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# Unravelling the fermentation potential of *Ulva fenestrata* by lactic acid bacteria and its impact on protein digestibility and mineral accessibility<sup>☆</sup>

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## ABSTRACT

Fermentation of *Ulva fenestrata* by isolated lactic acid bacteria (LAB) can be advantageous due to their adaptability to diverse substrates (e.g., ulvan), and high salinity compared to commercial strains. This study aimed at identifying LAB strains holding promise for both fermentation and enhanced nutrient digestibility of *U. fenestrata* focusing on proteins and microelements. Fifty LAB strains were selected for evaluation, of which thirty-six were revived and screened using water extracts from *U. fenestrata*. From the initial 36 LAB strains, 30 were able to reduce the pH to <4.5 within 24 h in *Ulva* substrate. Six strains were further screened with whole *Ulva* biomass, resulting in four being able to reach pH <4.5 within 48 h and presenting proteolytic activity, with *Lactiplantibacillus plantarum* NFICC19 being superior in both, followed by *Levilactobacillus brevis* NFICC1668 and *Lactiplantibacillus paraplantarum* NFICC1922. These three strains were then used for larger-scale fermentations, in which carbohydrate profiles indicated a relative reduction in glucose for all strains. After *in vitro* digestion according to INFOGEST protocol, the degree of protein hydrolysis (DH) of *U. fenestrata* fermented with *L. brevis* NFICC1668 presented a significantly higher value, 71%, compared to the fresh biomass or the unfermented control having DH of 29% and 42%, respectively. In addition, *L. paraplantarum* NFICC1922 gave the highest iodine (32% vs 18% in the control) and iron accessibility (38% vs 11% in the fresh biomass). Overall fermentation with *L. brevis* NFICC1668, can increase protein digestibility and iodine accessibility, whereas *L. paraplantarum* NFICC1922 can improve iodine and iron accessibility.

## 1. Introduction

Seaweed biomass is rich in carbohydrates, minerals and vitamins, with some species also reaching high protein levels (e.g., *Ulva fenestrata*), making it an attractive raw material for food applications (Steinhagen et al., 2021). However, the high water activity renders rapid deterioration after harvest of *U. fenestrata*, calling for methods to preserve both the quality and the nutritional value of the biomass (Vall-llosera et al., 2024).

Among available technologies, fermentation with lactic acid bacteria

(LAB) is promising for its acidification potential, and are considered to be a green choice based on its a resource-efficiency (Stévant et al., 2024). In the context of LAB-driven seaweed fermentation, research to date has predominantly focused on brown species, such as *Saccharina latissima* (Akomea-Frempong et al., 2021; Bruhn et al., 2019; Gupta et al., 2011; Gupta et al., 2012; Sandbakken et al., 2018; Sørensen et al., 2021; Stévant et al., 2024; Yen et al., 2022; Zioga et al., 2025), *Laminaria japonica* (Wei et al., 2024; Zhu et al., 2021), and *Alaria esculenta* (Allahgholi et al., 2023; Healy et al., 2024; Sørensen et al., 2021; Yen et al., 2022); as well as red species (i.e., *Pyropia* sp.), *Gracilaria*

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*chilensis* (Maiorano et al., 2022) and *Palmaria palmata* (Luo et al., 2025; Marrion et al., 2003; Yang et al., 2024). In contrast, fermentation of green seaweed biomass for food remains largely unexplored, with only two studies published, to our knowledge. One of these focused on commercial LAB coupled with yeast to ferment *U. rigida* hydrolysates (Brandão et al., 2025), while the other explored the use of isolated bacteria to ferment *U. facinata*, specifically using *Leuconostoc holzapfelli*, *Lactococcus lactis*, *Leuconostoc lactis*, *Leuconostoc mesenteroides*, *Wissella confusa* and *Weissella cibaria* (Admantin et al., 2023). However, in the later study, it was reported that there was a need to add glucose to aid growth and thus, to speed up the decrease of pH to 4.5 (Admantin et al., 2023).

In the northern part of Europe, *U. fenestrata* has received great interest for cultivation due to its resilience to nutrient, temperature and salinity changes (Steinhagen et al., 2021). Further, *U. fenestrata* can reach high protein content under some conditions (Stedt, Trigo, et al., 2022), can be a source of vitamin B12 (Cherry et al., 2019; Trigo, Chamlagain, et al., 2025) and is rich in ulvan, a sulphated polysaccharide (Kidgell et al., 2019). However, few commercial LAB strains possess the enzymes to breakdown such complex polysaccharides (Rubak et al., 2026), which can induce problems for commercial strains if no external substrate is available e.g. glucose. On the other hand, isolated bacteria from different niches, e.g. LAB, have been reported as promising microorganisms without requiring nutrients supplementation during fermentation (Stévant et al., 2024; Zioga et al., 2025).

For brown seaweed, LAB isolated from e.g., plants, beach cast seaweed and sourdough demonstrated strong acidification and ability to metabolise mannitol and rhamnose (Allahgholi et al., 2023; Sørensen et al., 2021; Stévant et al., 2024; Zioga et al., 2025). We hypothesise that the large diversity among the >2000 strains of LAB existing in libraries such as the National Food Institute Culture Collection (NFICC) (Zioga et al., 2025), possess great opportunities to find a range of organisms that are capable of utilising sugars present in *U. fenestrata* without the addition of other carbon sources, i.e., glucose. Among the LAB, *Levilactobacillus brevis* is of particular interest due to its reported ability to use xylose as a carbon source (Brandão et al., 2025; Sedó Molina et al., 2022), and amino acids as a nitrogen source (Allahgholi et al., 2023; Zioga et al., 2025). Another candidate is *Lactiplantibacillus plantarum* known for its fast acidification, proteolytic activity (Stévant et al., 2024), and reduction of bitter amino acids in fermented brown seaweed (Zioga et al., 2025).

Beyond acidification, fermentation has the potential to improve the nutritional value of *U. fenestrata*, particularly in terms of protein digestibility and elemental bioaccessibility. Higher protein digestibility has been shown for *Gracilaria gracilis* (Maiorano et al., 2022), pre-hydrolysed *U. rigida* and *P. palmata* (Marrion et al., 2003). One reason is that the microbial proteolytic activity occurring during fermentation, as reported for *L. plantarum*, may generate peptides with higher accessibility (Healy et al., 2024; Luo et al., 2025). Second, LAB degradation of carbohydrates, which can be in complexes with proteins, can result in proteins being more accessible for digestive enzymes (Li et al., 2025).

Although several studies have examined seaweed fermentation, there is still missing knowledge on the use of LAB to ferment whole green seaweed, specifically *U. fenestrata*, as well as the effect of fermentation on protein digestibility and additional nutritional aspects. Thus, this work aims to explore the capabilities of naturally isolated LAB strains to ferment *U. fenestrata*, including their use of seaweed-derived polysaccharides, and the subsequent effect of LAB fermentation on protein digestibility and elemental bioaccessibility. Candidate strains were selected based on their reported proteolytic activity, their preferred carbon source and their QPS (Qualified presumption of safety) status by EFSA (Panel et al., 2026).

## 2. Methods

### 2.1. Seaweed material

*U. fenestrata* was cultivated in tanks at the Tjärnö Marine Laboratory (TML), University of Gothenburg, Sweden (58°52'33.272"N, 11°47'20.2"E) and harvested in July 2024. It was frozen directly after harvest at −60 °C. Then it was moved to −80 °C until it was minced in a frozen state using a meat grinder with an 8 mm hole plate, and then again stored at −80 °C until fermentation. The biomass remained frozen during the grinding.

### 2.2. Screening of bacterial strains

#### 2.2.1. Strains for screening

A total of 50 bacterial strains were selected (Fig. 1), based on relevant literature and their availability in the in-house library from the National Food Institute Culture Collection (NFICC) at the Danish Technical University (DTU, Lyngby, Denmark). The strains were screened to investigate and assess their performance in seaweed fermentation. The selection included *L. plantarum* (n = 10), *Pediococcus pentosaceus* (n = 6), *Levilactobacillus brevis* (n = 7), *L. pentosus* (n = 4), *Latilactobacillus curvatus* (n = 3), *Lactocaseibacillus paracasei* (n = 3), *Latilactobacillus fuchensis* (n = 3), *Lactocaseibacillus rhamnosus* (n = 2), *Leuconostoc pseudomesenteroides* (n = 2), *Limosilactobacillus fermentum* (n = 2), *L. kimchii* (n = 2), *Latilactobacillus sakei* (n = 2), *L. paralimentarius* (n = 2), *Leuconostoc mesenteroides* (n = 1), *Leuconostoc citreum* (n = 1), *L. paraplantarum* (n = 1) and *L. delbrueckii* spp. *lactis* (n = 1). Strains were taken from −80 °C storage and streaked onto an MRS agar (CM1153R, Oxoid Fisher Scientific, Denmark) prepared in plates, which were then incubated at 30 °C for 48 h under atmospheric conditions.

