

Review

Seaweeds as a Fermentation Substrate: A Challenge for the Food Processing Industry

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Abstract: Seaweeds are gaining momentum as novel and functional food and feed products. From whole consumption to small bioactive compounds, seaweeds have remarkable flexibility in their applicability, ranging from food production to fertilizers or usages in chemical industries. Regarding food production, there is an increasing interest in the development of novel foods that, at the same time, present high nutritious content and are sustainably developed. Seaweeds, because they require no arable land, no usage of fresh water, and they have high nutritious and bioactive content, can be further explored for the development of newer and functional food products. Fermentation, especially performed by lactic acid bacteria, is a method used to produce functional foods. However, fermentation of seaweed biomass remains an underdeveloped topic that nevertheless demonstrates high potential for the production of new alimentary products that hold and further improve the organoleptic and beneficial properties that these organisms are characterized for. Although further research has to be deployed in this field, the prebiotic and probiotic potential demonstrated by fermented seaweed can boost the development of new functional foods.

Keywords: fermentation; seaweed; prebiotic; probiotic; functional food; bioactive compounds



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1. Introduction

Nowadays, many researchers are focused on seaweeds and their benefits to human health. Seaweeds are rich in several bioactive compounds, such as polyphenols, sterols, alkaloids, flavonoids, tannins, proteins with essential amino-acids, polyunsaturated fatty acids, etc. [1], which possess powerful characteristics, namely antioxidant and anti-inflammatory properties [2]. Seaweed bioactive elements can help us not only improve our health, but also live a more environmentally friendly lifestyle which includes the utilization of sustainable food sources. Since ancient times, marine algae have been consumed as a whole meal, particularly in Asian countries [3]. In fact, seaweeds are considered a low-caloric food, [4] which makes them attractive for the inclusion in the human daily diet. Furthermore, the inclusion of vitamins, fibers, and proteins provides a high nutritional value, as well as various health benefits. Polysaccharides from seaweeds, for example, have a beneficial effect on the intestinal tract, but, unlike fibers, they are calorie-free [4]. These biological molecules could be used to make novel and functional foods, as well as implemented in pharmacological and medicinal applications, due to their beneficial qualities [5]. Agar and carrageenan, extracted from red algae, and alginates, extracted from brown algae, are already employed in food and pharmaceutical industries as stabilizers [6]. Agar is present in food products such as candy, fruit juice, frozen food, bakery icing, or meringues, but it is also widely employed for the production of biological culture media [1].

Carrageenans from family Gigartinales (Rhodophyta) are already present in the food market since they exhibit high viscosity in drinks [7]. Nutraceutical products, such as seaweed-based food, give nutritional value while also assisting in the prevention of health problems. For example, green algae (Chlorophyta) possess several bioactive compounds that exhibit antioxidant, anticoagulant, antimutagenic, antibacterial, and anticancer activities which make them potential functional food supplements [8]. *Ulva lactuca*'s extract showed antimicrobial and photocatalytic activities [9], while an alkaloid called 'caulerpin', isolated from *Caulerpa* spp., showed antitumor activity [10]. Brown algae (Ochrophyta, Phaeophyceae) are considered powerful nutraceutical sources as well. For example, *Sargassum fulvellum* possesses health-promoting components, such as phlorotannins, fucoxanthin, and fucoidans. Fucoidan extracted from this species showed a stronger antioxidant activity when compared with synthetic antioxidants already present in the market [11].

These powerful properties may contribute to the formulation of innovative and natural alternatives that answer customers' search for healthier products. Due to this growing search, seaweed fermentation has gained considerable interest [12,13]. The process of seaweed fermentation answers challenges such as the provision of healthy alternatives to marketed products and can be beneficial for human health or have other applications, for example, in the animal feed, food safety, and food-coating industries. Notably, the beneficial properties of seaweed fermentation and its by-product applications in human nutrition and health demonstrate remarkable potential [12,13].

The purpose of this review is to highlight seaweed fermentation's prebiotic and probiotic potential. Moreover, the applicability of seaweed fermentation and its by-products in other industries, such as the food and feed ones, is also considered.

2. Fermentation's Basic Concepts

Fermentation is the process in which occurs the conversion of carbon molecules, such as glucose and other polysaccharides and sugars into smaller molecules, with lower molecular weight, such as acetic, lactic, and other organic acids. The process of molecular oxidation/reduction is mediated by specific microorganisms [14]. Usually, fermentative species commercially used belong to Gram-positive bacteria groups, which include the genera *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Streptococcus*, and *Pediococcus*. These are recognized for their fermentative potential and provide enrichment to products, boosting food organoleptic properties and health benefits, and ensuring food security [15].

