46. Chan ASH, Jonas AB, Qiu X, et al. Imprime PGG-mediated anti-cancer immune activation requires immune complex formation. PLoS One 2016;11(11):e0165909; doi: 10.1371/journal.pone.0165909
47. Chan ASH, Kangas TO, Qiu X, et al. Imprime PGG enhances anti-tumor effects of tumor-targeting, anti-angiogenic, and immune checkpoint inhibitor antibodies. Front Oncol 2022;12:869078; doi: 10.3389/fonc.2022.869078
48. Adams EL, Rice PJ, Graves B, et al. Differential high-affinity interaction of dectin−1 with natural or synthetic glucans is dependent upon primary structure and is influenced by polymer chain length and side-chain. J Pharmacol Exp Ther 2008;325(1):115–123; doi: 10.1124/jpet.107.133124
49. Bligh M, Nguyen N, Buck-Wiese H, et al. Structures and functions of algal glycans shape their capacity to sequester carbon in the ocean. Curr Opin Chem Biol 2022;71:102204; doi: 10.1016/j.cbpa.2022.102204
50. Zvyagintseva TN, Usoltseva RV, Shevchenko NM, et al. Structural diversity of fucoidans and their radioprotective effect. Carbohydr Polym 2021;273:118551; doi: 10.1016/j.carbpol.2021.118551
51. Colin S, Deniaud E, Jam M, et al. Cloning and biochemical characterization of the fucanase FcnA: Definition of a novel glycoside hydrolase family specific for sulfated fucans. Glycobiology 2006;16(11):1021–1032; doi: 10.1093/glycob/cwl029
52. Zueva AO, Silchenko AS, Rasin AB, et al. Expression and biochemical characterization of two recombinant fucoidanases from the marine bacterium *Wenyingzhuangia fucanilytica* CZ1127T. Int J Biol Macromol 2020;164:3025–3037; doi: 10.1016/j.ijbiomac.2020.08.131
53. Vuillemin M, Silchenko AS, Cao HTT, et al. Functional characterization of a new GH107 endo-α-(1,4)-fucoidanase from the marine bacterium *Formosa haliotis*. Mar Drugs 2020;18(11); doi: 10.3390/MD18110562
54. Trang VTD, Mikkelsen MD, Vuillemin M, et al. The endo-α(1,4) specific fucoidanase Fhf2 from *Formosa haliotis* releases highly sulfated fucoidan oligosaccharides. Front Plant Sci 2022;13:823668; doi: 10.3389/fpls.2022.823668
55. Vickers C, Liu F, Abe K, et al. Endo-fucoidan hydrolases from glycoside hydrolase family 107 (GH107) display structural and mechanistic similarities to -L-fucosidases from GH29. J Biol Chem 2018;293(47):18296–18308; doi: 10.1074/jbc.RA118.005134
56. Schultz-Johansen M, Cueff M, Hardouin K, et al. Discovery and screening of novel metagenome-derived GH107 enzymes targeting sulfated fucans from brown algae. FEBS J 2018;285(22):4281–4295; doi: 10.1111/febs.14662
57. Mikkelsen MD, Tran VHN, Meier S, et al. Structural and functional characterization of the novel endo-α(1,4)-fucoidanase Mef1 from the marine bacterium *Muricauda ecklonia*e. Acta Crystallogr D Struct Biol 2023;79(Pt 11):1026–1043; doi: 10.1107/S2059798323008732
58. Tran VHN, Nguyen TT, Meier S, et al. The endo-α(1,3)-fucoidanase Mef2 releases uniquely branched oligosaccharides from *Saccharina latissima* fucoidans. Mar Drugs 2022;20(5):305–325; doi: 10.3390/md20050305
59. Liu G, Shen J, Chang Y, et al. Characterization of an endo-1,3-fucanase from marine bacterium *Wenyingzhuangia aestuarii*: The First member of a novel glycoside hydrolase family GH174. Carbohydr Polym 2023;306:120591; doi: 10.1016/j.carbpol.2023.120591

