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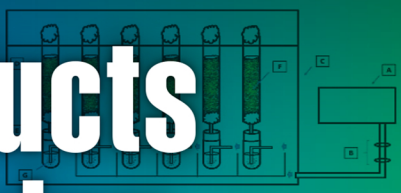

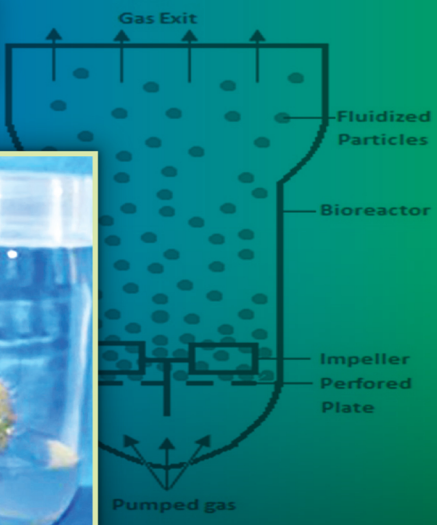

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Mónica L. Chávez-González, MSc, PhD, has been a full professor and researcher at the School of Chemistry of the Universidad Autónoma de Coahuila since 2016. She is member and leader of a research line in the Bioprocesses and Bioproducts Research Group of the Food Research Department.

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Prof. Chávez-González earned her master's and PhD in Food Science and Technology with an emphasis in valorization of food industrial waste. She is a member of Mexican Association for Food Science (AMECA), Mexican Society for Biotechnology & Bioengineering (SMBB, and the Mexican Association for the Protection of Food (AMEPAL) affiliate at the International Association for Food Protection (IAFP). She has received awards for her academic excellence and technological innovation. She has been recognized as a Member (Candidate) of the National Research Scientists System of México by the National Council of Science and Technology of the Government of México since 2016, and she has been recognized as a New Professor with desirable Profile by the Secretary of Education, Government of México. Prof. Chávez-González has published seven papers in indexed journals, 15 book chapters, and three editions of international books.

Nagamani Balagurusamy, PhD, has been a professor at the Universidad Autonoma de Coahuila, México since September 2001. Previously, he worked as an associate professor at Tamil Nadu Agricultural University, India.

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He has nearly 30 years of experience in teaching courses on biochemistry, microbial biochemistry, biotechnology and environmental engineering to undergraduate students of the Biochemical Engineering Program; and microbial physiology, bioremediation, and microbial molecular ecology to postgraduate students. Further, he is involved in guiding students on microbial diversity, microbial interactions, and microbial enzymes and their application in the food industry and in bioenergy production.

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Dr. Aguilar has been a member at level III of the National System of Researchers of México (S.N.I.) since 1998. He has been awarded with several prizes, including the Outstanding Researcher Award 2019 (International Bioprocessing Association, IBA-IFIBiop 2019); Prize of Science, Technology and Innovation Coahuila 2019; National Prize of Research 2010 of the Mexican Academy of Sciences; the Prize-2008 of the Mexican Society of Biotechnology and Bioengineering; National Prize AgroBio-2005; and the Mexican Price in Food Science and Technology, CONACYT-Coca Cola México 2003. He has been a member of the Mexican Academy of Science since 2014. Dr. Aguilar has developed more than 25 research projects, including six international exchange projects. He has been an adviser on 18 PhD theses, 25 MSc theses, and 50 BSc theses. He is member of the International Bioprocessing Association, the Mexican Academy of Sciences, the Mexican Society for Biotechnology & Bioengineering, and the Mexican Association for Food Science and Biotechnology. His research is in the areas of the design of bioprocesses for bioproducts production (of importance in Hort/Food/Pharma/Environ sectors) and in fermentation and enzymatic technologies. He is associate editor of *Frontiers in Sustainable Food Systems* (Section: Sustainable Food Processing) and is a member on several editorial boards, including *HELIYON* (Elsevier), *International Journal of Green Technology,* and *Food & Drug Research, Información Tecnológica*, among others.