#### 2.2.2. MALDI-TOF-Biotyper

The identity of the strains was confirmed by MALDI-TOF Biotyper® Sirius IVD system (Bruker, USA) using the method previously described by Sedó Molina et al. (2022). One microliter of a fresh colony was spread as a thin layer on the MALDI-TOF plate and allowed to dry. Afterwards, 1 µL of 70% formic acid was added to each spot with the sample. Then, a matrix mixture was prepared by adding 475 µL of water, 500 µL of acetonitrile, and 25 µL of trifluoroacetic acid, and dissolving 50 µL of α-cyano-4-hydroxycinnamic acid. Next, 1 µL of the matrix mixture was added to the target spot on the plate. Once all target spots were fully dry, the plate was placed in the MALDI-TOF Biotyper for analysis.

#### 2.2.3. *U. fenestrata* substrate

The 8.5 g of dried seaweed was mixed with 25 mL of water and autoclaved at 121 °C for 30 min to extract sugars. Afterwards, a centrifugation step at 1000 xg for 5 min was performed to recover the supernatant, which was then collected and used as the *Ulva* substrate. This process was selected based on a previous study in which carbohydrates were extracted from the biomass, in order to high-throughput screening of LAB strains (Zioga et al., 2025).

#### 2.2.4. pH and optical density (OD)

For growth based on OD<sub>600</sub> and pH monitoring, 200 µL of *Ulva* substrate was placed in a 96-well plate, with half containing 0.01% Bromocresol green (BCG) as a pH indicator and 2 µL of inoculum (from a stock of OD<sub>600</sub> = 10) of each strain. At the same time, the other half was only inoculated with water to monitor OD<sub>600</sub>. The plate was incubated at 30 °C for 24 h. The pH was determined using a predetermined colorimetric scale ranging from pH 7 to 3.5, at 620 nm. The scale was generated by adjusting the pH of the *Ulva* extract with L-lactic acid to obtain the standard curve, which is shown in Supplementary Fig. S1. For both pH and OD<sub>600</sub>, values obtained from blanks containing uninoculated substrate were subtracted from the corresponding measurements.

The six most promising strains were chosen for further testing. The

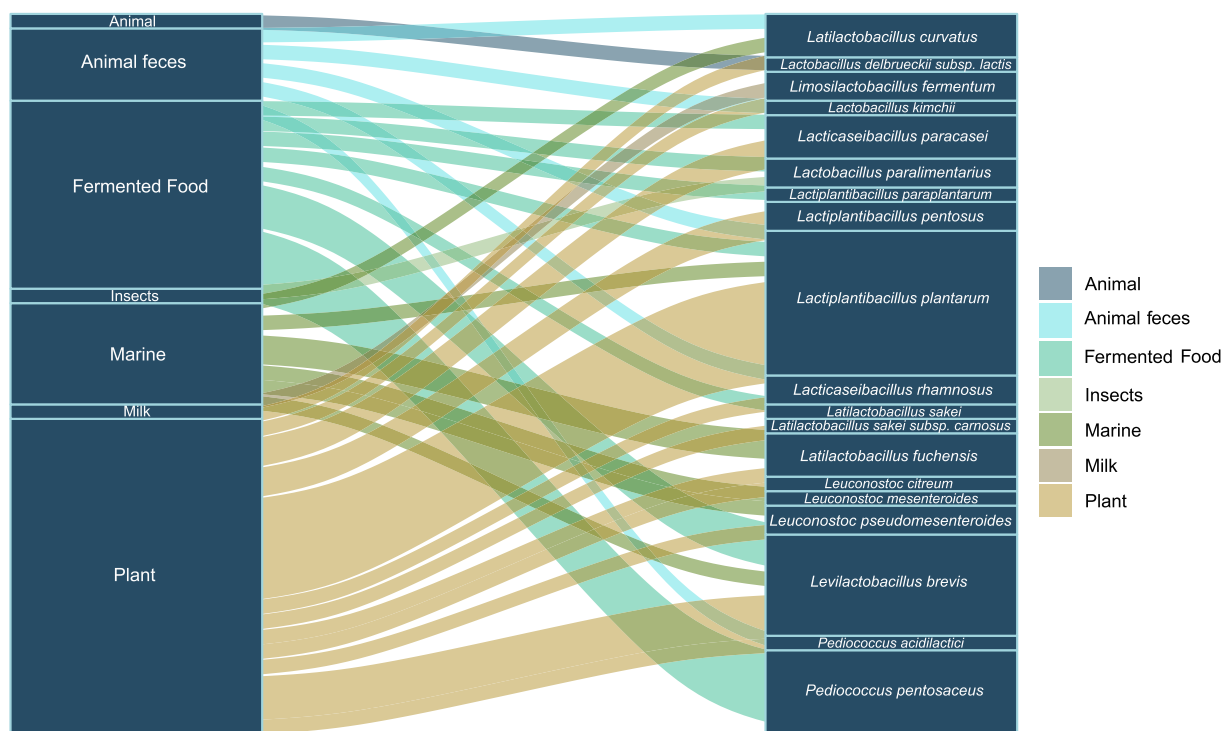


**Fig. 1.** Illustrative scheme of all 50 strains tested in this study, and the origin from where each strain was isolated by the National Food Institute Culture Collection (NFICC).

selection criteria for these strains were: i) their performance as acidifiers; ii) their growth curve; and iii) their ecological relevance, such as diversity of origins, representation of fermentative processes (i.e., kimchi) and diversity of strains.

### 2.3. Fermentation experiments

The fermentation of *U. fenestrata* was done in 2 steps. First, a strain selection for fermentation was done in order to pre-screen 6 strains down to 3 to study further the effects on the nutrient digestibility. A scale-up fermentation, in which larger volumes of biomass and 3 selected strains were further studied, including protein digestibility and elemental bioaccessibility. The terminology “selection of strains” and “scale-up fermentation” will be used throughout the rest of the article.

### 2.3.1. Preparation of inoculum for both fermentation processes

The process was done according to the previously described method by Sørensen et al. (2021) and Zioga et al. (2025). Individual strains were revived from glycerol stocks and grown overnight (18 h) in MRS broth CM0359 Oxoid, Fisher Scientific, Denmark), followed by centrifugation at 2100 xg for 5 min to remove the supernatant. The remaining pellet was washed with 500 µL of 0.9% sterile NaCl and centrifuged at 2100 xg for 5 min. The washed cells were dissolved in 900 µL of 0.9% NaCl to create a dense cell suspension (inoculum). After OD<sub>600</sub> measurements, the amount of inoculum added to each tube was calculated to achieve an OD of 0.1.

Appropriate serial dilutions were performed for each strain suspension ( $10^{-1}$  to  $10^{-6}$ ) and 100  $\mu$ L of each dilution was plated onto MRS plates to assess cell concentration. The plates were incubated for 24 h at 30 °C to enumerate CFU/mL of the original inoculum added.

### 2.3.2. Fermentation process

Twelve and a half g (Strain selection) or 25 g (Scale-up) of minced and frozen *U. fenestrata* were placed into a 50 mL Falcon tube or a 100 mL screw-cap bottle, respectively. Then, a 2% NaCl brine solution, previously autoclaved, was added to each tube or beaker at a 1:3 ratio of

seaweed to brine. The use of 2% NaCl brine was based on the wish to keep isotonic conditions and thereby avoid lysis of cells. The ratio of 1:3 was based on a pre-test with frozen biomass, resulting in the minimum water needed to fully submerge the minced seaweed biomass. The mixture was then inoculated, with inoculum from [section 2.3.1](#), ranging between 6.7 and 7.7 log CFU/mL for both strain selection and scale-up experiments. At the same time, two tubes were not inoculated; one containing only 2% NaCl brine (referred to as “Control”), included in both strain selection and scale-up fermentations, and the other only for the scale-up fermentations. In the latter, the pH was adjusted to pH 4.5, with L-lactic acid (referred to as “Acidified *Ulva*”). The acidified biomass served as a control to discern between the effects of acid per se, from those of LAB fermentation on protein digestibility and element accessibility.

The inoculated and uninoculated *U. fenestrata* biomass was placed in an incubator at 30 °C. This process was done in triplicate ( $n = 3$ ). During fermentation, pH was monitored at 10, 12, 20, 24, 28, 40, 42, and 48 h. Additionally, at 48 h, an aliquot was taken for further analysis of the degree of hydrolysis (DH), colony enumeration per mL, and carbohydrate composition.

After the strain selection for fermentations, three strains were selected for scale-up fermentations (section 3.3) based on: i) pH and OD from strain selection; ii) pH after 48 h strain selection for fermentation; iii) the origin of the strains (see section 3.1); iv) the highest serine equivalent.

#### 2.4. Nitrogen and amino acid content

The total nitrogen content of the *U. fenestrata* biomass was determined on 5 mg oven-dried (105 °C for 24 h) biomass using an Elementar Cube (Elementar, Germany) nitrogen analyser ( $n = 3$ ). The amino acid content was determined using frozen material sampled both before and after fermentation. The method used was previously as described by Trigo et al. (2011), with the main steps being acid hydrolysis and separation, plus detection using LC-MS.

