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Review

Marine prebiotics: Polysaccharides and oligosaccharides obtained by using microbial enzymes

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ABSTRACT

Utilization of marine algae has increased considerably over the past decades, since biodiversity within brown, red and green marine algae offers possibilities of finding a variety of bioactive compounds. Marine algae are rich sources of dietary fibre. The remarkable positive effects of seaweed dietary fibre on human body are related to their prebiotic activity over the gastrointestinal tract (GIT) microbiota. However, dietary modulation of microorganisms present in GIT can be influenced by different factors such as type and source of the dietary fibre, their molecular weight, type of extraction and purification methods employed, composition and modification of polysaccharide and oligosaccharide. This review will demonstrate evidence that polysaccharides and oligosaccharides from marine algae can be used as prebiotics, emphasizing their use in human health, their application as food and other possible applications. Furthermore, an important approach of microbial enzymes employment during extraction, modification or production of those prebiotics is highlighted.

1. Introduction

Health disorders related to modern lifestyle, and known as chronic diseases, are a major social and economic concern. Consciousness of the diet's role in these diseases (prevention over cure) and development of nutritional science led to an increased search for functional foods (Blatchford et al., 2013; Hasler, 2002; Plaza, Cifuentes, & Ibáñez, 2008; Siró, Kápolna, Kápolna, & Lugasi, 2008). Prebiotics are dietary ingredients classified as soluble or insoluble fibre that can selectively enhance beneficial components of the indigenous gut microbiota (Blatchford et al., 2013; Goñi & Martín-Carrón, 1998; Kedia, Vázquez, & Pandiella, 2008; Nawaz, Bakhsh Javaid, Irshad, Hoseinifar, & Xiong, 2018). Dietary fibre is a nondigestible fraction composed of resistant starches, polysaccharides and oligosaccharides, that may be associated with noncarbohydrate compounds, such as polyphenols, and resistant proteins (Corradini, Lantano, & Cavazza, 2013; Siró et al., 2008). Prebiotics can be found in a great variety of sources, such as milk, honey, fruits, vegetables and marine algae (Corradini et al., 2013). Marine algae are valuable sources of structurally diverse bioactive compounds presenting antibacterial activity, antioxidant potential, anti-inflammatory properties, anti-coagulant, anti-thrombotic, anti-viral activities among others (Gurpilhares et al., 2016). In addition, marine algae contain large amounts of complex carbohydrates that can be considered as prebiotics (Wells et al., 2017). Polysaccharides and oligosaccharides from marine algae have raised a great interest since they are easily cultivated and the production of some bioactive compounds can be controlled under optimized cultivation conditions or by using genetic engineering approaches (Ibañez & Cifuentes, 2013). In addition, the employment of microbial enzymes during extraction, purification or modification of these prebiotics represents an important tool to create novel structures with elucidation of their biological function (Gurpilhares et al., 2016; Liu, Willfor, & Xu, 2015). This review deals with the presentation of red, green and brown algae for potential use as functional food ingredients, together with the possibility of improving

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Table 1
Functional foods and their functional component with their possible effect.

Functional food	Functional Component	Possible effect	References
Tomato	Lycopene	Reduces risk of prostate cancer	Hasler (2002)
Red wine	Polyphenolic compounds	Reduce risk of cardiovascular disease	Zenebe, Pechánová, and Bernátová (2001))
Fatty fish	Fatty acids	Reduce risk of certain heart diseases	Hasler (2002)
Fermented dairy products	Probiotics	Support intestinal tract health, boost immunity	Hasler (2002)
Blueberry	Anthocyanin and other polyphenols	Antioxidant and anti-inflammatory actions (cardiovascular protection)	Lasekan (2014)
Spirulina platensis	Extracellular products (prebiotic)	Promote the growth of human gut microbiota	Parada, de Caire, de Mulé, and de Cano (1998)
Marine Algae	Soluble and insoluble fibre (prebiotic)	Growth of beneficial bacteria in the intestines	Holdt and Kraan (2011)

the polysaccharide and oligosaccharide production from these sources by using microbial enzymes, and their beneficial effects for health and other applications.

2. Functional food

For a long time, foods have been known to be sources of important nutrients, in addition to providing daily calorific value for humans (Corradini et al., 2013). Health and well-being demonstrate a close relationship with dietary quality (Beards, Tuohy, & Gibson, 2010). In this sense, many researchers have been evaluating foods and food components that provide specific health benefits beyond basic nutrition (Holdt & Kraan, 2011).

The correlation between diet and a lower incidence of chronic diseases, in addition to the prevention of age-related diseases, was demonstrated by several scientific studies in Japan, leading to a primary concept of functional foods as “a method to protect or improve consumer health” (Ibañez & Cifuentes, 2013). In 1991, Japanese Ministry of Health, Labor and Welfare established rules for the approval of FOSHU (Foods for Specified Health Uses), concerning health claims for the effect of this type of food in human body. In United States of America, the legal regulations are determined by Food and Drug Administration (FDA) (Ibañez & Cifuentes, 2013; Siró et al., 2008). Despite the existing regulations for each country, there is no official definition of functional foods (Vicentini, Liberatore, & Mastrocola, 2016).

In Europe, the European Commission supported and stimulated the scientific study on functional foods by the European Section of the International Life Science Institute (ILSI) inside the IV Framework Program (action FUFOSE – Functional Food Science in Europe). From this project, an appropriate definition for functional food could be established: a food can be considered as ‘functional’ if it has demonstrated beneficial effects on one or more target functions of human organism, beyond adequate nutritional effects, improving the state of health and well-being and/or decreasing the risk of disease. In addition, the amount of intake and form of functional food should be as expected for dietary purposes (Ibañez & Cifuentes, 2013; Plaza et al., 2008; Siró et al., 2008; Vicentini et al., 2016). However, European legislation considers functional food as a concept rather than a specific food category (Siró et al., 2008). According to a guideline approved in December 2006 by the European Union, the nutritional attributes and/or health properties of the new products need to be regulated (including their presentation, labeling and promotion) (Plaza et al., 2008).

Undoubtedly, there is a recognized demand for these products explained by the increasing cost of healthcare, the constant increase in life expectancy and change in lifestyle, in addition to the desire to improve life quality (Siró et al., 2008; Vicentini et al., 2016) This resulted in health claims being made for functional foods. According to the Codex Alimentarius (established by the United Nations through Food and Agriculture Organization – FAO and World Health Organization – WHO), a claim means “any representation which states, suggests or implies that a food has particular characteristics relating to its origin, nutritional properties, nature, production, processing, composition or any other quality”, this being the key factor for the development of the functional food market, which is dynamic and growing each year (Ibañez & Cifuentes, 2013; Siró et al., 2008; Vicentini et al., 2016).

The emergence of health conscious consumers and the development of nutritional science, which provides evidence on the role of certain food components by using valuable tools to unravel the mechanisms of action of the functional food ingredients, has driven not only the growth of functional foods markets but also the increase in the interest on discovering new functional food ingredients from different natural sources (Ibañez & Cifuentes, 2013).

2.1. Prebiotics versus probiotics

The importance of dietary factors on health status has been recognized since antiquity, but more recently, scientific studies have provided more information about the food habits and its relationship in supporting, or even improving, our health and disease prevention (Plaza et al., 2008). Based on the health criterion and on foods with specific health-enhancing characteristics, there has been growing interest in so-called functional food (Corradini et al., 2013).

Different categories of food may be included in the definition of “functional food”. Besides, the food recognized as functional can be natural, of animal, plant and microbial origin; or industrialized (Siró et al., 2008). Table 1 presents some recognized functional food ingredients with their possible effect on human health.