The term "prebiotic" is used to indicate a substrate that is used by selective microorganisms which confer health benefits to humans [16,17]. On the other hand, live-ingested microorganisms that live within the gastrointestinal tract of the animal host are called "probiotics". They have a positive effect on the organism by enhancing the immune response from the host, acting as a source of beneficial gut population inoculant that can increase the prevalence of the selected strain and also act in the production of several health-improving compounds, such as lactic or acetic acid, which positively modulate the production of health-improving compounds [18,19].

Relevant Fermentative Strains—Lactic Acid Bacteria

One of the most studied fermentative strains are lactic acid bacteria (LAB) [20]. Lactic acid bacteria are a group of microorganisms that perform anaerobic fermentation, where the main final product is lactic acid (Figure 1). Glucose metabolization by these organisms can occur in three specific ways: (i) Obligatory homofermentative metabolization, in which glucose is metabolized via the Embden–Meyerhorf–Parnas (EMP) pathway: these groups degrade hexoses to lactic acid, but do not possess the capacity to degrade pentoses or gluconate; (ii) Obligatory heterofermentative, in which glucose is decomposed via the pentose–phosphoketolase pathway: besides lactic acid as an end product, these groups also produce acetic acid, ethanol, and carbon dioxide; and (iii) Facultative heterofermentative, strains that perform mixed fermentation, which means both pathways can occur, but vary according to environmental cues, such as pH, temperature, or nutrient availability,

and metabolize hexoses via EMP and other substrates through pentose-phosphoketolase pathway [20–24].

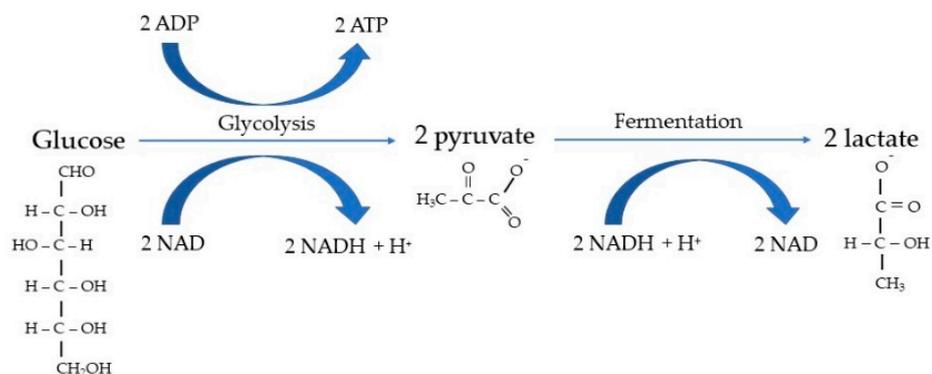


Figure 1. Schematic representation of lactic acid fermentation.

Lactic acid is already employed in the food and pharmaceutical industries. It is utilized as a food preservative because of its ability to suppress the growth of bacteria that causes food spoilage [25]. It is also used in chocolates and sweets for the flavour, as well as for obtaining the correct pH [26]. In the pharmaceutical industry, lactic acid is used as an electrolyte in various intravenous solutions to supplement bodily fluids [27]. It is also involved in mineral preparations, prostheses, controlled drug delivery systems, surgical sutures, and in the preparation of solutions for dialysis processes [26]. The approach of lactic acid in cosmetics is positive as well; indeed, it acts as a moisturizer, anti-acne agent, humectants, anti-tartar agent, pH regulator, skin-lightening agent, and skin-rejuvenating agent, preventing wrinkles [28]. Moreover, lactic acid could be applied in the production of biodegradable plastic for everyday use, such as food packaging, containers, trash bags, or protective clothing, for example [29,30].

Lactic acid bacteria (LAB) are used commercially in the food industry for the production of fermented products, such as kefir, cheese, yogurt, or milk, acting as preservatives. Studies demonstrated that the uptake of fermented foods (e.g., yogurt and dairy products) may reduce the development of cardiovascular disease and type 2 diabetes mellitus [31–34]. Moreover, they alleviate inflammatory bowel disease (IBD) symptoms [35].

Studies on LAB showed their (1) capacity to resist intact to the transit through the gastric tract, (2) ability to colonize the host, and (3) safety and potential health benefits, such as cholesterol reduction. LAB, during fermentation, probably degrade cyanobacterial cells via peptoglycan hydrolyses, consequently extracting and converting complex organic compounds, such as polysaccharides, lipids, and proteins, into smaller molecules that possess antioxidant, immunomodulatory, and anti-inflammatory activity [36].

However, the cost-production for lactic acid by microbial fermentation can be improved, and low-cost solutions, in order to increment the production of lactic acid to industrial levels, but also involving an eco-friendly approach, are required [37,38].