## 2.5. Moisture and ash

The moisture content was analysed as explained in 978.18 AOAC ("AOAC, 2023b). Following subjection to 600 °C overnight, as mentioned in 938.08 AOAC ("AOAC, 2023a) ash was determined gravimetrically.

## 2.6. Monosaccharides analysis by acid hydrolysis

The monosaccharide composition was determined before and after fermentation. The fermented samples were centrifuged at 2500 xg for 5 min, and supernatant and pellet were obtained, followed by freeze-drying. The following method was used as previously described by Johansson et al. (2022, 2024) with some modifications. Briefly, 30 mg freeze-dried biomass was hydrolysed with 0.45 mL 72% H<sub>2</sub>SO<sub>4</sub> and placed under vacuum for 15 min, followed by a heating step at 30 °C for 1 h with stirring at 20 min intervals. Then 12.6 g of deionised water was added, and the sample was autoclaved at 125 °C for 1 h. Thereafter, a vacuum filtering step was performed using a Whatman quantitative filter (Merck, Denmark), and the samples were diluted with deionised water to meet the calibration range of the monosaccharides. All samples were passed through a 0.45 µm filter before aliquoting them into HPLC vials. The monosaccharides were then analysed using high-performance anion exchange chromatography (HPAEC) with an amperometry detector (PAD) (ICS 3000 Dionex, Sunnyvale, USA), equipped with a column AEC (CarboPac PA 1 analytical 4 × 250 mm). The solvents used were Milli-Q water and 60% v/v 200 mM NaOH with 40% v/v 200 mM NaOH +170 mM NaOAc, the latter run as a washing solution between injections. The internal standard was fucose, while the standards for quantification were D(+)-glucose, D(+)-xylose, D (+) mannose, D(+)-galactose, L(+)-arabinose and L(+)-rhamnose, purchased from Merck (Denmark).

## 2.7. In vitro digestion

### 2.7.1. Enzymatic activity

The enzymatic activities were determined and calculated based on the INFOGEST protocol as described by Brodkorb et al. (2019), with some modifications to the trypsin activity procedure, based on Sousa et al. (2020). The pancreatic enzyme was dissolved in simulated intestinal fluid (SIF) and was incubated in a sonication bath for 5 min. Afterwards, the enzyme solution was centrifuged at 6000 xg at 4 °C for 6 min, and the supernatant was used to determine the enzyme activity. The different enzymes used, all purchase from Merck (Denmark), except RGE from Lipolytech (France), and their corresponding measured activities were the following: alpha-amylase from human saliva (A1031 Lot. 0000435893, 101.8 U/mg), pepsin (P720 Lot SLCJ3496, 2853 U/mg), pancreatin (P7545 Lot SLCQ9801, 6 U/ mg), and bile acids (B8631 Lot SCLR6286, 1.77 mmol of bile salts/ g bile). Rabbit gastric extract (RGE) was from Lipolytech (REG15-1G Lot #2701, 27.96 U/mg, with pepsin activity of 231 U/mg).

### 2.7.2. In vitro digestion protocol

*In vitro* digestions were performed on non-fermented/fermented *Ulva* which was pre-homogenised with liquid nitrogen, and both soy protein isolate (SUPRO® EX 33 IP, Solae, Denmark) and casein (C3400, Merck, Denmark) were used as reference materials. The digestions were done as suggested in INFOGEST 2.0 (Brodkorb et al., 2019). In order to harmonise the digestions, all fermented and unfermented samples had a 1.5 g wet weight (ww) at the start of the digestion, the unfermented samples included 1 part seaweed and 3 parts water. In case of casein and soy, a food like sample was prepared by dissolving each protein to 4% in Milli-Q water overnight under magnetic stirring; 1.5 g of solution was used in the digestions. In the oral phase, 1.5 g of the sample was placed into a digestion tube, where a mix of simulated salivary fluid (SSF) containing (15 µL of 0.3 mol/L CaCl<sub>2</sub> stock solution), added in a ratio of

1:1 to the samples. Further, α-amylase from human salivary (0.150 mL, 75 U/mL final volume (vf) of 3 mL) was added, after which the sample was incubated for 2 min at 37 °C. Afterwards, the pH was adjusted to pH 3, then 0.15 mL of both pepsin (final concentration 2000 U/mL) and RGE (final concentration 60 U/mL digesta was added to each tube (vf = 6 mL). Subsequently, SGF was added to reach 6 mL on the gastric phase, and the samples were incubated for 120 min at 37 °C. After that time, SIF was added, and the pH was adjusted to 7, followed by the addition of 1.375 mL pancreatin (to reach 100 U of trypsin/ mL digesta) and 0.688 mL bile acids (to reach 10 mM); both previously dissolved in SIF (vf = 12 mL). The samples were vortexed and incubated at 37 °C for 120 min. At the end of the digestion, 550 µL pefabloc (0.1 M) to inhibit the digestion enzymes. A 1 mL aliquot of the digesta was then taken, while the rest was submitted to centrifugation at 2000 xg for 10 min at 4 °C to separate the supernatant (soluble phase) from the pellet (insoluble phase). Immediately after sampling or from digesta or supernatant, all samples were snap-frozen with liquid nitrogen and placed at −80 °C.

## 2.8. Degree of protein hydrolysis (DH)

The DH of fermented samples, from both strain screening fermentation and scale-up fermentations, and the latter also before and after *in vitro* digestion, was determined using the OPA method described by Ghelichi et al. (2024). The calculations were based on Nielsen et al. (2001), and the DH was expressed as a percentage. The number of hydrolysed peptide bonds (h) was calculated as Eq. 1:

$$h = \frac{\text{SerineNH}_2 - \beta}{\alpha} \quad (1)$$

where Serine-NH<sub>2</sub> represents the concentration of OPA-reactive primary amino groups in the sample expressed as mmol serine equivalents per gram protein. For digested samples the contribution of the corresponding digestion blanks was subtracted before calculating (h). The constants α and β were set to 1 and 0.4, respectively according to Nielsen et al. (2001).

The degree of hydrolysis was subsequently calculated as seen in Eq. 2:

$$\text{DH}\% = \frac{h}{h_{\text{tot}}} \times 100 \quad (2)$$

where h<sub>tot</sub> represents the total peptide-bond equivalents per gram of protein, estimated from the total amino acids content determined after acid hydrolysis.

## 2.9. Elemental analysis and their accessibility

Elemental composition of fresh and *in vitro* digested samples was analysed following a microwave digestion. Two hundred µL of the whole sample or supernatant after INFOGEST digestion was placed into a Teflon vial (TFM vessel from Milestone, USA), then 7 mL of pure water, together with 1.75 and 0.35 mL of concentrated HCl Trace metal 34–36% (Fisher chemical, USA) and HNO<sub>3</sub> Trace metal 67–70% (Fisher chemical, USA) were added, respectively. The vials were sealed and placed into the microwave system Ethos Plus (Milestone, USA) equipped with MPR 300/ 125 medium-pressure segmented rotor (Soriso, Italy). The digestion was performed at 180 °C for 20 min, with an initial 20 min ramp to the set temperature. The microwave digested samples were then analysed for their content in 10 elements using ICP-MS (iCAP-Q, Thermo Scientific, USA), utilising dilutions of elemental standard solutions (Inorganic Ventures, USA) and internal standards (scandium, indium and bismuth, of to 2 ppb) to normalise the areas of the detected compounds. The external standard mix ranged from 2 to 20 ppb, and the standard curves for each element, with the corresponding limit of detection (LOD) and quantification (LOQ), are found in the supplementary data Supplementary Table S3.

Accessibility of the elements in percent was calculated as earlier described (Afonso et al., 2018), exemplified in Eq. 3:

$$\text{Accessibility\%} = \frac{([E] \times V_E) - ([B] \times V_B)}{[iE] \times V} \times 100 \quad (3)$$

where [E] is the concentration of the element measured in the supernatant, and  $V_E$  is the volume of the supernatant. [B] is the concentration of the element in the blank digestion, and  $V_B$  is the corresponding volume. [iE] is the initial concentration of the element in the sample at the start of the digestion, which is multiplied by the volume of sample added to the digestion system (V).

## 2.10. Statistical analysis

All results were submitted to statistical analysis using RStudio software version 4.5.0. The graphs and statistics were done using “corrplot”, “heatmap”, “dplyr”, “tidyverse”, “stats”, “multcomp”, “rstatix”, “FSA”, “PMCMRplus” and “car”. The correlation was done using Pearson's correlation.

The data was tested for normality and homogeneity using Wilcoxon and Levene's tests. If conformity, then an ANOVA with a Tukey HSD test was performed using a Bonferroni adjustment of the  $p$ -value. If no conformity was found, the data were evaluated using a non-parametric test (Dunn test) with Benjamini and Hochberg  $p$ -value adjustments.

## 3. Results and discussion

### 3.1. Screening results

Of the 50 selected LAB strains, 36 were successfully revived on MRS agar, and their identities were confirmed. The remaining 14 strains failed to grow, possibly due to longer lag phases or suboptimal conditions tested. The 36 viable strains originated from diverse environments, including seaweed beach cast, plants, and animal faeces, reflecting the biodiversity of strains.