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5 Biological Catalyzers of Brown Seaweed: Biochemical Properties, Production, and Applications

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CONTENTS

5.1 INTRODUCTION

Marine seaweeds are a variable group of photosynthetic organisms that are classified according to their morphological characteristics and mainly for the presence of structural pigments that will determine the category group to which they are going to belong: red, brown, or green seaweeds (Domínguez 2013; Ngo and Kim 2013).

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According to the database of the Food and Agriculture Organization of the United Nations, by 2014, the world's seaweed production had reached 25,665,934 tons. China produced more than 13 million tons, which is equivalent to 50% of this production. Other principal producers are Asian countries such as Indonesia, the Philippines, and Korea. Denmark is the principal non-Asiatic producer, with less than 0.1% of the world's production.

Throughout history, Asiatic countries have been recognized for taking advantage of seaweed, particularly Japan, China, and Korea. Seaweed cultivation has become a major industry—principally as a source of food and for the obtainment of hydrocolloids such as agar, carrageenan, and alginate (Smit 2004), which have been used as gelling agents and stabilizers in the food and pharmaceutical industries as animal feed, fertilizers, and for industrial applications (Guiry 2012; Mohamed et al. 2012; Stengel and Connan 2015; Cervantes-Cisneros et al. 2017).

In several coastal areas, seaweed are considered a waste because they represent a problem for the local ecosystem due to their high rates of growth and reproduction, which creates problems for fishing, recreational activities, and in other areas (Balboa et al. 2015). This means that in these regions, the seaweed biomass is not currently exploited.

Looking to increase the use of seaweed, there have been more studies and research on the extraction of components and compounds such as soluble and sulfate polysaccharides, peptides, vitamins, minerals, and phenolic compounds (Kadam et al. 2015a, 2015b; Argüello-Esparza et al. 2019). Traditional methods used for the extraction of these compounds are generally based on the use of heat with water, acid, and alkaline solvents, but these techniques usually report long extraction times, high energy consumption, and low extraction yields (Yin et al. 2014; Kadam et al. 2015b; Ruiz et al. 2015; Kadam et al. 2017). This has led to the development of novel extraction procedures in order to obtain higher yields with less energy used in shorter time periods, such as using specific enzymes to disrupt the cell wall structure of seaweed and to degrade or depolymerize internal compounds for an easier extraction. The aim of this chapter is to give an update on the seaweed biomass and the specific biological catalyzers of brown seaweed that are capable of simplifying the elucidation of the structure of the most relevant seaweed polysaccharides. The development of enzyme-assisted extraction is a promising technique not only because it is environmentally friendly but also because it is the most specific method available.

5.2 STRUCTURAL COMPOSITION OF SEAWEED

The taxonomical classification of seaweed is principally divided into four groups according to the presence of specific pigments. The characteristic color of green algae, or chlorophyte, is due to the presence of chlorophyll, which is the phylum Rhodophyta that gets its red tones from the abundance of phycoerythrin and phycocyanin, and the phylum Phaeophyta, in which the brown or yellow-brown hue characteristics are attributed to the presence of fucoxanthin (Quitral et al. 2012; Domínguez 2013; Kılınc et al. 2013). Many authors report that seaweed composition varies depending on factors such as growth and climatic conditions such as light, nutrients, temperature, geographical localization, the seaweed species, and

the season of harvesting (Banerjee et al. 2009; Schiener et al. 2014; Chakraborty et al. 2016; Tabassum et al. 2016).