The functional components of the food are non-conventional biomolecules and include phytochemicals, which are plant-derived present in whole grains, roots, fruits and vegetables; non-starch carbohydrates, such as those from marine algae; probiotics; conjugated linolenic acid, long-chain omega-3, -6 and -9 polyunsaturated fatty acids and bioactive peptides found in animal products (e.g. milk, fermented milk products and cold water fish) (Abuajah, Ogbonna, & Osuji, 2015). Accordingly to Siró et al. (2008) the most prominent types of functional products could be represented by prebiotics, probiotics, functional drinks, functional cereals, bakery products, spreads (cholesterol-lowering spreads), functional meat and functional eggs.

One of the most interesting areas of research and innovation in the food industry is related to the functional foods. In addition, since consumers have shown interest in natural bioactive compounds, as functional ingredients in the diet, there has been an increase on prebiotics and probiotics demand (Corradini et al., 2013).

Probiotics are defined as a live microbial feed supplement, administered in adequate amounts to survive in the intestinal ecosystem, which affects the host animal in a beneficial way (Hoseinifar, Ringø, Masouleh, & Esteban, 2016). Some criteria have been proposed to consider a microorganism as probiotic: safe for the host, resistant to gastric acidity and pancreatic secretions, adherent to epithelial cells, antimicrobial activity, resistant to antibiotics, tolerant to food additives and stable in the food matrix. The most commonly used probiotics are the strains of lactic acid bacteria, as *Lactobacillus* and *Bifidobacterium*, but also strains of the genera *Bacillus*, *Pediococcus* and some yeasts. They contribute to the intestinal microbial balance and play an important role in the protection of the organism against harmful microorganisms, helping to strengthen the host's immune system (Soccol et al., 2010).

On the other hand, prebiotics are non-digestible food components that can selectively enhance beneficial microorganisms of the indigenous gut microbiota (Gibson, 2004). Some criteria are also applied to select a prebiotic: resistant to gastric acidity; hydrolysis by mammalian enzymes and gastrointestinal absorption; fermented by intestinal microbiome; selectively stimulates the growth and/or activity of intestinal bacteria related to health and well-being (Blatchford et al., 2013). In general, prebiotics are dietary fibres that comprise the carbohydrates, both oligosaccharides and polysaccharides, which can be subdivided into soluble and insoluble types (Corradini et al., 2013). Soluble dietary fibre swells in the stomach and increases the viscosity of the digesta leading to a lower postprandial blood glucose and insulin response with the decrease of nutrients absorption in the intestinal mucosa and an increase of satiety. In this sense, soluble fibre could be useful to control non-insulin dependent diabetes mellitus and to treat obesity. In another way, insoluble dietary fibre increases the fecal bulk and reduce the gastrointestinal transit time due to the higher water-holding capacity, this being useful in the prevention and treatment of different intestinal disorders, which could have an impact in reducing the incidence of colon cancer and irritable bowel syndrome (Goñi & Martín-Carrón, 1998; Kedia et al., 2008) (Table 2).

Despite the fact that prebiotics and probiotics are distinct functional foods, there is a great potential for a synergistic effect, when combining them appropriately, since prebiotics promote the growth activities of probiotics (Soccol et al., 2010)

3. Marine algae and enzymes from microorganisms

Algae are photosynthetic organisms with different origin and evolutionary history, commonly separated into two groups: 1) unicellular or colonial microalgae that inhabit not only oceans, but fresh water lakes, rivers, ponds and soil; and 2) multicellular marine organisms (macrophytes, seaweeds) (Usov & Zelinsky, 2013). Marine organisms are considered a rich source of natural products and special attention has been given to marine algae. Seaweed is another term to denominate marine algae, which can be classified according to their pigmentation into red (Rhodophyta), brown (Phaeophyta) and green (*Chlorophyta*) algae (Chan, Ho, & Phang, 2006).

Marine algae have ecological importance, since they supply oxygen to the sea, act as a primary producer in the marine food chain and have the capacity to remove heavy metals from the water. Moreover, they are widely used as ingredients in cosmetics and fertilizers, in pharmaceuticals and food industries as source of phycocolloids, thickening and gelling agents (Chan et al., 2006; Gómez-Ordóñez, Jiménez-Escrig, & Rupérez, 2010).

Due to the fact that some marine algae can adapt to changing environmental conditions and are able to live in complex habitats submitted to extreme conditions (e.g. salinity, UV irradiation, temperature) producing a great variety of secondary metabolites, they can be a very interesting natural source of compounds with biological activity that could be used as functional ingredients (Ibañez & Cifuentes, 2013; Plaza et al., 2008). Although varying from one type to another, the content of bioactive compounds of marine algae includes polyphenols, polysaccharides (Wijesinghe & Jeon, 2012), proteins, low lipid content with high concentrations of certain long-chain polyunsaturated fatty acids, vitamins and minerals (Table 3) (Rodrigues et al., 2015; Gómez-Ordóñez et al., 2010).

The increase in marine algae consumption is related to their functional components and their special nutritional properties (Gómez-Ordóñez et al., 2010). Some of the most extensively studied bioactive compounds, namely sulfated polysaccharides, phlorotannins and diterpenes, have been reported to possess anti-viral and anti-cancer properties. At the same time, the prebiotic health potential of the polysaccharides from seaweeds is also increasingly being studied. Prebiotic health potential is due to the presence of dietary fibre in edible marine algae. These soluble and insoluble fibres differ chemically from those of the terrestrial species and may induce different fermentative patterns, being the fibre content of seaweed varieties higher than those found in most fruits and vegetables (Gupta & Abu-Ghannam, 2011). The differences are related to the fact that algal cell walls contain uncommon polyuronides and polysaccharides that may be methylated, acetylated, pyruvylated and/or sulfated (Wells et al., 2017).

The consumption of algal fibre has been proven beneficial to human health. Humans possess the enzymes that degrade algal starches, being unable to digest more complex polysaccharides. Therefore, algal fibre is fermented in the large intestine to degrees depending on the enzymatic competence of the microbiome. As an example, soluble fibre can be fermented to short-chain fatty acids as acetate, propionate and butyrate that nourish and modify the microbial consortia in the large intestine (exerting prebiotic effects) (Wells et al., 2017). On the contrary, insoluble fibre is not hydrolyzed by microorganisms, and water associated with these components all serve to increase faecal bulk (Kedia et al., 2008). There are about 10,000 species of known marine algae, but only few of them are used in animal and human feed (Makkar et al., 2016). Edible marine algae may contain 33–75% total fibre on a dry weight basis, particularly rich in the soluble fractions (Gómez-Ordóñez et al., 2010; Holdt & Kraan, 2011) (Fig. 1).

Traditionally, seaweeds are part of the Oriental diet, especially in Japan, China and Korea, with the most consumed being nori (red algae – *Porphyra*, 33 species), wakame (brown algae – *Undaria*, 2 species) and kombu (brown algae – *Laminaria*, 12 species) (Jimenez-Escrig & Sanchez-Muniz, 2000; Lange, Hauser, Nakamura, & Kanaya, 2015). However, over the last decades, there has been an increase in seaweed consumption as food in other countries (Gómez-Ordóñez et al., 2010).

Although the scientific literature has been demonstrating the bioactivity of algal polysaccharides, the molecular structures responsible for such observed physiological functions are poorly

Table 2
Prebiotics as soluble and insoluble dietary fibre and their sources.

Prebiotic	Compound	Solubility	Sources
Oligosaccharide	Fructooligosaccharides (FOS)	Soluble	wheat, honey, onion, garlic, banana, barley, tomato, rye and brown sugar
	Galactooligosaccharides (GOS)	Soluble	Mammalian milk
	Xylooligosaccharides (XOS)	Soluble	Wheat bran and wheat straw, barley hulls, brewery spent grains, almond shells and corn cob
	Mannan-oligosaccharides (MOS)	Soluble	yeasts
Polysaccharide	Hemicellulose	Insoluble	Oats, cereals, grains, bread, fruit, legumes and nuts
	Pectins	Insoluble	Fruit and vegetables
	Cellulose	Insoluble	Oats, cereals, grains, bread,fruit, vegetables, grains and nuts
	Resistant-starch	Insoluble	Raw potato and unripe green banana

Table 3
Bioactive compounds from marine algae and their possible effect on human health.