The monosaccharide composition of the *Ulva* extract revealed that the main carbohydrates present were rhamnose, glucose and xylose (Supplementary Table S1). Thirty of the 36 strains tested on *Ulva* extract showed potential to use these carbohydrates. It should be noted, however, that confirmation using whole biomass is needed to ensure their fermentability potential.

The 36 strains inoculated into *Ulva*-derived substrates presented different growth (OD) and acidification (pH) profiles (Supplementary Fig. S2 and S3). To determine the fastest acidifiers among the thirty strains, the derivative of their pH curve was normalisation and the top strains ( $n = 13$ ) are presented enclosed in a black squared (Supplementary Fig. S4). The fastest acidifiers were seven strains belonging to the *L. plantarum* group (*L. plantarum* NFICC19, NFICC798, NFICC983, NFICC2020, NFICC629, NFICC2157, NFICC22 and NFICC72) (Fig. S2), together with *L. paracasei* NFICC1882 and *L. paraplantarum* NFICC1922. These nine strains reached pH 4.5 within 6 h. A second group consisting of three strains reached pH 4.5 after 7 h, and they were the following: *L. brevis* NFICC1668, *L. brevis* NFICC2606, and *L. rhamnosus* NFICC2041. In contrast, the slowest acidifiers were *L. brevis* NFICC205, *L. pseudomesenteroides* NFICC202, and *L. fermentum* NFICC776. The growth profiles based on OD measurements aligned with the results on acidification, with strains of *L. plantarum* reaching the highest OD after 24 h at 30 °C (Fig. S3).

A similar dominance of *L. plantarum* during strain screening for fermentation was reported for brown seaweeds (Sørensen et al., 2021; Zioga et al., 2025). While strains such as *L. brevis* NFICC1723, *L. paracasei* NFICC1746 were found to provide slower acidification and/or were not able to acidify the *Ulva* substrate, the opposite behaviour was previously reported for *S. latissima*. This could be explained by the strain-specific preferences in carbon sources, e.g., mannitol (Zioga et al.,

2025), which is found in low concentrations in *U. fenestrata* (Jönsson et al., 2023; Olsson et al., 2020), compared to brown seaweeds.

The superior acidification and growth seen for the *L. plantarum* species on the *Ulva* extracts, could be due to this species ability to grow in the presence of polyphenols, which are enriched in seaweed (Zioga et al., 2025), although lower in *Ulva* than in e.g. *Fucus* (Sabeena Farvin & Jacobsen, 2013). Further, these species can shift its metabolism depending on the substrate available (Gupta et al., 2011), thus utilising diverse sugars (Allahgholi et al., 2023). When using, *A. esculenta* and *S. latissima* as fermentation substrates, respectively, *L. plantarum* NFICC22 or *L. rhamnosus* NFICC2041, were best suited (Sørensen et al., 2021; Zioga et al., 2025). However, in this study, with *U. fenestrata* biomass, *L. plantarum* NFICC19 and five other strains of the same species were superior to *L. plantarum* NFICC22, in terms of acidification rates and growth rates. This suggests that strains within the same species (*L. plantarum*) may be substrate-specific, therefore not achieving the same performance in acidification and/or growth rate. There are known differences between kelps and *Ulva* in terms of their monosaccharide profile, for instance kelps and specifically *S. latissima* contain fucose, mannose and glucose, while *Ulva* is rich in rhamnose, xylose and glucose (Rioux & Turgeon, 2015), as also verified in this study (Supplementary Table S1).

*L. rhamnosus* NFICC2041 did not acidify the medium nor show a high OD when *U. fenestrata* was used as substrate, which was unexpected since the *L. rhamnosus* group has earlier been associated with the ability to grow on rhamnose as a substrate, as the name indicates (Nagarajan et al., 2022; Zioga et al., 2025). We hypothesise there could be a genetic difference, since the specific strain used was isolated from squash. It remains to be proven whether the total carbohydrate composition, ulvan-rhamnose accessibility or other processes affected the strain performance.

Altogether, the following 6 strains were selected for *U. fenestrata* biomass fermentation based on earlier described criteria in the Methods section (2.2.4): (i) two from *L. plantarum*: NFICC19 (plant origin, dill), and NFICC2157 (marine fermentation, Kelp juice), (ii) two from *L. brevis*: NFICC 2696 (plant origin, broccoli) and NFICC1668 (fermented foods origin, sourdough), (iii) one *L. paraplantarum* NFICC1922 (fermented food products, Kimchi) and (iv) one *L. pentosus* NFICC2185 (plant origin, flower).

### 3.2. Strain selection for fermentation

Despite the variability in pH measurements (Supplementary Fig. S5), likely caused by the static position of the probe, most inoculated samples reached < pH 4.5 after 48 h. Exceptions were the control and samples containing *L. brevis* NFICC2696 or *L. pentosus* NFICC2185, which maintained the pH levels at 4.7 and 4.8, respectively (Table 1). The endogenous pH of the biomass was lower than in our previous study where we documented a pH of 6 (Vall-Ilosera et al., 2024). However, another recent study on fermentation of *U. fenestrata* also reported low starting pH (5.4), after mixing with water (Admantin et al., 2023). In our study, a likely reason is that the mincing and mixing of the *U. fenestrata* with 2% NaCl brine, could have induced displacement of  $H^+$  into the solution (Vall-Ilosera et al., 2024). This was confirmed in a separate trial (Supplement Table S2), although the documented decrease was small. However, even without salt brine, the biomass pH was strikingly low, and additional testing of *U. fenestrata* with a higher initial pH could be interesting to verify the performance of the strains tested in the present study.

After fermentation, viable counts for all the strains ranged between 6 and 7 log CFU/mL (Table 1), which was a slight decrease compared to initial levels of inoculum (6.7–7.7 log CFU/mL). Given that the pH was efficiently decreased, this could reflect that these strains had entered the death phase (Fu & Mathews, 1999), due to a limited amount of carbon source. However, both pH and counts indicate that all samples underwent fermentation (Stévant et al., 2024). Similarly, a decrease in viable

**Table 1**

LAB colony forming unit (CFU/mL) on MRS plates before and after fermentation (48 h at 30 °C) and the corresponding pH values for all strains, as well as the uninoculated biomass (Control) ( $n = 3$ ). The proteolytic activity is expressed as milliequivalents (mEq) of serine per litre ( $n = 3$ ). Different superscripts indicate significant differences ( $p < 0.05$ ).

| Strain                            | Time                | CFU/mL               | log CFU | pH   | mEq Serine/L               |
|-----------------------------------|---------------------|----------------------|---------|------|----------------------------|
| Control                           | before fermentation | 0                    | 0       | 5.00 |                            |
|                                   | after fermentation  | 0                    | 0       | 4.80 | 0.46 ± 0.03 <sup>ac</sup>  |
| <i>L. plantarum</i> NFICC19       | before fermentation | 4.68 10 <sup>7</sup> | 7.60    | 5.00 |                            |
|                                   | after fermentation  | 8.00 10 <sup>6</sup> | 6.90    | 4.12 | 0.66 ± 0.08 <sup>b</sup>   |
| <i>L. paraplantarum</i> NFICC1922 | before fermentation | 4.08 10 <sup>7</sup> | 7.61    | 5.01 |                            |
|                                   | after fermentation  | 5.00 10 <sup>6</sup> | 6.70    | 4.07 | 0.58 ± 0.17 <sup>ab</sup>  |
| <i>L. plantarum</i> NFICC2157     | before fermentation | 3.39 10 <sup>7</sup> | 7.59    | 5.00 |                            |
|                                   | after fermentation  | 1.90 10 <sup>6</sup> | 6.28    | 4.10 | 0.37 ± 0.12 <sup>c</sup>   |
| <i>L. brevis</i> NFICC1668        | before fermentation | 4.25 10 <sup>7</sup> | 7.63    | 5.00 |                            |
|                                   | after fermentation  | 3.95 10 <sup>6</sup> | 6.60    | 4.08 | 0.58 ± 0.04 <sup>ab</sup>  |
| <i>L. pentosus</i> NFICC2185      | before fermentation | 5.00 10 <sup>6</sup> | 6.70    | 5.00 |                            |
|                                   | after fermentation  | 8.84 10 <sup>6</sup> | 6.95    | 4.70 | 0.75 ± 0.15 <sup>b</sup>   |
| <i>L. brevis</i> NFICC2696        | before fermentation | 5.00 10 <sup>6</sup> | 6.70    | 5.00 |                            |
|                                   | after fermentation  | 5.46 10 <sup>6</sup> | 6.74    | 4.80 | 0.53 ± 0.11 <sup>abc</sup> |

counts after 48 h was observed in *U. rigida* hydrolysates under fermentation conditions with LAB (Brandão et al., 2025). However, other studies on brown seaweed have reported increased counts after fermentation (Allahgholi et al., 2023; Stévant et al., 2024; Zioga et al., 2025). This suggests that *U. fenestrata* carbohydrates were not as available as the carbohydrates from brown seaweeds.