Seaweed's cell walls are similar to land plants and other lignocellulosic materials and consist mainly of cellulose and hemicellulose but in a lower ratio. Also the cell walls are made up of complex biomolecules such as sulfated and branched polysaccharides, commonly associated with proteins and ions (Kadam et al. 2013; Raimundo et al. 2016) (Figure 5.1). These cell wall storage polysaccharides are specific according to the seaweed species. For example, red seaweed contains carrageenan, agar, xylan, floridean starch, and porphyrans; green seaweed contains ulvans, sulphated galactans, xylans, and sulphuric acid polysaccharides; and brown seaweed contains alginate, fucoidan, laminarin, and sargassan (Popper et al. 2011; Kraan 2012). The composition of these polysaccharides protects the seaweed against osmotic stress, pH and temperature changes, and metal toxicity, and also provides flexibility to resist strong ocean currents and antioxidant properties (Deniaud-Bouët et al. 2014; Ficko-Blean 2015). In recent years, these kinds of brown seaweed polysaccharides have attracted attention for their bioactive properties—such as being an antioxidant, anti-inflamatory, antiallergy, antiviral, antitumoral, among others (Berteau and Mulloy 2003; Ale and Meyer 2013; Ngo and Kim 2013; Yuan and Macquarrie 2015). These properties are closely linked with molecular weight, sugar composition, sulfation level, and the distribution of the sulfate groups along the polysaccharide backbone (Ale and Meyer 2013; Balboa et al. 2013; Mak et al. 2013; Shao et al. 2013). Due to this relation between the polysaccharide structure and properties and its molecular weight and composition, it is crucial to develop techniques and methods for the extraction of polysaccharide fractions in a more specific way.

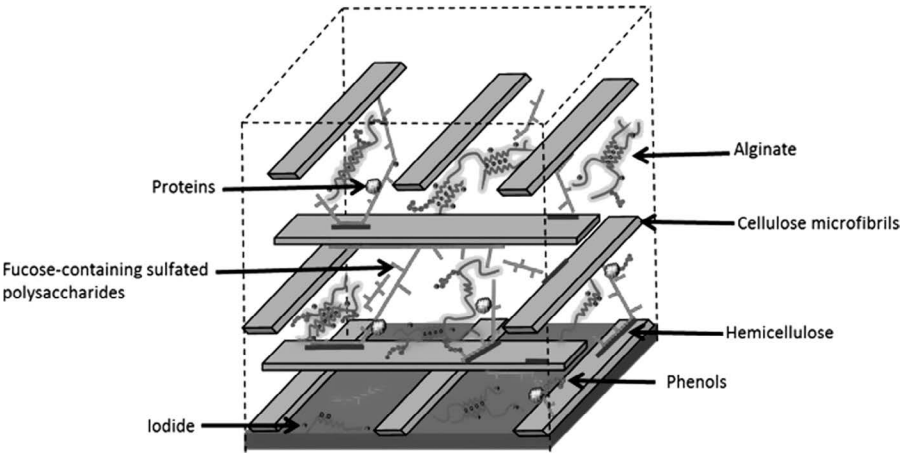

FIGURE 5.1 Hypothetical cell wall model from brown seaweed based on the model proposed by Deniaud-Bouët et al. (2014). The content of cellulose microfibrils is dispersed to a lesser extent than in terrestrial plants; these take on a flat ribbon shape. The fucose sulfate polysaccharides and alginates form the greater part of the cell wall polymers; the phenols are commonly associated with alginates and proteins.

5.3 EXTRACTION METHODS FOR SEAWEED POLYSACCHARIDES

The traditional methods for the extraction and/or modification of seaweed polysaccharides of different sizes and heterogeneity are frequently carried out by physicochemical methods, either by conventional techniques (solvent precipitation, acid/base reaction, soxhlet, etc.) or by emerging technologies (microwave, ultrasound, steam explosion, etc.) (Balboa et al. 2013; Kadam et al. 2015b; Yuan and Macquarrie 2015). Figure 5.2 shows a scheme of the main methods for obtaining the extraction compounds in seaweeds. The conventional extraction methods show some disadvantages, such as the long extraction times with low yields. As reported by Lim et al. (2014) for fucoidan extraction from *Sargassum binderi* with $CaCl_2$ at 85°C for periods of 24 h repeating six times, the environmental contamination with the use of chemicals as a solvent plays an important role in the negative aspect of these methods. On the other hand, the use of emerging technologies has developed extraction processes with more extraction yields, generating less contamination with lower costs, less time, and less consumption of energy. In spite of these advantages, with these kinds of technologies, it is unknown how they affect the biological properties and structure of the polysaccharides (Hahn et al. 2012). However, these compounds can be susceptible to these kinds of thermochemical methods where it is difficult to determine the hydrolytic point in the polysaccharide chain, thereby affecting the reproducibility of the production yield and the composition of the target compounds.