Algae species	Algae colour	Bioactive compound	Possible health effect	References
Mastocarpus stellatus	Red	Hybrid carrageenans (a type of sulphated-galactans)	Prevention of hyperlipidemia and thrombosis	Gómez-Ordóñez, Jiménez-Escrig, and Rupérez (2012)
Sargassum vulgare	Brown	Alginic acid	Antitumor	de Souza et al. (2007)
Ascophyllum nodosum	Brown	Polyphenols	Anti-diabetic effect	Nwosu et al. (2011)
Porphyra crispata	Red	Lipids (polyunsaturated fatty acids – PUFAs and sulfolipids)	Carcinoma inhibition	Tsai and Pan (2012)
Eisenia arborea	Brown	Phlorotannin Phlorofucofuroeckol-B	Anti-allergy	Sugiura et al. (2007)
Codium fragile	Green	Pigment (Siphonaxanthin, a specific keto-carotenoid)	Potent anti-angiogenic activity; suppression of cell viability and apoptosis induction in cancer cells	Sugawara, Ganesan, Li, Manabe, and Hirata (2014)
Undaria pinnatifida	Brown	Protein (peptide)	Antihypertensive	Suetsuna and Nakano (2000)

understood (Wells et al., 2017). In addition, structural modification methods and purification methods must be evaluated in order to enable the investigation of structure/activity relationship and facilitate the development of new compounds with similar or higher desirable bioactivities (Liu et al., 2015).

Based on the algae classification, red, green or brown polysaccharides and oligosaccharides with potential applications (such as prebiotic) may vary accordingly to the diversity of monosaccharides present in the molecule, the C1 anomery (α or β), the sugar absolute configuration (D or L), molecular weight, the linking position of glycosidic bonds and substituent groups (nature, yield, and position of substituents) (Courtois, 2009).

Enzymatic treatments have been used not only for extraction but also for modification of natural polymers, aiming structural evaluation and the enhancement of their biological activity, simplifying structural and structure/function studies. The higher efficacy of enzymatic methods for extraction is due to extraction time and energy consumption reduction, minimization of solvents usage, increase in yield, and, mainly, biological activities preservation. These advantages could be directed to the application of enzymatic treatments aiming structural modification of polysaccharides and oligosaccharides from marine algae (Gurpilhares et al., 2016), using different microbial sources (filamentous fungi, yeasts and bacteria) as enzymes producers. Microorganisms have been studied as important sources of a great variety of enzymes used for different purposes (Table 4).

Some types of microbial enzymes have been used for hydrolysis of polysaccharides from seaweeds, but they are mainly applied in lignocellulosic biomass conversion to fermented sugars aiming biofuel production (Reddy et al., 2013). In this sense, the conversion efficiency of algal biomass into fermented sugars overcome the disadvantage of lignocellulosic biomass since the former presents high content of lignin-free polysaccharide and lipids, leading to an energy conversion rate (bioethanol) over 80%. Besides the use of microbial enzymes in the sustainable generation of stock chemicals for biorefinement and bioenergy from marine algae, they are gaining importance for the application in producing oligosaccharides with various nutraceutical activities (Chi, Chang, & Hong, 2012). The difference between polysaccharides and oligosaccharides is due to the degree of polymerization (DP). In general, polysaccharides are molecules with a DP higher than 20–25, while oligosaccharides contain 2–10 residues of sugars. Oligosaccharides can occur naturally or be produced after chemical, physical or enzymatic treatments from polysaccharides (Courtois, 2009).

3.1. *Oligosaccharides and polysaccharides from brown algae and the use of microbial enzymes*

The three major types of soluble dietary fibre polysaccharides from brown algae are designated as alginate, fucoidan and laminaran. The insoluble fibre is substantially made of cellulose (Gómez-Ordóñez et al., 2010; Jin, Zhang, Liang, & Zhang, 2016).

Alginate is the main polysaccharide of brown algae, which contains polymannuronic acid, polyguluronic acid and a mixture of polymannuronic acid and polyguluronic acid with a linear structure (also referred as alginic acid). Alginate is available not only in acid, but also in salt form (considered an important cell wall component) (Holdt & Kraan, 2011; Jin et al., 2016; Wells et al., 2017).

Laminaran is a β-glucan consisting mainly of β-1,3-D-glucopyranose residue, branched with β-1,6-D-glucopyranose (Jin et al., 2016; Wang, Kim, & Kim, 2016) and comprises the main storage carbohydrates of this algae type.

Unlike alginate and laminaran, fucoidan have highly diverse and complex structures. Fucoidan is a term for a class of sulfated polysaccharide rich in fucose sugar, that may also contain several other monosaccharides (e.g. minor amounts of galactose, mannose, xylose, glucose and/or glucuronic acid), depending on the specific brown algae species (Senthilkumar, Manivasagan, Venkatesan, & Kim, 2013; Wang,

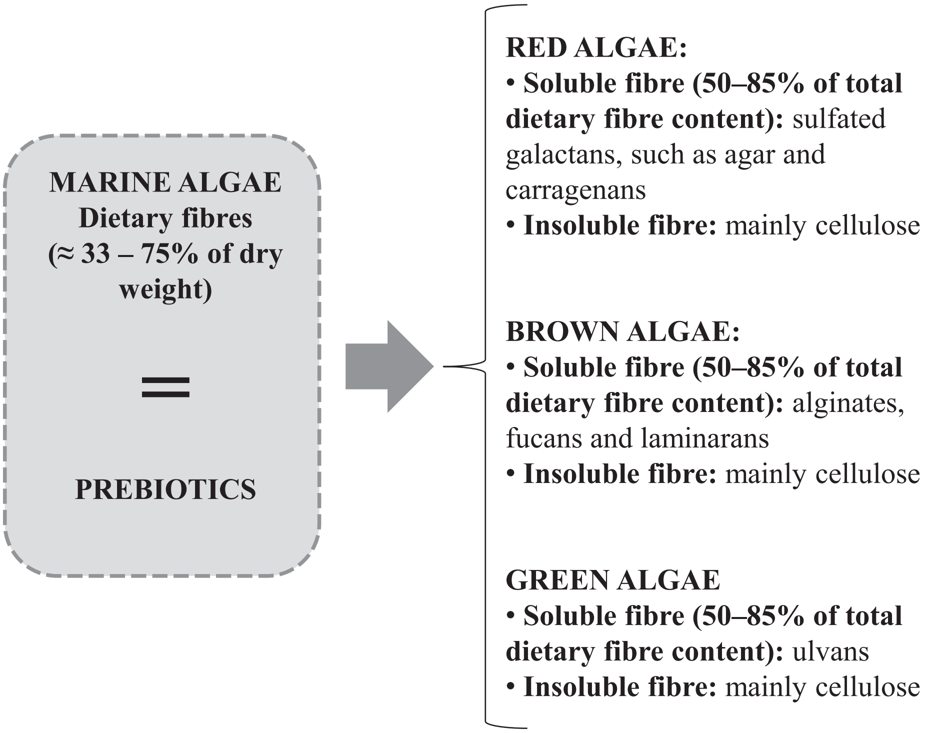

Fig. 1. Marine algae as prebiotics and the dietary fibre contents of red, brown and green algae (Gómez-Ordóñez et al., 2010; Liu et al., 2015).

Kim, et al., 2016).

The complex structure of brown seaweed polysaccharides makes them resistant to degradation by endogenous enzymes within the human intestinal tract (Ramnania et al., 2012). Thus, alginate, laminaran and fucoidan can be important sources of dietary fibre. Nevertheless, the potential prebiotic effects of poly- and oligosaccharides from seaweeds have not been effectively exploited. For this reason, great efforts have been placed on developing efficient methods for algal prebiotics extraction, purification and structural characteristics elucidation in order to improve the bioavailability of fibre (soluble and insoluble) (Rodríguez-Jasso, Mussatto, Pastrana, Aguilar, & Teixeira, 2010). In addition, some algal biomolecules, as proteins and cellulose, pose limitation to the extraction process feasibility (Charoensiddhi et al., 2016). Recently, several extraction approaches with seaweeds have been evaluated.