In contrast, *L. brevis* NFICC2696 and *L. pentosus* NFICC2185, which had a slightly higher log CFU/mL after fermentation, may have a longer lag phase or require longer fermentation times, due to inhibitory compounds in *Ulva* or suboptimal fermentation conditions (Brandão et al., 2025). The *L. brevis* NFICC2696 was derived from fermented broccoli, and as seen for a different *L. brevis* strain, minimal growth took place under salt stress assay at 2, 4 and 6% NaCl, resulting in longer lag phases with increasing salt content (Zioga et al., 2025). The ionic strength of the *Ulva* biomass was 2.5% NaCl equivalents, compared to <0.1% NaCl equivalents for broccoli (Supplementary Table S2). Further, the carbohydrates found in broccoli are glucose and fructose (Xu et al., 2015), while *U. fenestrata* contains more complex carbohydrates such as ulvan (Kidgell et al., 2019), thus the latter might require specific enzymes.

The other slow-growing strain, *L. pentosus* NFICC2185, was isolated from a garden flower, and a hypothesis is that *L. pentosus* was not able to utilise D-xylose, one of the major carbohydrates in *U. fenestrata*. This has previously been seen in *L. pentosus* isolated from fermented cucumbers (Anekella & Pérez-Díaz, 2020).

Among the six strains tested, *L. plantarum* NFICC19 and *L. paraplantarum* NFICC1922 gave the highest serine-equivalent concentration after 48 h (Table 1), and showed significantly ( $p < 0.05$ ) greater proteolytic activity than the control, which was not inoculated.

### 3.2.1. Monosaccharide composition in *U. fenestrata* during strain selection

Analyses of monosaccharides in *U. fenestrata* inoculated with the 6 selected strains (Supplementary Fig. S7) revealed that the relative glucose content was significantly lower compared to the control (uninoculated) for *L. plantarum* NFICC2157 and *L. paraplantarum* NFICC1922. In contrast, neither rhamnose nor xylose showed significant changes regardless of strain, suggesting utilisation of glucose by the inoculum but not of rhamnose and xylose.

The following three strains were selected for the scale-up fermentations, based on the criteria explained in the methods section 2.3:

- *L. plantarum* NFICC19, for its fast decrease of pH, higher viable counts of bacteria after fermentation and higher serine equivalent compared to other strains.
- *L. paraplantarum* NFICC1922, for its lower pH after fermentation, higher serine equivalent and a different origin (Kimchi).

- *L. brevis* NFICC1668, for its fast acidification and to diversify the strains, since the remaining candidates were either *L. plantarum*s or *L. paraplantarum*s.

### 3.3. Scale-up fermentation

Scale-up fermentations yielded pH curves similar to those observed in the strain selection experiments (Supplementary Fig. S5 and Fig. 2). All inoculated samples reached a pH < 4.5 after 48 h, while the uninoculated controls remained stable (Fig. 2). The strain *L. plantarum* NFICC19 achieved the lowest pH after fermentation with a 15% reduction from time 0 to time 48 h; however, it was the strain *L. brevis* NFICC1668 that yielded the fastest decrease in pH, reaching 4.5 after only 20 h, although it had a higher end pH at 48 h (4.43) and its total relative pH-decrease was only 10%.

Similar to the strain selection (section 3.2), the uninoculated *U. fenestrata* (Control) did not exhibit any viable LAB counts after 48 h, thereby confirming that neither contamination nor native LAB grew during 48 h at 30 °C (Table 2).

While all three inoculated *U. fenestrata* presented viable counts (CFU/ml) within the range of 6–8 log (Table 2), the control and the acidified *Ulva* presented no growth. Furthermore, all strains showed significantly higher CFU/mL after fermentation, with a similar increase of 0.3 log. The pH-monitoring during and after 48 h incubation (Fig. 2) confirms that there was a fermentation process, even though the starting pH of all samples was only 4.95, just as in the selection of strains for fermentation.

#### 3.3.1. Monosaccharide analysis

Fermentation of *U. fenestrata* with LAB resulted in statistically significant changes in the monosaccharide composition in the supernatant phase (Fig. 3), while the pellet remained unaffected (Supplementary Fig. S6). The total monosaccharide content for both the pellet and the supernatant is shown in Supplementary Fig. S8.

Rhamnose, xylose and glucose were the predominant monosaccharides in all samples, in both supernatant and pellet. The relative content of glucose was statistically significantly reduced when *U. fenestrata* was inoculated with *L. paraplantarum* NFICC1922 and *L. plantarum* NFICC19, compared to the control (Fig. 3), with 30% observed reduction for both (Fig. 3). These results are in line with previous studies that have reported a 41% depletion of glucose for the brown seaweeds *A. esculenta* and *S. latissima*, and a 43 and 26% reduction, respectively, of mannitol compared to initial values, when fermenting with *L. plantarum* NFICC22 (Sørensen et al., 2021). In another study of *S. latissima*, 88% reduction in mannitol was reported using a commercial *L. plantarum* strain (Bruhn et al., 2019).

The relative content of rhamnose and xylose was significantly higher

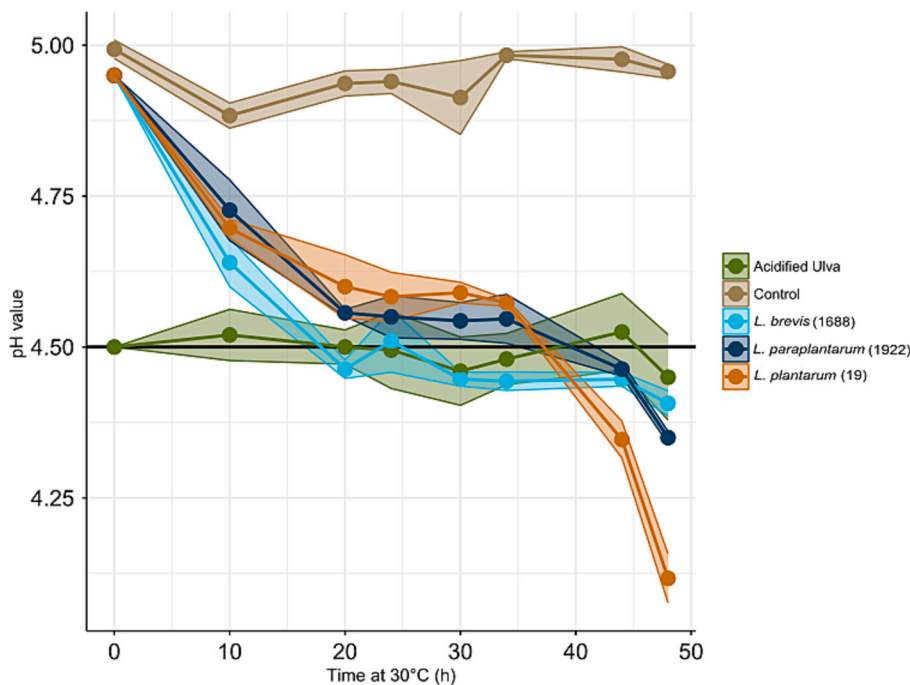


**Fig. 2.** pH curves during scale-up fermentation at 30 °C for 48 h with all strains; uninoculated biomass (Control), pH-adjusted sample to 4.5 (Acidified *Ulva*), inoculated samples with *Levilactobacillus brevis* (*L. brevis* NFICC1668), *Lactoplantibacillus paraplantarum* (*L. paraplantarum* NFICC1922), and *Lactoplantibacillus plantarum* (*L. plantarum* NFICC19), (*n* = 3). Values are represented by mean and ± SD. Each strain identifier appears as a numeric code from the National Food Institute Culture Collection (NFICC).

**Table 2**  
LAB colony forming unit (CFU/mL) on MRS plates before and after scale-up fermentation (48 h at 30 °C), and the corresponding pH values. Two controls were used, one with water (Control) and one adjusted to pH 4.5 with lactic acid (Acid control) (*n* = 3).

| Strain                            | Time                | CFU/mL               | Log CFU | pH          |
|-----------------------------------|---------------------|----------------------|---------|-------------|
| Control                           | before fermentation | 0                    | 0       | 4.95 ± 0.07 |
|                                   | after fermentation  | 0                    | 0       | 5.00 ± 0.07 |
| Acidified Ulva                    | before fermentation | 0                    | 0       | 4.50 ± 0.00 |
|                                   | after fermentation  | 0                    | 0       | 4.45 ± 0.01 |
| <i>L. plantarum</i> NFICC19       | before fermentation | 2.56 10 <sup>7</sup> | 7.40    | 4.95 ± 0.07 |
|                                   | after fermentation  | 5.16 10 <sup>7</sup> | 7.70    | 4.18 ± 0.02 |
| <i>L. paraplantarum</i> NFICC1922 | before fermentation | 3.60 10 <sup>7</sup> | 7.56    | 4.95 ± 0.07 |
|                                   | after fermentation  | 8.82 10 <sup>7</sup> | 7.95    | 4.27 ± 0.01 |
| <i>L. brevis</i> NFICC1668        | before fermentation | 4.71 10 <sup>7</sup> | 7.67    | 4.95 ± 0.07 |
|                                   | after fermentation  | 1.24 10 <sup>8</sup> | 8.09    | 4.47 ± 0.02 |

(14%) in *U. fenestrata* inoculated with either *L. plantarum* NFICC19 or *L. paraplantarum* NFICC1922 compared to the uninoculated control (%) (Fig. 3), with an increase of 14% each. One partial explanation might be the specific decrease in relative glucose content, which would increase the relative contributions of the remaining monosaccharides. Alternatively, a release of ulvan due to cell wall deterioration could skew the relative monosaccharide content (Reboleira et al., 2021), since ulvan primarily consists of xylose and rhamnose (Kidgell et al., 2019). In the acidified *U. fenestrata*, the composition remains comparable to the control, indicating that acid alone may not have been the main responsible factor for the increase of xylose and rhamnose in the solution. Future studies should however evaluate how the final pH-values yielded by the different strains (4.08–4.12) affect the monosaccharide composition.