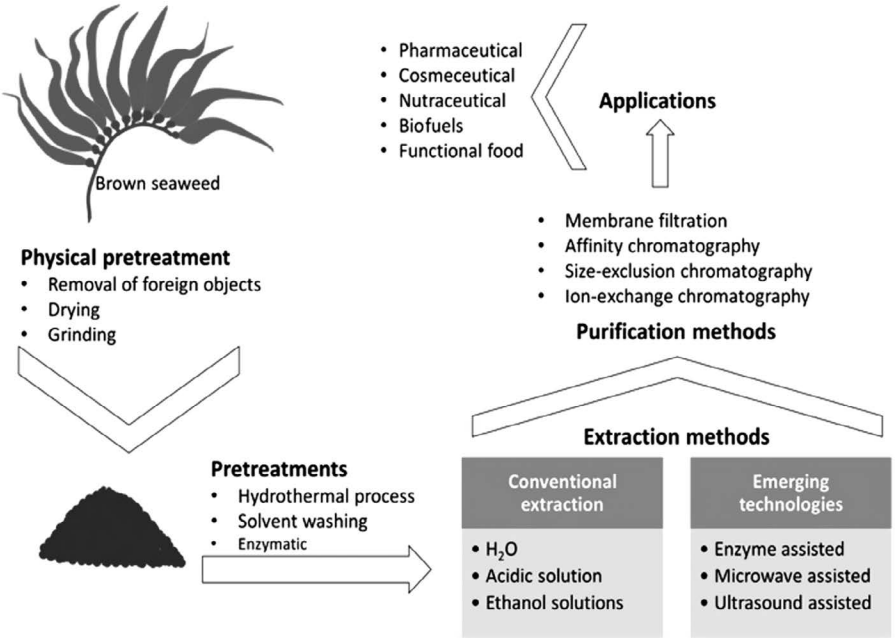

FIGURE 5.2 Sequencing scheme of the extraction methods for polysaccharides and other compounds in brown seaweed.

Moreover, the seaweed cell wall is more complex than the cell wall of terrestrial plants; the combination of sulfated and branched polysaccharides and other compounds is a characteristic that can affect the efficiency of general extraction procedures. To improve the use of cell wall degrading enzymes, an alternative extraction process can expedite the access to the seaweed compounds and derived metabolites. Actually, there are reports of the hydrolysis of the seaweed cell wall using enzymatic methods that show considerable yields of poly- and oligosaccharide fractions with relevant biological and chemical properties. Enzyme assisted extraction (EAE) is a promising alternative technology to the conventional methods that offers biotechnological procedures with many advantages, such as high catalytic efficiency and high specificity, and it is nontoxic and ecofriendly as well. In addition, the use of specific enzymes over seaweed substrate allows the extraction to be performed in mild conditions that can promote conserving the bioactivity of the extracts (Athukorala et al. 2006; Hahn et al. 2012; Wijesinghe and Jeon, 2012; Ale and Meyer 2013; Rodrigues et al. 2015; Siller-Sánchez et al. 2019).

5.4 ENZYME CATALYZERS OF BROWN SEAWEED

5.4.1 Alginate Lyases

The alginate lyases, or alginases, catalyze the degradation of alginate; alginate is the most abundant polysaccharide of brown seaweed (about 40% of dry weight). This polysaccharide is mainly composed of β-D-mannurate (M) and α-L-gluronate (G); its structure varies depending on the position of the monomer in the chain; and these units are linked in three kinds of segments or blocks—homopolymeric G blocks; poly α-L-guluronate (polyG), homopolymeric M blocks; and poly β-D-mannuronate (Yang et al. 2011; Lee and Mooney 2012; Pawar and Edgar 2012) (Figure 5.3).

The products of the depolymerization of alginate by the physicochemical method, or by the use of alginate lyase, are known as alginate oligosaccharides. These compounds and their derivatives have been attracting attention in recent years due to their bioactivity, such as the induction of tumor necrosis factor (TNF)-α^{37} and its wide use in the food, cosmetic, and pharmaceutical industries (Zhang et al. 2004; Li et al. 2011). Alginate lyase degrades alginate by β-elimination of glycosidic bonds and produces unsaturated oligosaccharides with double bonds at the nonreducing end (Wong et al. 2000; Lxa et al. 2008; Ogura et al. 2008; Hehemann et al. 2014). The alginases have been isolated from many different sources, such as marine mollusks, marine seaweed, terrestrial bacterial, and other microorganisms (Gacesa 1992; Sawabe et al. 1997; Ogura et al. 2008; Kim et al. 2013).