Chemical hydrolysis/pre-treatment followed by alkaline extraction is a possible methodology to obtain alginates. Acid pre-treatment promotes the solubilization of the alginate and, during the alkaline extraction, alginic acid is transformed to soluble sodium alginate that can be separated by centrifugation. However, this method is considered time-consuming and complicated (Mazumder et al., 2016). Another chemical extraction method is one using several organic solvents. It has been shown to be selective and efficient, but some solvents, such as hexane, diethyl ether, benzene and acetonitrile, are nonfood compatible and their residues cause environmental pollution. In contrast, water-based extraction is food compatible, nonexpensive, and environmentally friendly but has low selectivity with low extraction efficiency (Rodrigues et al., 2015).

An alternative physical method is the ultrasound-assisted extraction, which is based on sound wave migration that generates cavitations, leading to the disruption of cells and their walls (Rodrigues et al., 2015). The disadvantages of this method are related to highly energy consumption and low efficiency (Li, Zhang, Han, & Row, 2012) besides the high cost of the equipment.

Enzyme-assisted extraction has emerged as a promising tool to obtain extracts with attractive biological properties. The hydrolytic action of the enzymes over the seaweed cell walls and storage compounds is able to promote the intracellular biomolecules release, for example, antioxidant compounds from brown seaweeds (Heo, Park, Lee, & Jeon, 2005). Indeed, enzyme-assisted extraction improves bioactive compounds extraction rates and yields, being considered an economical and sustainable method, as well as safety for food applications (Rodrigues et al., 2015). Previous studies have shown that enzyme-assisted extractions performed in different conditions influence the biological activities of oligo- and polysaccharides from marine algae. However, the impact of the process parameters such as the type of enzyme, pH, and buffer on the sugar profiles and molecular weight distribution of marine algae extracts as the critical knowledge for process optimization and design are poorly reported (Charoensiddhi et al., 2016).

The enzymatic extraction of *Laminaria japonica* Aresch. polysaccharide was optimized by response surface methodology. Li et al. (2012) evaluated the enzyme concentration, the ratio of water to raw material and the extraction time as process parameters to obtain high polysaccharide extraction yields. The assays were carried out with a commercial enzyme namely Cellulase® that is generally produced by filamentous fungi, such as *Aspergillus* sp. or *Trichoderma* sp. Under optimal conditions, enzyme concentration of 997.03 mg/g, ratio water to raw material of 98.76 and extraction time of 117.69 min were achieved. The extraction yield was 32.47%. The authors proved that the use of an experimental statistical design is feasible to optimize the enzymatic extraction of polysaccharides from marine algae.

The impact of extraction processes on prebiotic potential of the brown seaweed *Ecklonia radiata* was investigated (Charoensiddhi, Conlon, Vuaran, Franco, & Zhang, 2016). The authors were able to understand the growth of selected bacterial genera of the large intestine and the production of short chain fatty acids, after 24 h *in vitro* anaerobic fermentation, by using six extracts of this seaweed. The extracts were obtained by enzymatic (with commercial enzymes carbohydrases Celluclast® 1.5 L and proteases Alcalase® 2.4 L FG,) acidic and water extraction processes. Although all seaweed samples contained variable amounts of potentially fermentable compounds, including fibre, starch, sugar, protein and polyphenols, there was a clear indication that dietary fibre content alone is not a primary predictor of short chain fatty acid. Short chain fatty acids are the primary products polysaccharide

Table 4
Enzymes produced from microorganisms and their potential use.

Enzyme	Microbial source	Microbial species	Application	References
α-galactosidases	Yeasts	*Debaryomyces hansenii* UFV-1	Hydrolysis of oligosaccharides in soybean products	Viana et al. (2007)
Xylanase	Filamentous fungi	*Gloeophyllum trabeum* CBS900.73	Hydrolysis of xylan used in the brewing, food and feed industries	Wang, Luo, et al. (2016)
xylanases 10 and 11	Filamentous fungi	*Trichoderma reesei*	Production of *xylo*-oligosaccharides from wheat arabinoxylans	Morgan, Wallace, Bedford, and Choct (2017)
Fructosyl transferase	Yeasts	*Rhodotorula* sp. LEB-10	Synthesis of fructo-oligosaccharides from sucrose	Hernalsteens and Maugeri (2010)
Transglutaminase TG-K (MTGase) ACTIVA® GS	Bacteria	*Streptoverticillium mobaraense*	Production of high quality gels from sea bass (*Dicentrarchus labrax*)	Cardoso, Mendes, Vaz-Pires, and Nunes (2011)
Endoglucanase, mannanase, and xylanase (enzyme extract)	Filamentous fungi	*Aspergillus terreus* K1	Release of prebiotic oligosaccharides from Palm Kernel Expeller	Chen et al. (2015)
Endo and exo types of alginate lyases	Bacteria	*Saccharophagus degradans*	Production of fermentable sugars from alginate	Wang, Kim, et al. (2016)
β-1,3-exoglucanases	Yeasts	*Candida albicans*	Enzymatic saccharification of laminarin	Wang, Kim, et al. (2016)

fermentation by gut microbiota, presenting multiple health benefits *in vivo*. It was noticed that 24 h fermentation was not sufficient to enable a more complete breakdown of some complex polysaccharides from the seaweed, but from all extracts tested, the most promising prebiotic potential was attained with those processed with carbohydrase-assisted extraction.

In another study, Charoensiddhi et al. (2016) evaluated the *Ecklonia radiata* polysaccharides enzyme-assisted extraction with the purpose to understand carbohydrate yield, composition, and molecular weight (MW) profiles of the obtained extracts. Once brown seaweed polysaccharides, such as fucoidan, are tightly associated with cellulose and proteins, the use of commercial carbohydrate hydrolytic enzymes and proteases may enable the release of the target polysaccharide without significant degradation. For instance, in this study, experiments were carried out with the use of Viscozyme® L, Celluclast® 1.5 L, Ultraflo® L (carbohydrate hydrolytic preparations from the microorganisms *Aspergillus aculeatus*, *Trichoderma reesei* and *Humicola insolens*, respectively) and Alcalase® 2.4 L FG, Neutrase® 0.8 L and Flavourzyme® 1000 L (three different proteases from *Bacillus licheniformis*, *Bacillus amyloliquefaciens* and *Aspergillus oryzae*, respectively). The authors concluded that enzyme type and pH had low influence on total sugar yield, although a different sugar composition of the extracts could be observed. Both process parameters affected the reduction of the molecular weight of the extracted polysaccharides, with the high molecular weight polysaccharides corresponding to alginates and fucoidans, and the low molecular weight fractions rich in laminaran. They also observed that high concentrations of buffer salts (acetate pH 4.5 and phosphate pH 6, 7 and 8) inhibit the polysaccharide extraction, the use of water with adjusted pH being an alternative (with HCl or NaOH). However, the study's purpose was to employ the enzymes to assist the extraction of seaweed carbohydrates, instead the seaweed polysaccharides hydrolysis. Furthermore, the authors reported that is relevant and important to find sources of seaweed polysaccharides-specific hydrolytic enzymes, such as fucoidanases and alginase, for the production of low molecular weight functional oligosaccharides.