The fermentation-induced increase in relative xylose content, found in the present study, also indicates that the strains did not use xylose as a carbon source, which is in line with the already reported inability of *L. plantarum* to utilise xylose (Zioga et al., 2025). In contrast, when fermenting *S. latissima* substrate inoculated with *L. brevis*, there was a depletion of xylose after 24 h (Zioga et al., 2025). Others have seen a small reduction in xylose when fermenting *A. esculenta* with a LAB

consortium containing *L. brevis* (Allahgholi et al., 2023), indicating *L. brevis* can utilise monosaccharides and simpler polysaccharides. However, the polysaccharide ulvan has been described to have a more complex structure compared to polysaccharides found in brown seaweed (Hurd et al., 2014), possibly making it unavailable for *L. brevis* in the present study.

Additional monosaccharides present in the supernatants and pellets from fermented *U. fenestrata*, although in smaller quantities compared to xylose, are mannose and galactose. Mannose is found in the cell wall of *Ulva* spp., and both *L. brevis* and *L. plantarum* have previously been reported to utilise mannose during fermentation of brown seaweeds *S. latissima* and *A. esculenta* (Sørensen et al., 2021; Zioga et al., 2025). However, no significant differences were found between the fermented samples and the control. Galactose levels were very low in all samples (0.5–1%); therefore, galactose was not included in the statistical analysis. Regarding arabinose, no detectable levels were found in the biomass used.

Future studies should determine the use of LAB *L. paraplantarum* NFICC1922 and *L. plantarum* NFICC19 in a more concentrated (e.g., 1:1) fermentation to confirm if the hypothesis that glucose is the only saccharide decreased during fermentation of *U. fenestrata*. Such a study

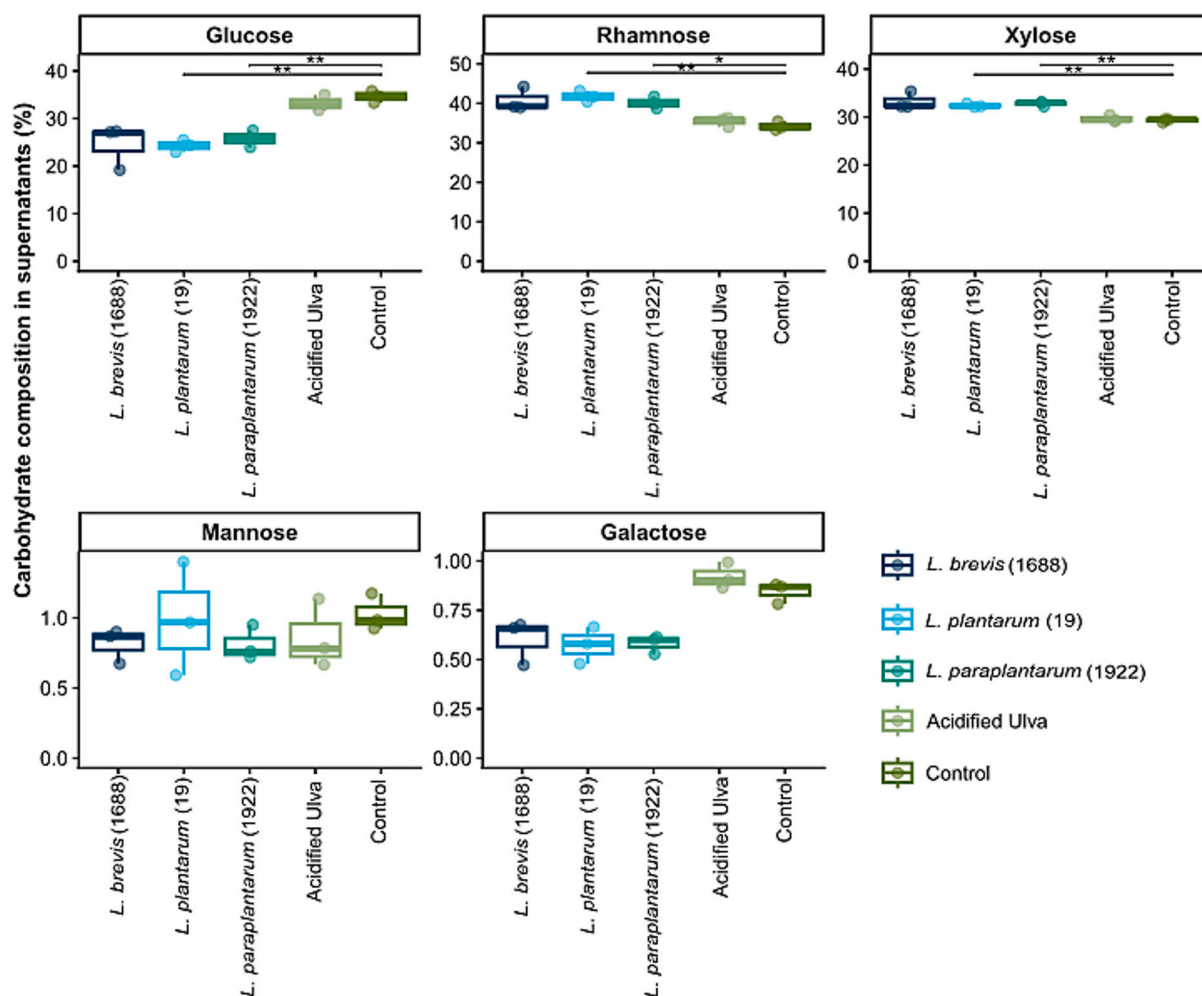


**Fig. 3.** Monosaccharide composition in the acid hydrolysed supernatant from fermented *U. fenestrata*, expressed as a percentage of the total monosaccharide ( $n = 3$ ). Control, which refers to the uninoculated biomass, and each strain identifier appears as a numeric code from the National Food Institute Culture Collection (NFICC). Significance represented as  $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)

should also explore potential negative effects from up-concentrating anti-microbial compounds, such as lectins (Brandão et al., 2025) or polyphenols (Zioga et al., 2025).

### 3.3.2. Total amino acids

The results from total amino acid analyses showed no significant differences between fermented samples and controls (Table 3). The total amino acid content ranged from 6.6 to 9.6% dry weight (DW), which is in the lower to mid end of the range previously reported for *U. fenestrata* (4.2–23.6% DW) (Stedt, Steinhagen, et al., 2022; Trigo, Steinhagen, et al., 2025; Trigo, Chamlagain, et al., 2025; Wirenfeldt et al., 2024).

### 3.3.3. In vitro protein digestibility

The DH was used as an indicator of protein digestibility, based on the cleavage of peptide bonds before and after *in vitro* digestion. Overall, fermentation significantly improved the total DH reached after digestion of *U. fenestrata*, however, the effect was strain-dependent (Fig. 4).

Samples fermented with *L. brevis* NFICC1668 exhibited the highest DH after digestion (71%), which was significantly greater than both the control and the fresh biomass (Fig. 4B). The final DH samples inoculated with *L. plantarum* NFICC19 was also significantly higher than for fresh biomass, however, not compared to the control. For *L. paraplantarum* NFICC1922, no significant differences were detected compared to either the fresh biomass or the control.

Previous studies investigating protein digestibility of fermented

seaweeds reported similar outcomes. Brandão et al. (2025) observed a tenfold increase in protein digestibility after fermentation and digestion of *U. rigida* using a consortium of four LAB, including *L. plantarum* and *L. brevis*, which can be compared with our 2.5-fold and 7.5-fold increase in DH with *L. plantarum* NFICC19 and *L. brevis* NFICC1668, respectively. However, that study used commercial strains and assessed the combined effect of LAB. Similarly, in the red seaweeds such as *P. palmata* and *Trichoderma pseudokoningii* protein digestibility was also improved by up to 65% compared to that of casein, set as reference (Marrion et al., 2003).