Gomaa et al. (2015) reported high specific alginase activity found in *acrophialophora* sp. and *Setosphaeria rostrata,* two algicolous fungi. The alginate lyases can be classified in two groups depending on their substrate specificities: (a) the G block-specific polyguluronate lyase (poly G) and (b) the M block-specific polymannuronate lyase (poly M) (Kim et al. 2012; Park et al. 2012). However, there are reports that show some enzymes having the ability to act on both links—the polyG and polyM. Likewise, these enzymes can be classified into endolytic and exolytic alginate lyase, depending on their mode of action (Balboa et al. 2013). Recent studies indicate that

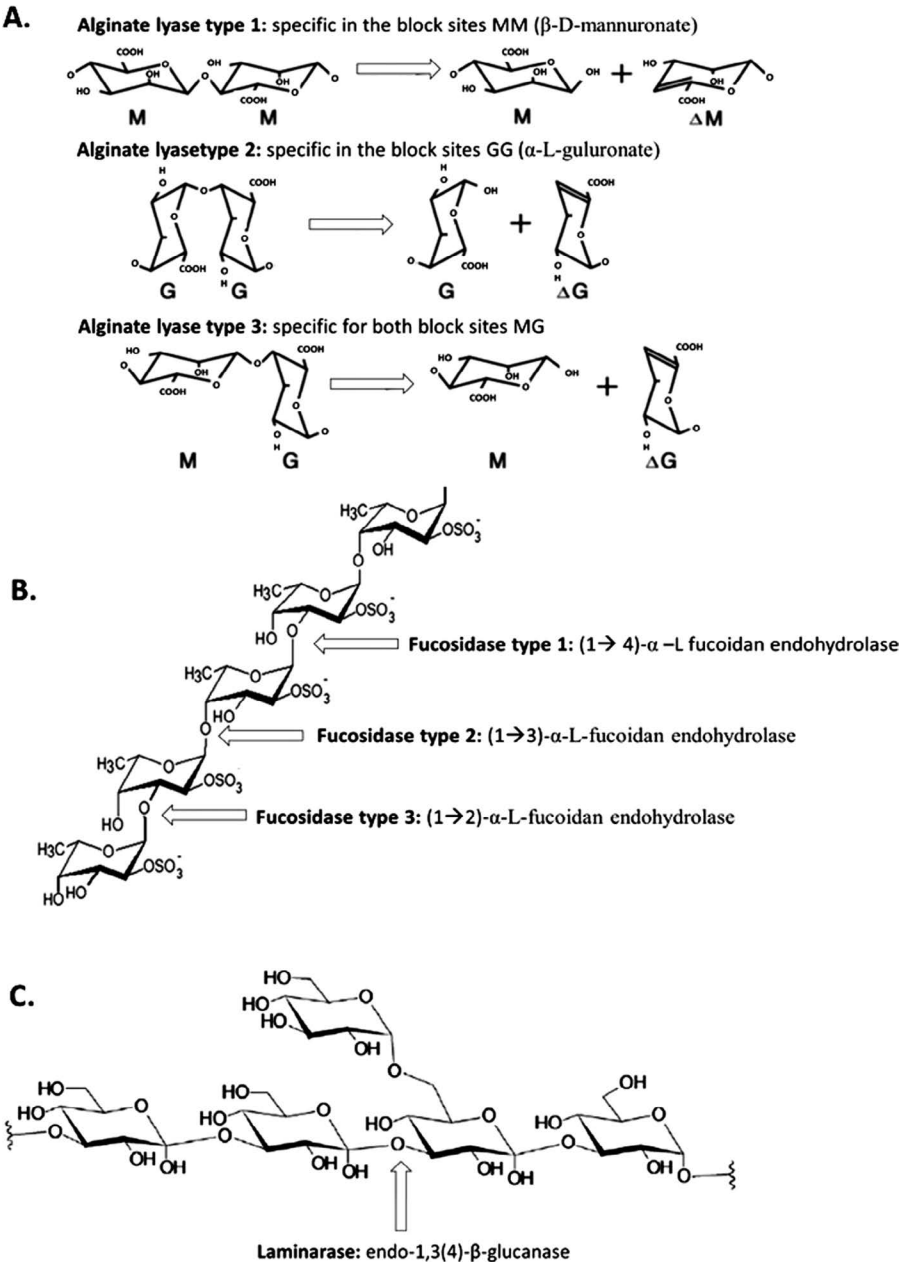

FIGURE 5.3 Chemical structure of the main polysaccharides in brown seaweed. (A) Alginate structure and alginate lyase block site action. (B) Common Fucoidan structure and fucosidase types depending on the specific site of cleavage. (C) Laminarin structure and laminarase cleavage action.

the alginate lyases have important biological applications such as the production of oligosaccharides with various biological functions and in determining the structure of alginate and alginases, which can be used in the treatment of some pathologies in combination with antibiotics (Zhu and Yin 2015).

5.4.2 Fucoidanases

The enzymes capable of degrading or modifying the fucoidan polysaccharides are called fucosidases or fucoidanases. The fucoidans are sulphated polysaccharides mainly consisting of sulfate L-fucose and the sulfate ester groups. These polysaccharides are principally found in brown seaweeds and in some marine invertebrates, such as sea urchins and sea cucumbers (Li et al. 2008; Ale and Meyer 2013). These polysaccharides are important for their high bioactive properties, such as anticoagulants, and their antithrombotic (Cho et al. 2010; Kwak et al. 2010), antiviral (Ahmadi et al. 2015), antitumoral, and anti-inflammatory aspects (Raghavendran et al. 2011; Yuan and Macquarrie 2015). The properties of the fucoidans