Many seaweed fibres are high molecular weight polymers that need to be hydrolyzed to oligosaccharides in order to increase their fermentability by gut microbial communities. Thus, it is imperative that researchers can find potential sources of specific enzymes, other than the current commercial enzymes that contained major activities of β-glucanase, cellulase, xylanase, and hemicellulase (Charoensiddhi et al., 2016). Sakai, Kawai, and Kato (2004) isolated a marine bacterial strain able to degrade fucoidan from *Kjellmaniella crassifolia* (class Phaeophyceae, order Laminariales, family Laminariaceae). The strain was namely SN-1009 and the phylogenetic analysis indicated that it was a member of the family Alteromonadaceae. The produced enzyme was characterized as an extracellular fucoidanase (an endosulfated fucan-digesting) with optimal activity in temperatures ranging from 30 to 35 °C, pH 6.5 to 8.0, in the presence of calcium ions. This novel fucoidanase demonstrated a preferential hydrolysis of fucoidans from brown algae within the Laminariales. Rodríguez-Jasso et al. (2010) also evaluated potential microorganisms able to produce fucoidan hydrolyzing enzymes with the screen of ten different fungal strains (within the genus *Aspergillus*, *Penicillium*, and *Mucor*). From the ten terrestrial fungus strains, *A. niger* PSH, *P. purpurogenum* GH2, and *Mucor* sp. 3P were able to grow on different fucoidan-urea media, whereas *A. niger* was the only to show importance for the synthesis of sulfated fucan-degrading enzymes.

3.2. Oligosaccharides and polysaccharides from red algae and the use of microbial enzymes

Red algae contain different types of nutritionally important polysaccharides such as floridean starch, an amylopectin-like glucan, carrageenan, agar and agar-carrageenan intermediate (Holdt & Kraan, 2011; Wells et al., 2017). Carrageenans are high molecular weight sulfated galactans composed of disaccharide units with alternating 3-linked β-D-galactopyranose (G-units) and 4-linked α-galactopyranose (D-units) or 3,6-anhydro-α-galactopyranose (AnGal-units). Based on the concentration of the 3,6-anhydrogalactose, the position and number of sulfate groups, they are classified into κ, λ, ι, ν, μ and θ types, the most industrially relevant carrageenans being κ, ι and λ forms (Jiao, Yu, Zhang, & Ewart, 2011; Raman & Doble, 2015). Agars present 4-linked α-L-galactopyranose residues. All the α-galactopyranose residues, of both carrageenans and agars, can exist in the form of 3,6-substituted by methyl groups, pyruvic acid ketal, besides sulfate ester (Jin et al., 2016).

The most studied red algae polysaccharides are those belonging to the orders Gelidiales, Gigartinales, and Gracilariales, but their potential use for human health remains to be established (Wells et al., 2017). Marine algae are interesting natural sources of compounds with biological activity that could be used as functional ingredients. Therefore, the authorization for their use in human nutrition is dependent on a detailed evaluation of the chemical composition and variations among species. In this sense, several studies have been performed in order to measure, mainly, ash, protein, total lipids, fatty acids, minerals, vitamins and dietary fibre contents. A useful protocol for total fibre content analysis in seaweed is the AOAC enzymatic gravimetric method. This is an official method of analysis that employs microbial enzymes such as protease, α-amylase and amyloglucosidase, in the case of red algae. α-amylase and amyloglucosidase are omitted for brown seaweeds, because they contain negligible amounts of starch (Denis et al. (2010); Gómez-Ordóñez et al., 2010; Rodrigues et al., 2015; Syad, Shunmugiah, & Kasi, 2013). Denis et al. (2010) studied the chemical composition of the edible red macroalgae *Grateloupia turuturu*, being the dietary fibre constitution of approximately 60% on the dry weight basis. Gómez-Ordóñez et al. (2010) analyzed the dietary fibre and physicochemical properties of the red edible seaweeds, *Mastocarpus stellatus* and *Gigartina pistillata*, from the northwestern Spanish coast, besides three other species of brown algae. The total dietary fibre content of red algae was around 30.5% dry weight, soluble dietary fibre of 22.4% and insoluble dietary fibre of 8.1%. In brown algae, the AOAC method used for dietary fibre analysis presented some limitations. The authors demonstrated that red seaweeds contain higher proportions in the soluble fraction of dietary fibre than brown seaweeds, which could be explained by the differences in each polysaccharide. Since the physiological effects of dietary fibre are related to their physicochemical properties, the comparison of the swelling capacity (SC), water retention capacity (WRC) and oil retention capacity (ORC) of both red and brown seaweeds becomes important. The values of SC and WRC of the seaweed samples had no statistically significant differences between the two groups. On the other hand, ORC was slightly lower in red seaweeds, indicating that the nature of their constituents was much more hydrophilic than lipophilic.

Agar has long been used in a variety of laboratory (microbiological and biochemical) and industrial applications (food, medical and cosmetic). However, recent reports have revealed new applications of oligosaccharides prepared from agar. Agarases include different enzymes, such as α-agarase, β-agarase, and β-porphyranase, which hydrolyze agar. α-agarases (EC 3.2.1.158) hydrolyze the α-(1,3) glycosidic linkages and produce agaro-oligosaccharides with 3,6-anhydro-α-L-galactose residues at their reducing ends. In contrast, β-agarases (EC 3.2.1.81) hydrolyze the β-(1,4) glycosidic bonds, producing agaro-oligosaccharides with 3-*O*-linked β-D-galactopyranose residues, while β-porphyranase (EC 3.2.1.-) hydrolyze the β-(1,4) glycosidic linkages of the porphyran and produce oligosaccharides with 3-*O*-linked β-D-galactopyranose residues at their reducing ends. Microorganisms of various genera have been identified as good sources of numerous agarases. In a review published by Chi et al. (2012), the authors presented several studies about agarolytic microorganisms and their isolation sources as seawater, marine sediments, marine algae, marine mollusks, fresh water, and soil. As examples, α-agarases have been reported in two marine microorganisms, *Alteromonas agarlyticus* GJ1B and *Thalassomonas* sp. JAMB-A33; β-agarases in many taxonomically diverse microbial genera, including *Cytophaga*, *Pseudomonas*, *Vibrio*, *Alteromonas*, *Pseudoalteromonas*, *Flammeovirga* and *Agarivorans*; β-porphyranases in a Gram negative bacterial specie, *Zobellia galactanivorans*, but the production of agarases in addition to carragenases is still scarce compared to the enzymes that degrade alginate and starch (Ziayoddin, Manohar, & Lalitha, 2010). Other authors have published their findings about potential microbial sources of these enzymes. Ziayoddin et al. (2010) isolated a Gram negative marine bacterium, identified as *Pseudomonas aeruginosa* ZSL-2, which was capable to utilize both agar and carrageenan by producing extracellular agarase and carrageenases, respectively. Li et al. (2014) proved that the variations in colonic microbiota composition exist among individuals, which leads to changes in the ability to degrade and utilize complex polysaccharides by different persons. For this study, authors noticed that the extension of degradation of agarose (AP) and their agaro-oligosaccharides (AO) varied among the six human fecal samples tested. The authors also determined the effect of AO on changes in the populations of major bacteria from the six fecal samples (prebiotic effect over the composition of fecal microbiota), including *Bacteroides*, *Bifidobacterium*, *Clostridium* cluster XIVab, *Lactobacillus*, and *Enterobacter*. Microorganisms isolated from human feces showed a pronounced ability to degrade AO and generate D-galactose as its final end product, although it could not growth in k-carrageenan oligosaccharides, guluronic acid oligosaccharides, and mannuronic acid oligosaccharides. The results indicated that *Bacteroides uniformis* L8 is a special degrader of AO in the gut microbiota.