3. Seaweed as a Fermentation Substrate

In that scenario, seaweed presents a possible solution for several industries (Figure 2). Moreover, fermentation has the potential to cut total costs and encourage innovation in novel food and feed products based on this technique [39]. Studies revealed that seaweeds' bioactive compounds can be utilized as a substrate for lactic acid bacteria which are specialized in lactic acid production [40,41]. This colourless and odourless monocarboxylic acid [42] has been positively evaluated not only for food application, but also for pharmaceutical and industrial applications [26,43].

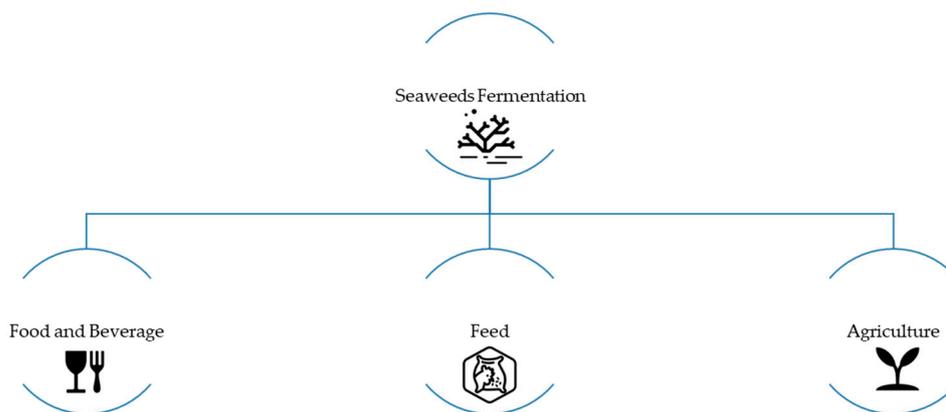


Figure 2. Schematic representation of seaweed fermentation biotechnological applications.

The use of seaweeds as a prebiotic element may overcome the cost issues. Additionally, the ingestion of non-digestible seaweeds' polysaccharides may help with the development of lactic acid bacteria within our intestinal tract, favouring the growth of beneficial bacteria instead of the proliferation of pathogenic bacteria present in our organism [44].

Seaweed fermentation is poorly reported in literature, although LAB and yeast have been employed to produce fertilizers from red seaweed waste. For example, fermentation of *Undaria pinnatifida* (Phaeophyceae) has been reported as an alternative feeding strategy for aquaculture and a fermented beverage from *Gracilaria fisheri* (Rhodophyta) has been developed within the framework of human consumption [45].

3.1. Green Seaweeds

Several investigations have been carried out to evaluate the potential of seaweeds as a fermentation substrate (Tables 1 and 2).

Initially, fermentation products were discovered after *Ulva* sp. (Chlorophyta) fronds were left with cellulase for 17 months. Several lab and yeast strains were discovered when the strains in the fermented *Ulva* were examined. It was later discovered that the addition of a small quantity of cellulase (1% w/v) aids in the breakdown of algae's unfavourable polysaccharides, and that yeast strains are not required to promote fermentation. The addition of salt until 5% is also beneficial, as lactic acid bacteria (LABs) must be halophytes [46].

The existence of 11 LAB species was studied in seaweed fermentation with and without NaCl. After 11 days at 20 °C, the species *Lactobacillus brevis*, *L. plantarum*, *Lactobacillus casei*, and *Lactobacillus rhamnosus* were found to grow in the saline substrate. While species such as *B. cereus* and *B. fusiformes* grew, no LABs were found to develop in the non-saline substrate, and the agal biomass had perished [46].

Subsequently, each of the LAB strains and yeast strains detected were evaluated for induction of fermentation. Fermentation was successfully initiated by LAB strains; however, yeast strains produced mediocre results and bacterial contamination. Inoculation with yeast is not required. When LAB is employed as a beginning culture, it outperforms other cultures in terms of growth. If not employed as a starter culture, there is no apparent LAB growth and the culture spoils, with *Bacillus* strains dominating [46], thus demonstrating that *Bacillus* strains can produce other compounds, which are not good.

3.2. Red Seaweeds

One of the most cultivated red seaweeds is *Kappaphycus alvarezzi*, a promising resource for biorefinery feedstock [47]. Previous research has shown the potential of this seaweed as a bifidogenic factor, increasing *Bifidobacterium* populations in a similar way to the well-known prebiotic inulin, and increasing overall short chain fatty acids production [48].