The initial DH prior to digestion was significantly largest for *U. fenestrata* fermented with *L. brevis* NFICC1668 compared to the fresh and control (Fig. 4A). This result indicates that proteolysis also occurs during the fermentation, likely due to the proteolytic activity of the strains (Christensen et al., 2022). Thus, the final DH, as an indicator for total protein digestibility, was influenced not only by digestive enzymes, but also by protein hydrolysis during fermentation. Regarding the digestion-induced hydrolysis, *L. brevis* NFICC1668 was significantly different to the control. In future studies, additional microbial blank controls should be included to confirm that DH measurements were not influenced by peptides originated by microbial biomass.

After subjection to low pH alone, i.e., acidified *Ulva*, the DH reached after digestion was significantly higher than that of fresh biomass, and similar to the fermented samples. This suggests that no further digestion occurred based on the fermentation per se. A hypothesis could be that

**Table 3**  
Amino acid composition for all fermented samples and controls (i.e., after dilution 1:3 in salt brine) after 48 h of fermentation/incubation, as well as in fresh non-diluted *U. fenestrata* in mg per gram DW biomass. Data are shown as mean  $\pm$  standard deviation ( $n = 3$ ). Total amino acids (TAA) and total essential amino acids (TEAA) are shown. Significant differences between samples are calculated on a DW basis, and indicated with different subscripts ( $p < 0.05$ ).

| Sample                         | LYS**           | ARG             | HIS**           | GLY             | SER             | ALA              | THR**            | GLU             | ASP              | PRO             | VAL**           | MET**           | TYR             | ILE**           | LEU**           | PHE**           | TAA                            | TEAA                          |
|--------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|------------------|------------------|-----------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|--------------------------------|-------------------------------|
| Fresh                          | 4.65 $\pm$ 0.11 | 1.37 $\pm$ 0.03 | 4.09 $\pm$ 0.09 | 5.04 $\pm$ 0.27 | 8.06 $\pm$ 0.56 | 6.48 $\pm$ 0.42  | 17.65 $\pm$ 1.37 | 6.39 $\pm$ 0.34 | 16.88 $\pm$ 1.08 | 3.75 $\pm$ 0.17 | 3.74 $\pm$ 0.29 | 1.39 $\pm$ 0.06 | 3.83 $\pm$ 0.13 | 1.90 $\pm$ 0.19 | 7.66 $\pm$ 0.33 | 3.89 $\pm$ 0.41 | 96.78 $\pm$ 5.78 <sup>a</sup>  | 44.98 $\pm$ 2.83 <sup>a</sup> |
| Acidified <i>Ulva</i>          | 4.58 $\pm$ 0.39 | 0.79 $\pm$ 0.06 | 2.54 $\pm$ 0.20 | 6.83 $\pm$ 0.43 | 6.61 $\pm$ 0.78 | 11.29 $\pm$ 1.45 | 10.79 $\pm$ 1.10 | 6.38 $\pm$ 0.67 | 11.17 $\pm$ 1.53 | 5.27 $\pm$ 0.69 | 6.50 $\pm$ 0.90 | 1.96 $\pm$ 0.26 | 2.57 $\pm$ 0.30 | 3.91 $\pm$ 0.68 | 7.87 $\pm$ 0.97 | 6.97 $\pm$ 0.95 | 96.06 $\pm$ 10.61 <sup>a</sup> | 45.13 $\pm$ 5.23 <sup>a</sup> |
| Control                        | 3.77 $\pm$ 0.81 | 0.75 $\pm$ 0.04 | 2.41 $\pm$ 0.16 | 6.31 $\pm$ 1.01 | 5.87 $\pm$ 1.25 | 7.42 $\pm$ 5.59  | 9.91 $\pm$ 2.02  | 5.58 $\pm$ 0.81 | 10.26 $\pm$ 2.42 | 4.74 $\pm$ 1.04 | 5.94 $\pm$ 1.19 | 1.60 $\pm$ 0.42 | 2.23 $\pm$ 0.42 | 3.66 $\pm$ 0.70 | 6.15 $\pm$ 1.56 | 5.98 $\pm$ 1.74 | 80.86 $\pm$ 17.64 <sup>b</sup> | 37.68 $\pm$ 5.51 <sup>a</sup> |
| <i>L. brevis</i> (1668)        | 3.16 $\pm$ 0.25 | 0.78 $\pm$ 0.07 | 2.40 $\pm$ 0.15 | 5.90 $\pm$ 0.08 | 5.30 $\pm$ 0.18 | 1.05 $\pm$ 0.02  | 9.73 $\pm$ 0.73  | 5.15 $\pm$ 0.51 | 8.77 $\pm$ 0.53  | 3.78 $\pm$ 0.38 | 5.15 $\pm$ 0.51 | 1.31 $\pm$ 0.19 | 1.97 $\pm$ 0.09 | 2.86 $\pm$ 0.39 | 4.25 $\pm$ 0.39 | 4.82 $\pm$ 0.14 | 66.35 $\pm$ 3.42 <sup>a</sup>  | 33.63 $\pm$ 2.72 <sup>a</sup> |
| <i>L. paraplantarum</i> (1922) | 3.94 $\pm$ 0.60 | 0.90 $\pm$ 0.01 | 2.95 $\pm$ 0.04 | 6.64 $\pm$ 0.64 | 6.24 $\pm$ 0.87 | 4.46 $\pm$ 5.84  | 10.45 $\pm$ 5.85 | 6.05 $\pm$ 0.68 | 9.05 $\pm$ 1.05  | 4.63 $\pm$ 0.52 | 6.16 $\pm$ 0.84 | 1.66 $\pm$ 0.27 | 2.44 $\pm$ 0.43 | 3.32 $\pm$ 0.68 | 5.10 $\pm$ 0.53 | 5.51 $\pm$ 1.52 | 79.72 $\pm$ 16.82 <sup>a</sup> | 38.65 $\pm$ 7.37 <sup>a</sup> |
| <i>L. plantarum</i> (19)       | 3.54 $\pm$ 0.73 | 0.79 $\pm$ 0.05 | 2.56 $\pm$ 0.17 | 5.28 $\pm$ 1.31 | 4.83 $\pm$ 1.21 | 4.54 $\pm$ 5.12  | 8.79 $\pm$ 1.02  | 8.80 $\pm$ 1.02 | 5.49 $\pm$ 1.81  | 4.01 $\pm$ 1.01 | 5.22 $\pm$ 1.11 | 1.42 $\pm$ 0.35 | 2.03 $\pm$ 0.55 | 3.30 $\pm$ 0.80 | 6.11 $\pm$ 1.56 | 5.02 $\pm$ 1.66 | 79.46 $\pm$ 19.07 <sup>a</sup> | 35.98 $\pm$ 7.38 <sup>a</sup> |

\*TAA and TEAA, calculated by summing up the individual amino acids. \*\* indicates TEAA.

acidic conditions can aid the liberation of proteins which are cross-linked to cell wall polysaccharides, thus becoming more accessible for enzymes (De Souza Celente et al., 2023). That an acidic environment can lead to cell wall deterioration has been shown during ensilaging *S. latissima* and *A. esculenta* (Yen et al., 2022).

Interestingly, acidification alone did not result in a higher initial DH. Therefore, the improved protein digestibility reached in the acidified samples was induced by the digestion, and may reflect enhanced accessibility of digestive enzymes to proteins.

Significant differences were observed in DH after digestion between the inoculated *Ulva* samples, and the soy protein isolates, which both reached a DH of 42% and 55%, respectively (Fig. 4B). Casein, however, showed lower DH than soy; 27%, which was still higher than what others have reported, 15% (Marmon & Undeland, 2013). A reason could be that the casein powder partly remained non-dissolved, with observable particles detected by the authors, hampering the access of the enzymes to the proteins. This was also seen in our previous study (Cavonius et al., 2016).

The digestibility of the fresh *U. fenestrata* (29% DH) was in line with previous values reported for freeze-dried *U. fenestrata* using a rat model; 26% (DH) (Juul et al., 2022), as well as an *in vitro* digestion protocol adapted for Caco-2 cell studies (Trigo et al., 2021);  $27.7 \pm 2.6\%$ .

The absence of significant differences in digestibility observed between the fermentation control and the fresh *Ulva* indicated that 30 °C for 48 h alone did not improve the digestibility (Fig. 4A and B). This was likely since such mild heating is not sufficient to break down the plant cell wall (Kidgell et al., 2019), which would rise accessibility of the digestion enzymes to the proteins (Marion et al., 2003).

Overall, fermentation with *L. brevis* NFICC1668 improved *in vitro* digestibility both before and after digestion of fresh *U. fenestrata* biomass under the conditions of this study. The higher DH observed prior to digestion for this strain, together with the higher DH post digestion, as well as the significant increased in the relative content of xylose and rhamnose in the supernatant, may reflect partial degradation of cell wall polysaccharides and higher proteolysis of intracellular proteins (Luo et al., 2025). To confirm whether the main cell wall polysaccharide of *U. fenestrata*, ulvan, was degraded during acidification and/or fermentation would require further investigations, for instance, using imaging analysis or size-exclusion chromatography of the soluble fraction, or by determining specific enzymatic activities, i.e. ulvan lyase (Reisky et al., 2019).