are related to their molecular weight, sulfate content, the position of their sulfate ester groups, and their monosaccharide composition (Li et al. 2008; Hahn et al. 2012; Morya et al. 2012). However, almost all the methods used for the extraction of fucoidan can cause structural alterations, and fucoidanases are useful for the partial depolymerization of these polysaccharides without modifying the structural units of the fucoidan (Hahn et al. 2012; Ale and Meyer 2013; Rodrigues et al. 2015).

The main natural sources for obtaining these fucoidan-degrading enzymes are several marine microorganisms such as marine bacteria—for example, members of the bacterodetal *Flavobacteria* (Mann et al. 2013; Ficko-Blean 2015), *Vibrio sp., Alteromonadaceae sp.* (Sakai 2004), *Pseudoalteromonas sp., Cytophaga, Fucanobacter lyticus* (Holtkamp et al. 2008; Ale and Meyer 2013), and some marine fungus *Dendryphiella arenaria* (Wu et al. 2011). Likewise, it is reported in marine mollusks and invertebrates. Silchenko et al. (2014) reported that the isolation of fucoidanase from the digestive glands of the marine mollusk *Lambis* sp. is capable of hydrolysating the fucoidan from *Fucus vesiculosus* and *Fucus evanescens*; other authors report the isolation from *littorina kurila* (Kusaykin et al. 2006) and several species of abalone *(Haliotis sp.),* scallops *(pectinidae sp.)* (Berteau and Mulloy 2003), *Chamalea gallina,* and *pomacea canaliculata*. But, in the last decade, a few papers reported the presence of fucoidanases in the digestive systems of arachnids and ticks (Moreti et al. 2003). There are very few reports of the production of these enzymes by solid-state fermentations, such as those reported by Qianqian et al. (2011) using *Fusarium* sp., a marine fungus isolated from sand in Germany's North Sea. Rodriguez-Jasso et al. (2013) reported the production capacity of these enzymes in solid-state fermentation using seaweed biomass with some terrestrial organisms, such as filamentous fungi as *Aspergillus niger* and *Mucor sp.*

