Seaweeds as a Fermentation Substrate

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The interest in marine macroalgae are increasing as novel and functional food and feed products. Marine macroalgae show a high flexibility and applicability due to the bioactive properties of the compounds produced. In the nutraceutical sector, the development of novel food products with high nutritious value and sustainable development raises the interest in this biological group. Indeed, seaweeds mainly need exposure to sun, aeration, artificial or naturally, and seawater rich in nutrients to grow and obtain a high amount of biomass to then develop novel functional food products that can be produced by the fermentation method, mainly performed by lactic acid bacteria. Still, this is a topic that needs to be further developed in order to improve the beneficial and organoleptic properties of seaweeds.Here is proposed to the potential of prebiotic and probiotic fermentation and the potential of seaweed in food and feed industrial sectors.

Keywords: seaweed ; fermentation ; Food products ; polysaccharides ; technological properties

1. Seaweed as a Fermentation Substrate

In that scenario, seaweed presents a possible solution for several industries (**Figure 1**). Moreover, fermentation has the potential to cut total costs and encourage innovation in novel food and feed products based on this technique [1]. Studies revealed that seaweeds' bioactive compounds can be utilized as a substrate for lactic acid bacteria which are specialized in lactic acid production [2][3]. This colourless and odourless monocarboxylic acid [4] has been positively evaluated not only for food application, but also for pharmaceutical and industrial applications [5][6].

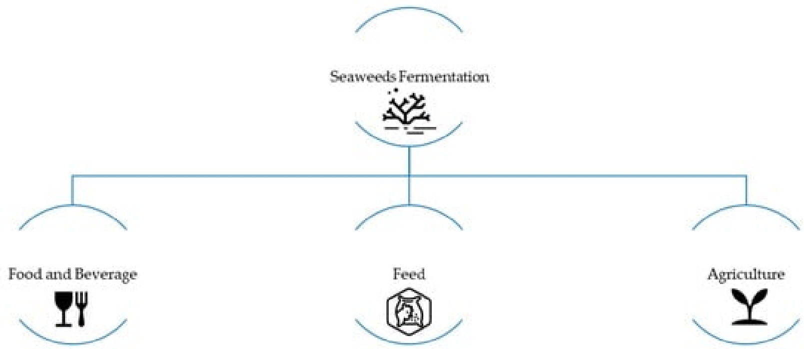

Figure 1. Schematic representation of seaweed fermentation biotechnological applications.

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 [7].

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 [8].

1.1. Green Seaweeds

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

Table 1. Seaweed species investigated as fermentation substrate.

Seaweed Species		Strains of Bacteria Involved in Seaweed Fermentation	Reference
Chlorophyta	*Ulva* sp.	*Lactobacillus brevis*, *L. plantarum*, *Lactobacillus casei*, and *Lactobacillus rhamnosus favour fermentation in* non-saline substrate	[9]
	Monostroma nitidum	Oligosaccharide extracts are potential substrate for *Lactobacillus* sp.	[10]
Rhodophyta	*Kappaphycus alvarezzi*	Potential substrate for *Bifidobacterium* populations	[11]
	Palmaria palmata (dulse)	*Trichoderma pseudokoningii* fermentation increase casein digestibility of 65.5%	[12]
	Gracilaria vermiculophylla	Potential substrate for *L. casei* B5201, *D. hansenii* Y5201 and *Candida* sp. Y5206 for lactic acid and ethanol production	[9]
	Gracilaria sp.	Potential substrate for *Lactobacillus acidophilus and Lactobacillus plantarum* for lactic acid production	[13]
	Gelidium sp.	Agar is a potential substrate for *Bifidobacterium* populations. An increase of short-chain fatty acids (SCFAs) has been detected	[14]
	Grateloupia filicina *Eucheuma denticulatum* (formerly *Eucheuma spinosum*)	Extracted polysaccharides are potential substrate for *Bifidobacterium* populations	[15]
Phaeophyceae	*Undaria pinnatifida*	Potential substrate for LAB for lactic acid and ethanol production (thallus)	[9]
	Saccharina japonica	Fermentation with *Monascus purpureus* and *Monascus kaoliang* showed an increase in phenolic, flavonoid, anti-diabetic and antioxidant effects	[16]
Angiosperm	*Lonicera japonica*	Potential substrate for growth and survival of gut microbial lactic acid bacteria in humans	[17]

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.
Ulva sp. (Chlorophyta)	26.64	36–43	Cultivated;	[18]
Undaria pinnatifida (Phaeophyceae)	12.4	8.7	Cultivation and wild harvest; also, invasive species;	[19] [20]
Saccharina latissimi (Phaeophyceae)	35.2	17	Cultivated and wild harvest;	[21]
Gracilaria fisheri (Rhodophyta)	79.31	13.33	Cultivated;	[21]
Kappaphycus alvarezzi (Rhodophyta)	7	58.8	Cultivated;	[22] [23]
Palmaria palmata (Rhodophyta)	11.6	35.4	Cultivated;	[24] [25]
Gracilaria vermiculophylla (Rhodophyta)	ND	24–33	Cultivated and wild harvest. Invasive species;	[26]

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 [9].

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 [9].

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 [9], thus demonstrating that *Bacillus* strains can produce other compounds, which are not good.

1.2. Red Seaweeds

One of the most cultivated red seaweeds is *Kappaphycus alvarezzi*, a promising resource for biorefinery feedstock [11]. 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 [27].

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 [28][29]. The water-soluble xylan, which is abundant in dulse, could be responsible for the protein's poor digestion [30]. 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* [12]. 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 [12].

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 [9].

Lin et al. [13] 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. [13].

1.3. 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 [9].

Researchers found that heat treatment allied to the fermentation process can, in fact, enhance sugar kelp (*Saccharina latissima*) organoleptic characteristics [2]. 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 [2].

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 [31]. 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 [16].

2. 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 [16].

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 [16].

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. plantensis*'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 [32]. 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 [32][33].

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 [34][35].

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 [36][32].

3. 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 [37][38].

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 [14].

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 [39][40][41].

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 [42][43][44].

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 [45][46].

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 [45].

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 [47].

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 theses 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.

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