Palmaria palmata (dulse) is an edible red alga that is well known by its protein content. Nonetheless, previous research has shown that due to the cell wall encasing cytoplasmic

proteins and the presence of fibers, the digestibility of dulse proteins is poor [49,50]. The water-soluble xylan, which is abundant in dulse, could be responsible for the protein's poor digestion [51]. In this context, fermentation can be an efficient method to increase the protein availability of this red seaweed. Nevertheless, the results can vary according to the starting culture used. For instance, the results of fermentation with *Rhizopus microsporus* var. *chinensis*, *Aspergillus oryzae*, and *Trichoderma pseudokoningii* showed unequally enhanced digestibility when compared to crude *Palmaria palmata* [52]. The degree of improvement varied depending on the strains employed. The best digestibility improvement came from *Trichoderma pseudokoningii*, while the lowest came from *Rhizopus microsporus* var. *chinensis*. *Palmaria palmata* fermented with *Trichoderma pseudokoningii* had a digestibility of 65.5% of casein after 6 h [52].

Researchers found that 0.5 g of *Gracilaria vermiculophylla* pre-treated with cellulase, allied to a saline solution of 3.5% of NaCl and with the strains *L. casei* B5201, *D. hansenii* Y5201, and *Candida* sp. Y5206 as starting culture, proved to be a good substrate to produce lactic acid and ethanol, achieving values of 0.31 and 0.23 g 100 mL/L, respectively [46].

Lin et al. [53] tested lactic acid bacteria for the fermentation of *Gracilaria* sp. for lactic acid production. Previous studies showed that galactose, the main sugar present in red seaweeds, has not been able to be fermented by lactic acid bacteria such as *Lactobacillus bulgaricus*, *Lactobacillus delbreckii*, *Lactobacillus lactis*, and *Lactobacillus brevis*. Thus, *Lactobacillus acidophilus* and *Lactobacillus plantarum*, which can ferment galactose, were selected. As a result, the preliminary test showed that the combined use of *Lactobacillus acidophilus* and *Lactobacillus plantarum* had the best lactic acid production for *Gracilaria* sp. [53].

3.3. Brown Seaweeds

Researchers found that the fermentation of different parts of the brown seaweed *Undaria pinnatifida*'s thallus resulted in different yields of lactic acid and ethanol. For instance, with the stem, concentrations of 0.25 and 0.12 g 100 mL/L of acid lactic and ethanol, respectively, were obtained, while in the blade, the values varied between 0.18 and 0.23 g 100 mL/L for acid lactic production and between 0.07 and 0.38 g 100 mL/L for ethanol synthesis [46].

Researchers found that heat treatment allied to the fermentation process can, in fact, enhance sugar kelp (*Saccharina latissima*) organoleptic characteristics [40]. Fresh sugar kelp collected in June was fermented with *Lactobacillus plantarum* to obtain a product with a milder, less salty taste, a reduced sea scent, and a less slimy visual appearance. The fermentation had no effect on the protein content of the seaweed biomass, but it improved the mineral composition of the product by lowering the levels of two toxic metals (Cd and Hg), and also the Na content [40].

Suraiya's research looked at algae fermentation (*Saccharina japonica* and *Undaria pinnatifida*) as a technique to improve their bio-properties. Red mould *Monascus purpureus* and *Monascus kaoliang* fermented these algae [54]. They found that seaweed extracts fermented with *Monascus* species had more phenolic, flavonoid, anti-diabetic, and antioxidant effects than controls. Furthermore, the fermented extracts showed DNA protection activities and no harmful effects on CACO-2 cells intestinal epithelium. They could be utilized to treat patients with oxidative stress, hyperglycemia, or hyperlipidemia [22].

Table 1. Seaweed species investigated as fermentation substrate.

	Seaweed Species	Strains of Bacteria Involved in Seaweed Fermentation	Reference
Chlorophyta	<i>Ulva</i> sp.	<i>Lactobacillus brevis</i> , <i>L. plantarum</i> , <i>Lactobacillus casei</i> , and <i>Lactobacillus rhamnosus</i> favour fermentation in non-saline substrate	[46]
	<i>Monostroma nitidum</i>	Oligosaccharide extracts are potential substrate for <i>Lactobacillus</i> sp.	[55]

Table 1. Cont.