Little is known about the mechanisms that these enzymes possess. The fucosidases are able to cleave special glycosidic bonds in the polysaccharide chain, and this cleavage pattern depends on the source of the production of the enzyme. Presently, these enzymes are classified in three groups: α-L-fucosidases, which cause the release of the L-fucose from the nonreducing end of the polysaccharide;

and the fucoidanases or fucansulphatehydrolases, which have two kinds of cleavage patterns—the first is the endo-fucoidanase that cleaves the core of the polysaccharide producing oligosaccharides, and the second is the exo-fucoidanase that cleaves the edge of the polysaccharide with a slow decrease of the molecular weight-producing monomers (Figure 5.3) (Berteau and Mulloy 2003; Wu et al. 2011; Silchenko et al. 2013).

5.4.3 Laminarinases

The laminarinases are also known as β-(1→3)-glucanases and are enzymes with the ability to hydrolyze β-1,3- and β-1,4-glycosidic bonds of the laminarin (Cano-Salazar et al. 2011) (Figure 5.3). This class of enzyme is typical in bacteria, archaea, and some eukaryotic organisms, mainly fungi. Giese et al. (2006) investigated the hydrolysis of laminarin from *Lamianria digitate* by the preparation of β-1,3-glucanases from two filamentous fungi, *Botryosphaeria rhodina* and *Trichoferma harzianum*. They have already classified some structural and chemical β-1,3-glucanases. But, in the case of β-1,3-glucanases, organisms that are in contact with genuine laminarin from macroalgae reports are scarce. In recent years, research on these enzymes has increased. Kobayashi et al. (2016) reported the purification and homogeneity of β-1,3 glucanase produced by the sub-seafloor bacteria *Laceyella putida*, and Zhang et al. (2004) found a β-glucosidase capable of degrading the laminarin precedent from *Vibrio campbellii*.

Laminarin is present in the cell walls of brown algae. This is a reserve polysaccharide that provides strength and flexibility, maintains ionic equilibrium, and prevents desiccation. This low molecular weight polysaccharide is composed of β-(1,3) glucans with occasional β-(1-6) branches (Lynch et al. 2010; Hou et al. 2015). The beta-glucans are composed of monomers of D-glucose. These heterogeneous carbohydrates differ from each other by their structural properties, such as their molecular weight, length, and degree of branching (Volman et al. 2010; Fuentes et al. 2011). Likewise, some investigations show that these polysaccharides have immune-modulating effects and can be considered as dietary fiber. Not only does the structure affect these bioactive properties, isolation methods can also influence the properties of β-glucans (Volman et al. 2010).

5.4.4 Other Enzymes Capable of Degrading the Cell Walls of Brown Seaweed

With the rise in the use of enzymatic hydrolysis of brown seaweed, studies to increase and improve yields have led to the use of nonspecific enzymes of seaweed polysaccharides—either in combination with various enzymes or using only specific enzymes such as alginases, laminarinases, fucosidases, etc. The vital factors for an efficient cell wall degradation include the selection of a mixture of enzymes suitable for the optimal conditions of the reaction, such as temperature and pH that are specific for each kind of enzyme (Wijesinghe and Jeon 2012; Heo et al., 2015). The proteases, classified as endopeptidases or exopeptidases depending on their hydrolyzing mechanism, and the carbohydrate degrading enzymes are the principal types

TABLE 5.1
Optimal Conditions of Some of the Most Commonly Used Commercial Enzymes

Enzyme	Enzyme Characteristics	Seaweed	Optimal Conditions		Reference
			Temperature (°C)	pH	
Vizcozyme	Arabanase, cellulase, β-glucanase, hemicellulase, and xylase	*Ecklonia cava*	50	4.5	(Heo et al. 2005; Kim et al. 2006)
Celluclast	Cellulase	*Ecklonia cava*	50	4.5	
Termamyl	Thermos-stable α-amylase	*Ecklonia cava*	60	6.0	
Alginate lyase	β-(1,4)-glucosiase	*Macrocystis pyrifera*	37	6.3	(Ravanal et al. 2016)
Kojizyme	Endoprotease and exo-peptidase	*Ecklonia cava*	40	6.0	(Ahn et al. 2008)
Laminarase	Endo-1,3-β-glucanase	*Laminaria digitate* *Luminaria hyperborean* *Saccharina latissima*	45	5.1	(Schiener et al. 2015)

of enzymes used for the enzymatic hydrolysis of the seaweed cell wall. The most common enzymes used for cell wall hydrolysis and the extraction of biocompounds are summarized in Table 5.1.