Despite the microbial enzymes use in seaweed polysaccharides structural modifications, the polymers and their oligosaccharides can have been arisen from enzyme-assisted extraction. A comparison of the impact of enzyme- and ultrasound-assisted extraction methods on biological properties of the red alga seaweed *Osmundea pinnatifida* (Ceramiales) was reported by Rodrigues et al. (2015). According to the authors, the extraction yields were higher when assisted by two types of enzymes (carbohydrases and proteases) than in hot water-based extracts or ultrasound-assisted extracts. Cellulase and Flavourzyme (commercial microbial enzymes) were responsible for the statistically significant highest extraction yields (54–55% of dry weight, respectively). The endoprotease and exopeptidase in the Flavourzyme enzymatic complex from *Aspergillus oryzae* hydrolyzed peptide linkages of *O. pinnatifida* proteins and could be responsible, in part, for the higher extraction yield (due to an elevated protein content of 23.8%). The prebiotic effect was observed with a significant increase of the viable cells growth of the two probiotic strains, *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* BB-12, in a medium containing Viscozyme and Alcalase (other commercial microbial enzymes) extracts of *O. pinnatifida*.

3.3. Oligosaccharides and polysaccharides from green algae and the use of microbial enzymes

The most abundant and the best studied polysaccharide of green seaweeds are ulvans, heteropolysaccharides extracted from members of Ulvales. Those polymers are mainly composed of rhamnose, xylose, glucose, glucuronic acid and sulfate, with smaller amounts of mannose, arabinose and galactose (Pengzhan et al., 2003; Wells et al., 2017). As an example, the water-soluble polysaccharide xylogalactoarabian presents a backbone of 4-linked or 5-linked arabinopyranose residues sulfated at C3, 3-linked or 6-linked galactopyranose residues sulfated at C4 or C6, and 4-linked xylopyranose residues. Another type of green algae polysaccharide is the glucuronoxylomannan, which consists of 4-linked rhamnopyranose sulfated at C3 or C2, a 4-linked glucuronic acid residue and a 4-linked xylopyranose residue (Jin et al., 2016). Green algae also contain starch and cellulose.

The use of green seaweed as a dietary supplement by humans is rather widespread, but the potential health benefits of the whole algae or their derivatives are not well understood and further work is required to explore the structural diversity of the members *Enteromorpha*, *Monostroma*, *Codium,* and *Caulerpa*, besides *Ulva* (Taboada, Millán, & Míguez, 2010; Wang, Wang, Wu, & Liu, 2014).

Several algae have been authorized for human consumption, including species of *Ulva* and *Enteromorpha*, since they contribute to an augmentation of dietary fibre intake. Lahaye and Jegou (1993) measured the dietary fibre concentration of *Ulva lactuca* (L.) Thuret and *Enteromorpha compressa* (L.) Grev., commonly known as sea lettuce and A.O. nori, respectively; by using two methods: a “standard” and a “physiological” protocol, simulating the gastric and intestinal environments (pHs were set at 3.0 and 7.3, respectively). Both soluble and insoluble fibre fractions were treated with the commercial enzymes: protease from *Bacillus licheniformis* and amyloglucosidase from *Aspergillus niger*, being added Termamyl® L120 to the insoluble fibre fraction. Total dietary fibre contents varied from 36.6% to 44.6% in these two green algae (obtained values are similar to the other edible seaweeds). Both methods yielded similar total fibre contents to *U. lactuca* (40.0 and 40.6%), although lower amount for *E. compressa* was obtained by the 'standard' method (36.6% compared to 44.6%). Such difference could be due to the harsh extraction conditions of the 'standard' method that, probably, led to degradations and losses of *E. compressa* polysaccharides. Both algae were composed of xylorhamnoglycuronans, a water-soluble polysaccharide, and insoluble glucans mixed with xylose, rhamnose, mannose, uronic acids and sulfate. Physical-chemical properties were different for the whole algae in comparison to the insoluble fibre. Higher values of water holding capacity were observed for the insoluble fractions, indicating a modification in fibre porosity (reorganization of the cell-wall polymers) after extraction. In regard to the low intrinsic viscosities, the values obtained for *U. lactuca* soluble fibre were higher than that of *E. compressa* and they were increased with pH elevation from 3.0 to 7.3 (147.5 to 175.0 mL/g for *U. lactuca* and 35.9 to 36.6 mL/g for *E. compressa*). Thus, authors concluded that the soluble fibre from *U. lactuca* and *E. compressa* is not expected to demonstrate the physiological and metabolic effects classically associated with viscosity, but further studies are necessary to evaluate the nutritional effects of these fibres.

It is important to search for microorganisms with the capability to produce polysaccharides degrading enzymes and the microbial communities that live on diverse algal species are potential sources of such enzymes. In regard to this fact, a review was published by Martin, Portetelle, Michel, and Vandenbol (2014). An overview of bacteria living on macroalgae and their complex interactions, besides the production of seaweed polysaccharides hydrolytic enzymes of biotechnological interest, were specifically described. In the case of green seaweeds, the authors mentioned about the first isolated ulvanolytic bacterium, able to produce an ulvan lyase. The enzyme was shown to cleave the β-(1,4) linkage between L-rhamnose-3-sulfate and D-glucuronic acid, releasing an oligosaccharide with an unsaturated uronic acid at the non-reducing end. Another ulvan lyase was obtained from a flavobacterium nominated *Persicivirga ulvanivorans*. The activity of this enzyme is based on the cleavage of the glycosidic bond between the sulfated rhamnose and a glucuronic or iduronic acid.

4. Mode of action of prebiotics

The colon is the most metabolically active organ in the human body. Moreover, the human intestinal microbiota is one of the most densely populated microbial ecosystems in nature, even though it is characterized by a relatively low level of phylogenetic diversity (harbours around 500 different bacterial species) (Candela, Maccaferri, Turroni, Carnevali, & Brigidi, 2010; Gibson, 2004). Some substances, such as resistant starch, non-digestible carbohydrates, oligosaccharides and proteins, cannot be digested by the host in the small gut and remain unchanged in the first phase of digestion. Nevertheless, the resident gut microbiota is able to ferment them through saccharolytic and proteolytic fermentations. The majority of carbohydrate entering the colon is fermented in the proximal colon that is considered a saccharolytic environment. As digesta moves through the distal colon, carbohydrate availability decreases and protein and amino acids become the main metabolic energy source for bacteria in this region. The main end products of saccharolytic fermentation are the short chain fatty acids (SCFA), which contribute towards the host’s daily energy requirements. On the other hand, the end products of proteolytic fermentation include nitrogenous metabolites (such as phenolic compounds, amines and ammonia) some of which are carcinogens (Gibson, 2004). This means that the intestinal microbiota exerts a key contribution to the human energy balance and nutrition, by extending the host metabolic capacity to indigestible polysaccharides, the regulation of fat storage and the biosynthesis of essential vitamins (Candela et al., 2010). In addition, intestinal microorganisms develop and maintain the host immune system tolerance against harmless antigens with a fast responsiveness towards harmful pathogens, defending the host from colonization by opportunistic pathogens. Finally, prebiotics represent a useful dietary method for influencing the composition of the human gut microbial community (Candela et al., 2010).

4.1. Beneficial health effects of oligosaccharides and polysaccharides from marine algae

Over the past few decades, marine algae have been considered as a promising living organism for providing both novel biologically active substances and essential compounds for human nutrition (Jiménez-Escrig, Gómez-Ordóñez, Tenorio, & Rupérez, 2013; Wijesekara, Pangestuti, & Kim, 2011). Algal polysaccharides have been reported to possess anticoagulant (Zeid, Aboutabl, Sleem, & El-Rafie, 2014), antitumor (Fedorov, Ermakova, Zvyagintseva, & Stonik, 2013; Menshova et al., 2014), anti-inflammatory (Wijesinghe & Jeon, 2012), antiviral (Wang et al., 2007), antihyperlipidemic (Pengzhan et al., 2003; Qi et al., 2012) antioxidant activity (Fleita, El-Sayed, & Rifaat, 2015), and others. Hence, research has been focused on prebiotics, including poly- and oligosaccharides from seaweeds, as their consumption contributes to limit the occurrence of diseases (e.g. obesity, diabetes, heart, diseases, cancers) (Lahaye & Jegou, 1993).