	Seaweed Species	Strains of Bacteria Involved in Seaweed Fermentation	Reference
Rhodophyta	<i>Kappaphycus alvarezzi</i>	Potential substrate for <i>Bifidobacterium</i> populations	[47]
	<i>Palmaria palmata</i> (dulse)	<i>Trichoderma pseudokoningii</i> fermentation increase casein digestibility of 65.5%	[52]
	<i>Gracilaria vermiculophylla</i>	Potential substrate for <i>L. casei</i> B5201, <i>D. hansenii</i> Y5201 and <i>Candida</i> sp. Y5206 for lactic acid and ethanol production	[46]
	<i>Gracilaria</i> sp.	Potential substrate for <i>Lactobacillus acidophilus</i> and <i>Lactobacillus plantarum</i> for lactic acid production	[53]
	<i>Gelidium</i> sp.	Agar is a potential substrate for <i>Bifidobacterium</i> populations. An increase of short-chain fatty acids (SCFAs) has been detected	[56]
	<i>Grateloupia filicina</i> <i>Eucheuma denticulatum</i> (formerly <i>Eucheuma spinosum</i>)	Extracted polysaccharides are potential substrate for <i>Bifidobacterium</i> populations	[57]
Phaeophyceae	<i>Undaria pinnatifida</i>	Potential substrate for LAB for lactic acid and ethanol production (thallus)	[46]
	<i>Saccharina japonica</i>	Fermentation with <i>Monascus purpureus</i> and <i>Monascus kaoliang</i> showed an increase in phenolic, flavonoid, anti-diabetic and antioxidant effects	[22]
Angiosperm	<i>Lonicera japonica</i>	Potential substrate for growth and survival of gut microbial lactic acid bacteria in humans	[58]

Table 2. Monosaccharide and polysaccharide percentages of example seaweeds used in fermentation processes and their commercial status.

Species	Monosaccharide (%DW)	Polysaccharide (% DW)	Commercially Exploited	Ref.
<i>Ulva</i> sp. (Chlorophyta)	26.64	36–43	Cultivated;	[59]
<i>Undaria pinnatifida</i> (Phaeophyceae)	12.4	8.7	Cultivation and wild harvest; also, invasive species;	[60,61]
<i>Saccharina latissimi</i> (Phaeophyceae)	35.2	17	Cultivated and wild harvest;	[62]
<i>Gracilaria fisheri</i> (Rhodophyta)	79.31	13.33	Cultivated;	[62]
<i>Kappaphycus alvarezzi</i> (Rhodophyta)	7	58.8	Cultivated;	[63,64]
<i>Palmaria palmata</i> (Rhodophyta)	11.6	35.4	Cultivated;	[65,66]
<i>Gracilaria vermiculophylla</i> (Rhodophyta)	ND	24–33	Cultivated and wild harvest. Invasive species;	[67]

4. Food Industrial Applications of Fermentation Procedures on Algal Biomass

A balanced nutritional composition, nutrient availability, and good digestion are among the most important requirements for the formulation of functional foods.

Lactic acid fermentation is used as a food preservative method and to ameliorate the aroma of food and beverages, exploiting the ability of lactic acid bacteria (LAB) to produce volatile compounds during fermentation. LAB fermentation also improves the nutraceutical profile of products, making it a beneficial food-oriented technology that increases food safety, shelf life, and sensory qualities.

Functional Food Production

Algae, due to their qualities, can be used in the manufacture of fermented foods. Combining fermented products with high LAB and algae content can boost the nutritional value of food products. Combining the health benefits of fermented foods with the beneficial nutraceutical ingredients from algae, as well as the advantage of a high LAB load, may result in the production of high nutritional quality products, while also broadening the variety of macroalgal applications. Fermented food containing algae is called “dairy,” and it has the prebiotic and probiotic potential that these products are known for [22].

Because of the expanding prevalence of lactose intolerance and veganism, there is a growing demand for dairy-free products. Moreover, there is a growing concern about healthy and sustainable nutritional sources, thus seaweed and microalgae are suitable substrates for the development of probiotic, lactose-free goods by fermentation with lactic acid bacteria [22].

Because of their high nutritional content and/or valuable components, some algae are a good substrate for the creation of probiotic lactose-free food and beverages via lactic acid fermentation.

Arthrospira platensis's (Cyanobacteria) biomass (a commercial microalgae), one of the most common examples, is increasingly being employed as a food additive, appearing in gluten-free recipes, baking, chocolates, dairy products, and soft drinks. For instance, *A. platensis*'s formulation obtained a concentration of 8.8 to 10.7 CFU mL⁻¹, indicating the potential of *A. platensis* for the formulation of probiotic beverages. The findings in this investigation are comparable to those found with other algae species [36]. Thus, *A. platensis*'s biomass demonstrated to be a suitable substrate for LAB growth. The amount of protein was more than 50% of the dry biomass. Additionally, total phenolic content and in vitro and in vivo antioxidant activity increased, while phycocyanin content decreased [36,68].

To obtain a positive health effect from probiotic consumption, levels between 8 and 10 CFU must be consumed daily for at least 2 weeks. There is increasing evidence in the health benefits derived from these products, which are associated with improvements of intestinal health, enhancement of immune response, reduction of serum cholesterol, and cancer prevention [69,70].

Nevertheless, the yield of lactic acid obtained is explained by some authors, who tested different algae or food matrices, as the speed in which sugars are released through enzymatic hydrolysis, higher carbohydrate content or different carbohydrate profile [13,36].