60. Shen J, Chang Y, Zhang Y, et al. Discovery and characterization of an endo-1,3-fucanase from marine bacterium *Wenyingzhuangia fucanilytica*: A novel glycoside hydrolase family. Front Microbiol 2020;11:1674; doi: 10 .3389/fmicb.2020.01674

61. Wang J, Liu Z, Pan X, et al. Structural and biochemical analysis reveals catalytic mechanism of fucoidan lyase from *Flavobacterium* Sp. SA-0082. Mar Drugs 2022;20(8):533; doi: 10.3390/md20080533

62. Sakai T, Kimura H, Kojima K, et al. Marine bacterial sulfated fucoglucuronomannan (SFGM) lyase digests brown algal SFGM into trisaccharides. Mar Biotechnol (NY) 2003;5(1):70–78; doi: 10.1007/s10126-002-0056-3

63. Cao HTT, Mikkelsen MD, Lezyk MJ, et al. Novel enzyme actions for sulphated galactofucan depolymerisation and a new engineering strategy for molecular stabilisation of fucoidan degrading enzymes. Mar Drugs 2018;16(11); doi: 10 .3390/md16110422

64. Silchenko AS, Rubtsov NK, Zueva AO, et al. Fucoidan-active α-L-fucosidases of the GH29 and GH95 families from a fucoidan degrading cluster of the marine bacterium *Wenyingzhuangia fucanilytica*. Arch Biochem Biophys 2022;728: 109373; doi: 10.1016/j.abb.2022.109373

65. Silchenko AS, Rasin AB, Zueva AO, et al. Fucoidan sulfatases from marine bacterium *Wenyingzhuangia fucanilytica* CZ1127T. Biomolecules 2018;8(4); doi: 10.3390/biom8040098

66. Mikkelsen MD, Cao HTT, Roret T, et al. A novel thermostable prokaryotic fucoidan active sulfatase PsFucS1 with 0. Sci Rep 2021;11(1):19523; doi: 10 .1038/s41598-021-98588-3

67. Jönsson M, Karlsson EN. Chemical food safety of seaweed: Species, spatial and thallus dependent variation of potentially toxic elements (PTEs) and techniques for their removal. Appl Phycol 2023; doi: 10.1007/s10811-023-03131-8

68. WHO/World Health Organization. 10 chemicals of Public Health Concern. WHO, Geneva, CH. 2020. Available from: https://www.who.int/news-room/photo-story/photo-story-detail/10-chemicals-of-public-health-concern

69. Brownlee IA, Seal CJ, Wilcox M, et al. Applications of alginates in food. In: Alginates: Biology and Applications, Rehm B (ed.), 2009, pp 211–228, Springer, Berlin.

70. Krook JL, Duinker A, Larssen WE, et al. Approaches for reducing the iodine content of the brown seaweed *Saccharina latissima*—Effects on Sensory Properties. J Appl Phycol 2023; doi: 10.1007/s10811-023-02974-5

71. Trigo JP, Stedt K, Schmidt AEM, et al. Mild blanching prior to pH-shift processing of *Saccharina latissima* retains protein extraction yields and amino acid levels of extracts while minimizing iodine content. Food Chem 2023;404(Pt A): 134576; doi: 10.1016/j.foodchem.2022.134576

72. Noriega-Fernandez E, Sone I, Astrain-Redin L, et al. Innovative ultrasound-assisted approaches towards reduction of heavy metals and iodine in macroalgal biomass. Foods 2021;10(3):649; doi: 10.3390/ foods10030649

73. Ohmes J, Mikkelsen MD, Nguyen TT, et al. Depolymerization of fucoidan with endo-fucoidanase changes bioactivity in processes relevant for bone regeneration. Carbohydr Polym 2022;286:119286; doi: 10.1016/j.carbpol.2022.119286

Address correspondence to:
Anne S. Meyer
Department of Biotechnology and Biomedicine
DTU Bioengineering
Building 221
Technical University of Denmark
Lyngby
Denmark

E-mail: asme@dtu.dk