In fact, prebiotics use is directed to the enhancement of microorganisms, such as lactobacilli or bifidobacteria, which are part of the gut microbiota. These bacteria inhabit the intestinal tract of humans and are responsible for the regulation of fat storage and the biosynthesis of essential vitamins, exerting an important contribution to the human energy balance and nutrition. Moreover, intestinal microorganisms improve the host immune system against antigens and pathogens and inhibit the growth of harmful bacteria preventing gut infections (Blatchford et al., 2013; Candela et al., 2010). Although health promotion by prebiotics are often confined to the intestinal tract, studies have been suggesting benefits outside the gut indirectly by compositional or metabolic changes in the large intestinal microbiota, or directly by changes in the native microbiota in areas outside of the intestinal tract (e.g. mouth or vaginal tract) (Candela et al., 2010).

When seaweed fibre is ingested with the diet, it reaches the colon without any change and then the microbial consortia of the human gastrointestine promote its degradation. Thus, the bacterial fermentation process produces short chain fatty acids (SCFA), acetate, propionate, butyrate, gases (carbon dioxide, methane and hydrogen), microbial cell mass and lactate (Candela et al., 2010; Goñi & Martín-Carrón, 1998). SCFAs can reduce the pH level in the stomach and in the small intestine and also inhibit the growth of Gram-negative bacteria through the dissociation of the acids and production of anions in bacterial cells (Hoseinifar, Sun, & Caipang, 2017). Great part of SCFA is absorbed from the colonic lumen and metabolized by various body tissues. Butyrate constitutes an important energy source for colonic epithelium and has been found to act as a protective agent against experimental tumorigenesis of the epithelial cells. Propionate is cleared by the liver and

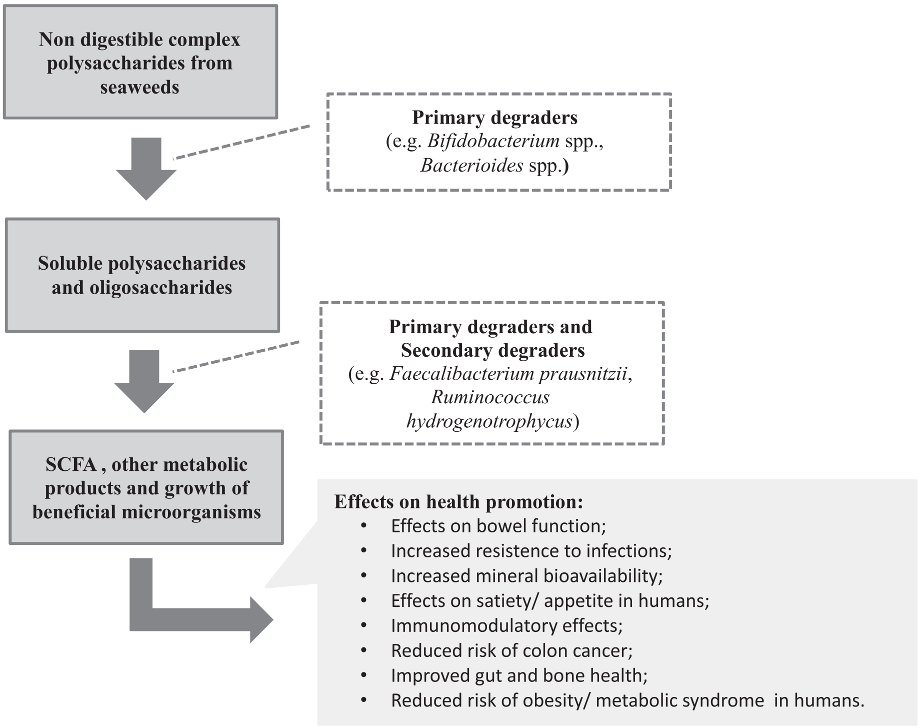

Fig. 2. Metabolic pathway of poly- and oligosaccharides in the gastrointestinal tract and health benefits in human with these prebiotics consumption (Candela et al., 2010; O'Sullivan et al., 2010).

may modulate hepatic carbohydrate and lipid metabolism that could be related to hypocholesterolemic effects. Acetate largely escapes colonic and hepatic metabolism and is utilized by peripheral tissues. Indeed, the human intestinal microbiota possesses a plenty of carbohydrate-active enzymes (CAZymes), many of which are not present in the human glycobiome and in some non-gut microorganisms (e.g. glycoside hydrolases, polysaccharide lyases) (Candela et al., 2010; Goñi & Martín-Carrón, 1998) (Fig. 2).

To evaluate the functional properties of potential prebiotics *in vitro* and *in vivo* studies must be carried out. Usually, *in vitro* studies demonstrate the algal polysaccharides resistance to hydrolysis by digestive enzymes in the upper gastrointestinal tract and the possibility of their fermentation by the flora of the large intestine (Zaporozhets et al., 2014). From this point of view, and since the physical properties of dietary fibre are dependent on the chemical nature of its components, the fermentation of either poly- or oligosaccharides by the gut microbiota will be also influenced by it. In addition, physical-chemical characteristics of dietary fibre will allow a specific local response and the associated systemic reactions, which may be expected with the ingestion of a certain fibre. The commonly studied physicochemical characteristics include water-holding capacity (dispersibility in water), viscosity, binding and absorptive capacity, faecal bulking capacity and fermentability (Jimenez-Escrig & Sanchez-Muniz, 2000).

Hyperlipidemia is associated to a decrease of serum high density lipoprotein cholesterol (HDL-cholesterol), increments of low density lipoprotein cholesterol (LDL-cholesterol) and triglyceride (TG), and therefore can be considered a risk factor to atherosclerosis development. According to some researches, acidic polysaccharides (e.g. alginate, carrageenan and ulvan) may produce non digestible ionic colloids, which exert antihyperlipidemic effects. The ionic groups of algae polymers are able to lower blood cholesterol levels due to their water dispersibility, their capacity to retain cholesterol and related physiologically active compounds with bile acids, increasing fecal bile acid excretion, beyond their ability to inhibit absorption in the gut (Jimenez-Escrig & Sanchez-Muniz, 2000; Pengzhan et al., 2003; Qi et al., 2012).

The molecular weight of dietary fibres may have direct effects on lipid metabolism. Based on these observations, Pengzhan et al. (2003) performed studies to determine the antihyperlipidemic effects of sulfated polysaccharides from *Ulva pertusa* (Chlorophyta) with different molecular weights, after microwave degradation process. The molecular weights of ulvan and its fractions were determined: ulvan presented a molecular weight of 151.6 kDa and their two low molecular weight fractions, U1 and U2, 64.5 and 28.2 kDa, respectively. Intrinsic viscosity was measured and values varied with the molecular weight reduction from 69.3 to 19.0. The rats' ulvan-based diet promoted a decrease of serum total cholesterol and low density lipoprotein cholesterol (LDL) levels, while U1- and U2-based diets increased serum high density lipoprotein cholesterol (HDL) (not significant for U1) and reduced triglyceride (TG) levels in comparison to the control. U1 and U2 were strongly able to inhibit the liver damage by hyperlipidemia and, along with ulvan, increased the amounts of daily bile acid excretion in rats. In fact, with the ulvan consumption, other nutrition components are firstly digested and then adsorbed from the small intestine and this green polysaccharide becomes the main component in the gut lumen. In addition, augmentation of ulvan viscosity by its association with calcium ions in the body interferes with bile acid absorption from the ileum. Consequently, ulvan degradation into smaller fractions, as occurred with U1 and U2, tends to diminish the viscosity and capability of interfering with bile acids and the action of lowering cholesterol levels in blood can be minimized or even disappear. Although ulvan and its fractions had exhibited an antihyperlipidemic effect, the usage of U1 and U2 were considered more beneficial to hyperlipidemia associated with diabetes.