5. Challenges to the Fermentation-Based Manufacture of Food Seaweed Products

Macroalgae have unique carbohydrates, which constitutes a challenge, when compared with terrestrial biomass, especially due to the presence of mannitol and laminarin. Based on this knowledge, developed technologies for terrestrial biomass cannot be directly applied to macroalgae biomass and the selection of appropriate microorganisms is vital for the success of seaweed fermentation [71,72].

Owing to the structural complexity, seaweed extracts are resistant to degradation by gut bacteria. Salyers suggested that none of the human colonic *Bifidobacterium* or *Lactobacillus acidophilus* was capable of fermenting alginate, fucoidan, or laminarin in vitro [56].

Drying methods are impractical for large scale seaweed processing. Sun drying requirements, although low-cost and used as the current practice for feed production, require large areas and are meteorologically dependent. Oven drying (or convective air drying) is energetically demanding and expensive for the industrial scale. Other methods, such as lyophilization, are mainly used in other types of industries, where the target compounds are small molecules and not the algae itself [73–75].

There are also reported limitations to the fermentation process through lactic acid bacteria, due to the high buffering capacity of fresh seaweeds. Due to the complexity and the high content of carbohydrates/polysaccharides resistant to microbial degradation, and the buffering capacity, the growth of natural lactic acid bacteria may be compromised on fresh seaweeds [24,76,77].

The presence of phlorotannins is proposed as a hindrance to fermentation processes due to the suppression of microbial degradation. It is important to establish the effects of ensiling mechanisms on phlorotannins content. Additionally, phlorotannins have antibiotic/antimicrobial/antiproliferative effects, which may restrict the growth of lactic acid bacteria in addition to spoilage of microorganisms, characterized by the presence of butyric and propionic acid [1,78].

The addition of microbial inoculants is dependent on several factors, such as original forage conditions, epiphytic microflora, ensiling conditions, and type of inoculant used. The addition of inoculant to these species demonstrated limited fermentation quality. This can provide evidence that seaweeds carry endogenous enzymes required for the hydrolysis of complex carbohydrates [1].

The absence of lignin in macroalgal biomass reduces the costs, time, and the difficulty of the bioconversion processes, comparing with other feedstock in which the need to remove lignin is a limiting step [79].

Thus, the high potential of seaweed polysaccharides in the development of novel fermented products is demonstrated, although there is still a long way to fully exploit these natural compounds efficiently. Future steps to the development of functional beverages to advance the production and commercialization of these products are the determination of technological properties (stability of components, consumer safety, etc.) and sensorial aspects.

6. Seaweed Polysaccharides as Prebiotics for Gut Bacteria

As demonstrated before, the seaweed polysaccharides are the principal compounds that can be fermented by bacteria or fungi, to produce new products or in the human digestive tract.

Accumulating evidence links several metabolic diseases, such as diabetes type 2, obesity, and colon cancer, to disruptions of gut microbiota [80].

As previously demonstrated that seaweeds have potential as a bifidogenic factor, increasing *Bifidobacterium* populations in a similar way to the well-known prebiotic inulin, and increasing the overall short chain fatty acid production [48].

A study by Chen et al. [57] demonstrated the prebiotic effect of polysaccharides in vitro conditions from four different seaweeds: *Grateloupia filicina*, *Eucheuma denticulatum* (formerly *Eucheuma spinosum*) (Rhodophyta), *Ulva australis* (formerly *Ulva pertusa*) (Chlorophyta), and *Ascophyllum nodosum* (Phaeophyceae). The results demonstrated that the polysaccharide extracted from *Grateloupia filicina* and *Eucheuma denticulatum* showed a strong prebiotic activity which promoted the growth of *Bifidobacterium*, widely used as probiotic [81].

Another case study observed a reduction in oxidative stress in healthy adults after a daily consumption of 1.5 g/day of fermented *Lonicera japonica* for 4 weeks, indicated by decreased serum γ -glutamyltransferase (GGT) and malondialdehyde. An increase of antioxidant enzymes (superoxide dismutase and catalase) was also noticed compared to the placebo test [82]. The decrease of oxidative stress is challenging in medicine and pharmaceutical industries, since oxidation damage influences several diseases, for example, Alzheimer's disease [83,84], arthritis [85], cancer [86,87], cardiovascular diseases [88], diabetes [89,90], and Parkinson's disease [91].

Thus, through the involvement of seaweeds and their prebiotic activities, it would be possible to find new solutions and new natural drugs with powerful properties for the treatment of several diseases.