Rodrigues et al. (2015) reports the use of two carbohydrate-degrading enzymes, Vizcozyme L, a multi-enzyme complex of carbohydrate hydrolases—such as arabanase, cellulase, β-glucanase, hemicellulose, xylanase, Cellulase, and two proteases, Alcalase and endopeptidase. The second compound is Flavourzyme, an endoprotease and exopeptidase. Both complexes are used for the extraction of bioactive compounds. The results show that the higher extraction yields correspond to Vizcozyme L and cellulase. The extraction efficiency and advantages provided by the cellulases are due to the extraction efficiency of targeted compounds and the robustness.

5.5 CURRENT APPLICATIONS OF THE ENZYME CAPABLE OF DEPOLYMERIZING SEAWEED POLYSACCHARIDES

Since the seaweed's biomass is a recognized as an adequate source of polysaccharides and other value compounds, as well as an alternative to the high demand for fuels. In the development of the use of green processes, the use of enzymatic hydrolysis leads one of the most appropriate methods for the depolymerization of compounds, such as those present in the cell wall of seaweed. For that reason, studies in this area have improved over the years. Nowadays, enzymatic hydrolysis and enzyme assisted extraction (EAE) techniques generally are still used on pilot and laboratory scales. Current studies on the use of enzymes in seaweed focus on their use in biorefineries

as a method of converting seaweed biomass into bioethanol via enzymatic hydrolysis, where sugars released by enzymes are used in fermentative process. Due to the absence of lignin in seaweed biomass, bioethanol production with this solid waste does not require pretreatment—unlike most commonly used raw materials (Tan and Lee 2014), which is an advantageous attribute. There are many reports of other compounds of interest, such as the investigation of Hou et al. (2015), who performed an integrated bioethanol and protein production using the brown seaweed *Laminaria digitate*. These investigations try to increase economic feasibility by recovering an important dietary complement from an unexploited source. However, enzyme application is not limited to biofuel production. There are many reports showing that the extracts obtained by enzyme-assisted extraction have a greater antioxidant capacity compared to the extracts obtained by conventional methods. Furthermore, Athukorala et al. (2006) reported that the cell proliferation inhibition on cancer cell lines of, and enzymatic extract from, *Ecklonia cava*, show the capacity of the enzymatic-assisted extraction for breaking the complex bonding between phenolics and proteins in the seaweed cell wall. In the same way, in vitro studies performed by Charoensiddhi (2015) showed that the enzyme extracts from *Ecklonia cava* processed with a carbohydrase mix have a promising prebiotic potential. There are reports of the use of EAE of a sulfated polysaccharide from *E. cava* with a potential modulation of anti-inflammatory agents (Lee et al. 2012). In all the cases, the use of hydrolytic enzymes has been combined for the rupture of cell walls, breaking down the structure and liberating the metabolites of interest from the seaweed.

5.6 PERSPECTIVES AND CONCLUSIONS

Seaweeds make up a significant source of novel compounds. The use of enzymatic-assisted extraction shows a potential for enhancing the extraction yield and improving the extractability of bioactive compounds such as peptides, carotenoids, fucoidans, and phlorotannins—all with promising applications in areas such as pharmaceuticals, functional foods, cosmetics, etc. With the capacity of enzymes to convert water-insoluble materials into water-soluble materials without using any other toxic chemicals and removing the most common mechanical barriers, EAE can be a useful method as an alternate stage in the solvent extraction process to improve yields and conserve bioactivity of compounds. In the same way, the use of enzymes in biofuel production in a biorefinery concept can be more profitable not only environmentally but also economically, with the recovery techniques of metabolites of interest. For that reason, aspects such as the investigation of optimal conditions of reaction of each enzyme, the correct use of enzymatic cocktails, as well as the search for new sources of enzymes each plays an important role in the development of this technology.

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