In another study, Qi et al. (2012) evaluated the antihyperlipidemic activities of high sulfate content derivative of polysaccharide extracted from *Ulva pertusa* (Chlorophyta). For this purpose, the sulfate content of ulvan was modified, increasing from 19.5% in the natural ulvan (U) to 32.8% for the high sulfated content ulvan (HU). The U and HU-based diet in mice did not negatively affect the weight gain. HU-fed group showed a decrease in LDL in comparison to the hyperlipidemic group, but a slight reduction on the levels of TG, TC and HDL. Although U and HU have demonstrated a high antihyperlipidemic activity in mice, the mechanism by which they regulate TC, TG, LDL and HDL blood levels in hyperlipidemia mice is not clear. Nevertheless, HU exhibited stronger antihyperlipidemic activity than U, probably due to the more effective antioxidant activity of the former (sulfate content of ulvan increases the antioxidant activity).

Other potential therapeutic benefit of seaweed consumption is the management of body weight and obesity. Seaweed dietary fibre can decrease energy intake by providing a satiety sensation. Mechanisms proposed for these effects include stimulation of gastric stretch receptors, delay in gastric emptying, increased viscosity of digesta and attenuated nutrient absorption (Lange et al., 2015). Jensen, Kristensen, and Astrup (2012) investigated the effects in obese subjects of alginate supplementation in conjunction with energy restriction (2300 kcal/day) on loss of body weight and on metabolic risk markers in comparison with a placebo group. As dietary fibre supplementation is thought to modulate human-appetite sensation, when consuming sodium alginate fibre, in combination with calcium, gel formation and viscosity augmentation may change gastric contents. Consequently, it may promote a decrease in gastric emptying and nutrient absorption with attenuation of postprandial glucose and insulin responses, leading to greater satiety. Although a weight loss enhancement and body composition improvement could be observed, there were no remarkable changes in metabolic risk markers (glucose, insulin, ghrelin, TC, HDL, LDL, among others) in comparison with control subjects. It is worth mentioning that occurred a suppression of active plasma ghrelin, which could result in a perception of less hunger. Indeed, the hypocaloric diet-induced weight loss often produces a coordinated decrease in plasma leptin and an increase in plasma ghrelin. When ghrelin, an orexigenic hormone, is secreted to the bloodstream by the stomach and duodenum, it increases food intake in humans.

Furthermore, the consumption of sulfated polysaccharides from marine seaweeds as prebiotics, may lead to anti-inflammatory effects on colitides, peptic-ulcer disease, and other disorders of the gastrointestinal tract by blocking the leucocyte adhesion to the epithelium of blood vessels and preventing the migration of these cells to the inflammation site (Zaporozhets et al., 2014).

Current literature provides some revisions about key biological activities of dietary fibre from seaweeds and the knowledge regarding the mode of action and the structural requirements necessary to elicit these effects (Cian, Drago, de Medina, & Martínez-Augustin, 2015; Jiao et al., 2011; Jimenez-Escrig & Sanchez-Muniz, 2000; Wang et al., 2014; Zaporozhets et al., 2014).

5. Application in food for human and others

For centuries, marine algae have been consumed whole among the East Asian populations (Paiva, Lima, Patarra, Neto, & Baptista, 2014). As they are low-calorie food, rich in vitamins, oligoelements, minerals and dietary fibre, seaweeds most used in foods include brown algae from the genera *Laminaria* (Kombu), *Undaria* (Wakame) and *Hijiki* (Hiziki) and species of the red algae *Porphyra* (Nori), being the later cultured and processed into a sheet-style dried food and served as traditional wrapping for sushi (Jimenez-Escrig & Sanchez-Muniz, 2000; Makkar et al., 2016; Muraoka et al., 2008). In the same way, seaweed polysaccharides are considered valuable additives in food industry because of their rheological properties as gelling and thickening agents (e.g. alginates, carrageenans and agar) (Jiao et al., 2011; Paiva et al., 2014).

Considering that seaweeds contain a number of complex carbohydrates and polysaccharides, a potential application could be in the livestock diets. In ruminants, the use of seaweeds in the diet may enable rumen microbes to adapt and thus enhance energy availability from these complex carbohydrates. According to *in vivo* studies on ruminants, pigs, poultry and rabbits and despite of the potential contribution for the protein and energy requirements of livestock, the prebiotic effect of seaweeds may enhance production and health status of both monogastric and ruminant livestock (Makkar et al., 2016).

Seaweeds and seaweed extracts have been shown to offer potential as alternatives to nutritional antibiotics in pigs after weaning. To assess the antibiotic effect of intact marine brown algae *Ascophyllum nodosum* on the gut flora of piglets, Dierick, Ovyn, and De Smet (2010) performed *in vitro* studies. The antimicrobial activity was evaluated under conditions prevailing *in vivo* in the jejunum and caecum of piglets. From the results, it was noticed that the use of *A. nodosum* depressed *Escherichia coli*, *Streptococcus* spp., *Lactobacillus* spp. and total anaerobes growth, both in small intestinal and caecal, probably, because this brown algae is not a suitable substrate for fermentation (production of small amounts of lactate and volatile fatty acids). Hence, the inclusion of *A. nodosum* in piglet nutrition may improve their gut health.

In addition to examining antimicrobial effect of seaweed prebiotics on gut microbial ecology of pigs, the antibiotic activity of another brown alga was noted in the olive flounder, one of the most important commercial marine finfish species cultured in Korea and Japan. Lee et al. (2016) evaluated how the brown alga *Ecklonia cava* affected the growth rate of olive flounder and its immune response to pathogenic bacteria. Therefore, experimental fish were divided into four groups after infection with the three administered bacteria, *Edwardsiella tarda*, *Streptococcus iniae* and *Vibrio harveyi*. The control group was fed a ‘ diet containing only 1.0% *Lactobacillus plantarum* (probiotic), group I was fed 1.0% *L. plantarum* and 1.0% EC (*E. cava* powder), group II was fed 1.0% *L. plantarum* and 0.1% EE (ethanol extract of *E. cava*, rich in polyphenols with antibacterial effects), and group III was fed 1.0% *L. plantarum* and 0.5% EE. Results indicated that the supplementation with EC enhanced the growth of olive flounder by increasing body weight, whereas the mortality decreased by 18% and 13% in fish supplemented with 1.0% EC and 0.1% EE, respectively. The biochemical profiles of olive flounder, characterized by the levels of total protein, triglycerides, aspartate aminotransferase, alanine aminotransferase, glucose, phosphorus, cholesterol and hematocrit; remained unchanged with the supplementation of 1.0% EC, 0.1% EE, and 0.5% EE. On the contrary, the supplementation of 1.0% EC improved the growth of olive flounder by enhancing innate immunity related to increases in superoxide anion production and respiratory burst activity, in lysozyme activity and myeloperoxidase activity. A decrease in the cumulative mortality of olive flounder infected by pathogenic bacteria was also observed with the supplementation of 1.0% EC, 0.1% EE, or 0.5% EE. Overall, these results demonstrated the potential value of *E. cava* as a beneficial prebiotic.

6. Conclusions and future trends

Undoubtedly, functional foods generate one of the most promising and dynamically developing segments of food industry. Prebiotics have been extensively studied and explored commercially, but a detailed knowledge of dietary fibre from marine algae remains scarce. In fact, the great variety of seaweed soluble and insoluble fibre, the composition in different species, their chemical and physicochemical properties, the relationship between structure and biological activities must be elucidated. In this sense, the use of known and new microbial enzymes could help mainly during optimization of downstream operations, such as harvesting, cellular disruption, extraction and purification of biological active compounds, besides the elucidation of chemical composition of seaweed poly- and oligosaccharides. Furthermore, due to the high demand for functional food consumption, the search and development of methods to modify or even improve the activities of the carbohydrates from seaweeds became mandatory. In this regard, the application of microbial enzymes shall be stimulated, since they could be considered suitable and safe for foodstuffs, environment-friendly and cost-effective.

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Conflict of Interest

All the authors declare that there is no actual or potential conflict of interest.

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