The most commonly used seaweeds as prebiotic food ingredients belong to Laminariales (commonly known as kelp). These macroalgae are present in fermented foods (e.g., kimchi), where the microbial content provides a source of probiotics, nutrients, and bioactive metabolites, which are reported to have antibacterial, antioxidant, and anti-obesogenic activities [92–94]. A study from Ko et al. [58] observed that four weeks of consumption of

kimchi made with *Lonicera japonica* promoted the growth and survival of gut microbial lactic acid bacteria in humans [58].

Low molecular weight polysaccharides (LWMP) and oligosaccharides derived from these hydrocolloids act as a source of dietary fiber and can act as prebiotics. However, the fiber component is very high in molecular weight, which makes it pass through the gut too fast for the microbiota to use it. It is then necessary to break these heavy polysaccharides into smaller molecules, more soluble, and which can be added in high concentration [56].

One of the principal advantages of the seaweed polysaccharides is the improved metabolization of short chain fatty acids (SCFA) by the intestinal microflora, resulting in a better status of the cardiometabolic, immune, bone, and mental health [95–99].

Short chain fatty acids (SCFA) profiles in the study of Ramnani et al. [56] indicated that most of the low molecular weight seaweed extracts were highly fermentable, producing more SCFA than cellulose. *Gelidium* sp. (Rhodophyta) exerted a selective increase in *Bifidobacterium* and a consistent increase in total SCFA, suggesting a prebiotic potential. Similar low weight polysaccharides, such as *Gracilaria*-derived agar (Rhodophyta) and *Ascophyllum nodosum*'s (Phaeophyceae) alginate, do not exert a prebiotic potential compared to *Gelidium* [56]. However, it was not observed an increase in bacterial populations with a decrease on the weight of the oligosaccharides, which makes it doubtful that the prebiotic potential of *Gelidium* is derived from the molecular weights of the oligosaccharides, and not from the chemical composition of the saccharides [56]. Additionally, other reports demonstrate that oligosaccharides have better potential for fermentation, consequently demonstrating prebiotic activities due to the end products of fermentation, when compared to their parent polysaccharides [100–102].

Wu et al. [55] demonstrated a growth increase of lactobacilli in seaweed oligosaccharide extracts of *Gelidium*, *Gracilaria* (Rhodophyta), *Monostroma nitidum* (Chlorophyta), and *Neoporphyra dentata* (formerly *Porphyra dentata*) (Rhodophyta), when compared to their polysaccharides.

6.1. The Biochemical Explanation for the Nutraceutical/Prebiotic Effect

The breakdown of glycosidic bonds results in the increase of reducing ends. The decrease of total carbohydrate content during fermentation is mainly due to bacterial consumption. The content change of total carbohydrate and reducing sugar is often used as the indication of fermentation. SCFA production during fermentation, such as acetic, propionic, and butyric acids, is the result of the fermentation metabolism of dietary carbohydrates by microbiota. Fermentation of galactose, mannose, and galacturonic acid can produce butyric acid. Increased production of SCFA is mainly attributed to the fermentation of arabinose, galactose, mannose, and galacturonic acid. Acetic acid has been found to improve glucose tolerance and insulin secretion in high-fat (HF) diet fed rats. Propionic acid has been found to reduce obesity-induced inflammation, and increase glucose uptake and lipogenesis. Butyric acid has a positive effect on diabetes and insulin resistance [103].

SCFA act as players in maintaining the barrier function of intestines, epithelial proliferation regulation, colorectal cancer prevention, immune response modulation, and helping with the metabolism, having such functions as the regulation of gluconeogenesis, lipid storage, and improvement of insulin sensitivity. Results indicated that fermentation can improve the production of these metabolites, which is consistent with the fact that non-digestible carbohydrates and prebiotic ingredients can be fermented by intestinal microbiota, accelerating the production of SCFA [104].

Overall, marine algal polysaccharides (MAPs) have been found to support a wide range of health promoting beneficial properties and prebiotic effects. In turn, these prebiotic properties are mainly determined by the digestion and fermentation behaviour of MAPs in the digestive tract. In vitro and in vivo mechanism studies are often used to establish hypothesis and regulate the effects of specific MAP components, which exhibit a variety of physical, chemical, and biological activities under controlled environmental conditions [104].

6.2. How Are the Prebiotics Studies Performed?

In vitro studies include laboratory-based mimic/models for one or more conditions encountered by these compounds in the digestive tract, such as oral cavity, esophagus, stomach, and small and large intestine. On the other hand, *in vivo* studies aim to assess detailed descriptions of digestion and fermentation mechanisms of MAPs by the gut microbiota [104].

For MAPs to be considered prebiotic 3 criteria have to be met: (i) they must not be digested in the upper gastrointestinal tract; (ii) MAPs must be a selective substrate for the growth of beneficial gut microbiota; and (iii) the metabolites produced by the gut microbiota must be beneficial to the host organism [104].

Most MAPs resist degradation in the stomach and small intestine. Oral processing of marine algae reveals no digestion of marine polysaccharides, with no release of oligosaccharides due to enzymatic reactions from the saliva. They are not degraded in the stomach either, with *in vitro* demonstration that no monosaccharides are detected under gastric juice. Similarly, there was no detection of small oligosaccharides or monosaccharides in the small intestine, revealing no degradation of MAPs in the small intestine. MAPs, carbohydrate polymers that are neither digested nor absorbed, are subjected to bacterial fermentation in the colon, consequently impacting and modulating bacterial populations in the gut. The large intestine contains a vast number of bacterial species, involved in a range of activities related to human health, such as metabolism transformation modulation [104].

The two major methods of evaluating gut microbiota diversity and proposed fermentation are through *in vitro* fermentation in fecal samples and *in vivo* oral administration of MAPs (Figure 3). Gut microbiota possess multiple carbohydrate-active enzymes (CAZymes) that display activity with MAPs. CAZymes can be divided in three groups according to their catalytic activity: Glycoside hydrolases (GH), polysaccharide lyases (PL), and carbohydrate esterases (CE). GH are a group of enzymes that cleave glycosidic linkages between two or more carbohydrate units, PL hydrolyse acidic sugar unit linkages, and CE catalyse breakage of ester linkages. Differences in CAZymes of various gut microbiota suggest a specific consumption of polysaccharides, and there is an influence in the bacterial gut population according to the MAP diet [104,105].

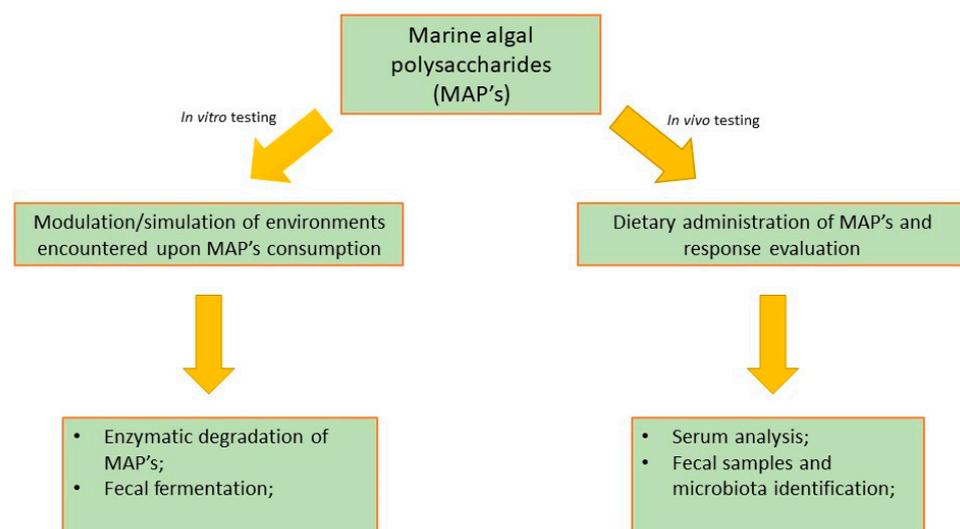


Figure 3. Schematic representation of prebiotic potential analysis using seaweed polysaccharides.

Prebiotic MAPs are utilized in the metabolism of gut microbiota, secreting beneficial metabolites, especially SCFA, such as acetate, propionate, and butyrate, which are the predominant SCFA in the human gut. However, they contain low amounts of isobutyrate, isovalerate, valerate, and caproate. The different types and concentrations of SCFA depend on the MAP diet and the microbiota composition [104].

7. Conclusions

The fermentation industry/exploitation is already using terrestrial plants, but this process is still unused/underdeveloped in regard to macroalgae.

At the level of feed formulation, the use of macroalgae is already generally accepted, but at the level of the use of macroalgae fermentation, there are no conclusive studies about the results obtained. However, industrial development of such technology would require large amounts of biomass for production and commercialization, ensuring the viability of seaweed fermentation products. In this scenario, harvesting wild organisms would consequently have an impact on the sustainability of the harvested shore, due to the removal of the first level of the trophic chain. Ideally, such industry would develop with access to large amounts of biomass from aquaculture productions or by utilization of invasive algal species, in this way developing the growth of circular economy.

Seaweed fermentation presents two important factors: (i) it can be used as a suitable fermentative substrate to promote the growth of LAB, and (ii) the end products of the algae polysaccharides' fermentation can act as potent prebiotics. Such characteristics can make the development of dairy products, such as kefir or fermented products, clear of animal raw sources, adding the beneficial characteristics of macroalgal bioactive compounds to the production and formulation of functional food.

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