### 3.3.4. Elemental composition and accessibility

The most abundant elements in all samples were iron and iodine (Table 4). Among the five elements, all were found in the range of previous values for *Ulva* spp. (Afonso et al., 2018; Flores et al., 2015; García-Sartal et al., 2013; Rauf Dahlstedt et al., 2026), with the exception of iron content, which was half of that previously reported for tank-cultivated *U. fenestrata* (Rauf Dahlstedt et al., 2026).

Almost all the fermented and acidified samples exhibited significantly lower iodine content compared to the control. However, large variation between replicates was observed for *L. plantarum* NFICC19, which may have contributed to the lack of significant differences. Below pH 5–6 iodine can become volatile (Cook et al., 2022; Winger et al., 2005), which could potentially explain the loss seen in this study. Manganese concentrations were also significantly reduced during incubation compared to fresh biomass, but only for the control and the acidified *Ulva*.

The accessibility of elements, was assessed after *in vitro* digestions, except for total arsenic which was found to be below the detection limit in digests from all samples (Supplementary Table S3). The low arsenic concentrations reported correspond to total arsenic only, as no arsenic speciation was performed. Consequently, the suitability of these samples for human consumption with respect to arsenic cannot be assessed, as regulatory limits are based on inorganic arsenic.

Among the quantified elements, manganese and copper exhibited the

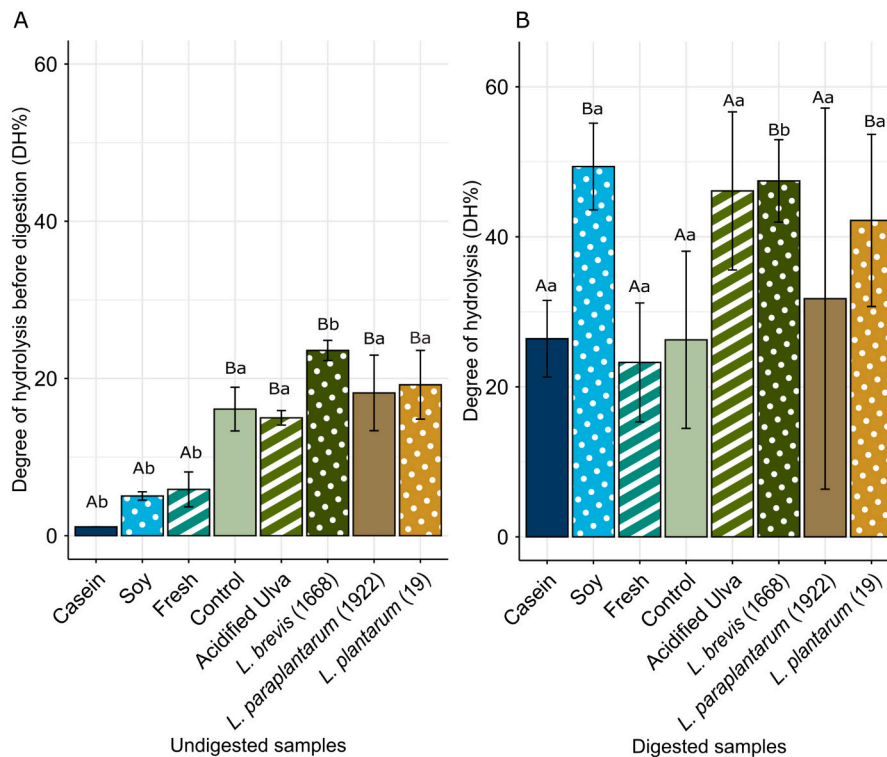


**Fig. 4.** Degree of protein hydrolysis (DH) determined by the OPA-method (Ghelichi et al., 2024; Nielsen et al., 2001). Panel A indicates DH prior to digestion and panel B after digestion ( $n = 4$ ). Different uppercase letters indicate statistical significance ( $p < 0.05$ ) compared to fresh *U. fenestrata*, whereas different lowercase letters indicate significant differences ( $p < 0.05$ ) relative to the control, uninoculated biomass.

**Table 4**

Elemental composition of fresh and fermented samples not subjected to *in vitro* digestion, expressed as a mean  $\pm$  SD ( $n = 3$ , except where indicated by (\*), where  $n = 2$ ). Different letters indicate significant differences among samples ( $p < 0.05$ ).

| Samples                           | Elemental composition (mg/kg dry weight biomass) |                                  |                               |                               |                              |
|-----------------------------------|--------------------------------------------------|----------------------------------|-------------------------------|-------------------------------|------------------------------|
|                                   | Iron                                             | Iodine                           | Manganese                     | Arsenic                       | Copper                       |
| Fresh                             | 138.02 $\pm$ 13.61 <sup>A</sup>                  | 115.42 $\pm$ 3.48 <sup>AB</sup>  | 9.51 $\pm$ 0.49 <sup>A</sup>  | 3.96 $\pm$ 0.35 <sup>A</sup>  | 5.76 $\pm$ 3.53 <sup>A</sup> |
| Control (uninoculated)            | 149.00 $\pm$ 15.49 <sup>A</sup>                  | 142.54 $\pm$ 20.82 <sup>A</sup>  | 6.57 $\pm$ 0.55 <sup>B</sup>  | 2.16 $\pm$ 0.18 <sup>B</sup>  | 3.40 $\pm$ 0.28 <sup>A</sup> |
| Acidified Ulva                    | 108.86 $\pm$ 3.54 <sup>A</sup>                   | 75.24 $\pm$ 3.42 <sup>B</sup>    | 5.74 $\pm$ 0.49 <sup>B</sup>  | 1.45 $\pm$ 0.00 <sup>B*</sup> | 3.48 $\pm$ 1.24 <sup>A</sup> |
| <i>L. plantarum</i> NFICC19       | 146.67 $\pm$ 32.19 <sup>A</sup>                  | 104.08 $\pm$ 20.39 <sup>AB</sup> | 9.29 $\pm$ 0.25 <sup>AB</sup> | 2.57 $\pm$ 0.21 <sup>B*</sup> | 5.44 $\pm$ 1.44 <sup>A</sup> |
| <i>L. paraplantarum</i> NFICC1922 | 118.80 $\pm$ 12.75 <sup>A</sup>                  | 82.06 $\pm$ 10.28 <sup>B</sup>   | 7.90 $\pm$ 1.25 <sup>AB</sup> | 1.68 $\pm$ 0.02 <sup>B*</sup> | 5.12 $\pm$ 2.42 <sup>A</sup> |
| <i>L. brevis</i> NFICC1668        | 123.48 $\pm$ 19.92 <sup>A</sup>                  | 73.31 $\pm$ 14.42 <sup>B</sup>   | 7.61 $\pm$ 1.16 <sup>AB</sup> | 1.87 $\pm$ 0.34 <sup>B*</sup> | 4.49 $\pm$ 1.47 <sup>A</sup> |

\* Values correspond to samples prepared by acid microwave digestion.

highest accessibility; 70% and 75%, respectively (Fig. 5), the former falling within the range reported for *U. lactuca* (95% and 67% after *in vitro* digestion) (Afonso et al., 2018; Moreda-Piñeiro et al., 2012).

In contrast, iodine accessibility did not exceed 32% in any sample (Fig. 5), in agreement with previously reported accessibility (24%) for *U. lactuca* (Afonso et al., 2018). The relatively low iodine accessibility in some seaweeds has been attributed to the presence of polysaccharides, which hinder accessibility for digestive enzymes (Melse-Boonstra et al., 2026). Further, iodine can be bound with other macromolecules such as proteins (Hou et al., 2000). Fermentation significantly increased the iodine accessibility from 25% up to 32%, with the exception of *U. fenestrata* inoculated with *L. plantarum* NFICC19. For *L. plantarum* NFICC1922, the increase may be attributed to the partial degradation of polysaccharides and proteins during fermentation, thereby facilitating iodine release during digestion, although additional studies on carbohydrate degradation are required to confirm this hypothesis. For *L. brevis*

NFICC1668 and the acidified *Ulva* sample, the high DH after fermentation and digestions (section 3.3.3) could point at disruption of protein-iodine interactions (Hou et al., 2000).

Iron accessibility after digestion of fresh biomass was 11%, and in line with previously reported iron accessibility for sea lettuce of 10.6%, based on dialysates (Moreda-Piñeiro et al., 2012) and 8%, based on Caco-2 cell experiments (Flores et al., 2015). Fermentation significantly increased iron accessibility when applying *L. plantarum* NFICC1922 (38%) compared to fresh biomass, but not relative to the control, 19%. As for iodine, increased iron accessibility may be related to polysaccharides and/or proteins degradation during fermentation, but further studies are needed to confirm the differences between *L. paraplantarum* NFICC1922 and other strains.

Our results indicate that while a daily serving of 5 g (dry weight) for the *U. fenestrata* fermented with *L. paraplantarum* NFICC1922, would provide approximately 131  $\mu$ g accessible iodine (Supplementary

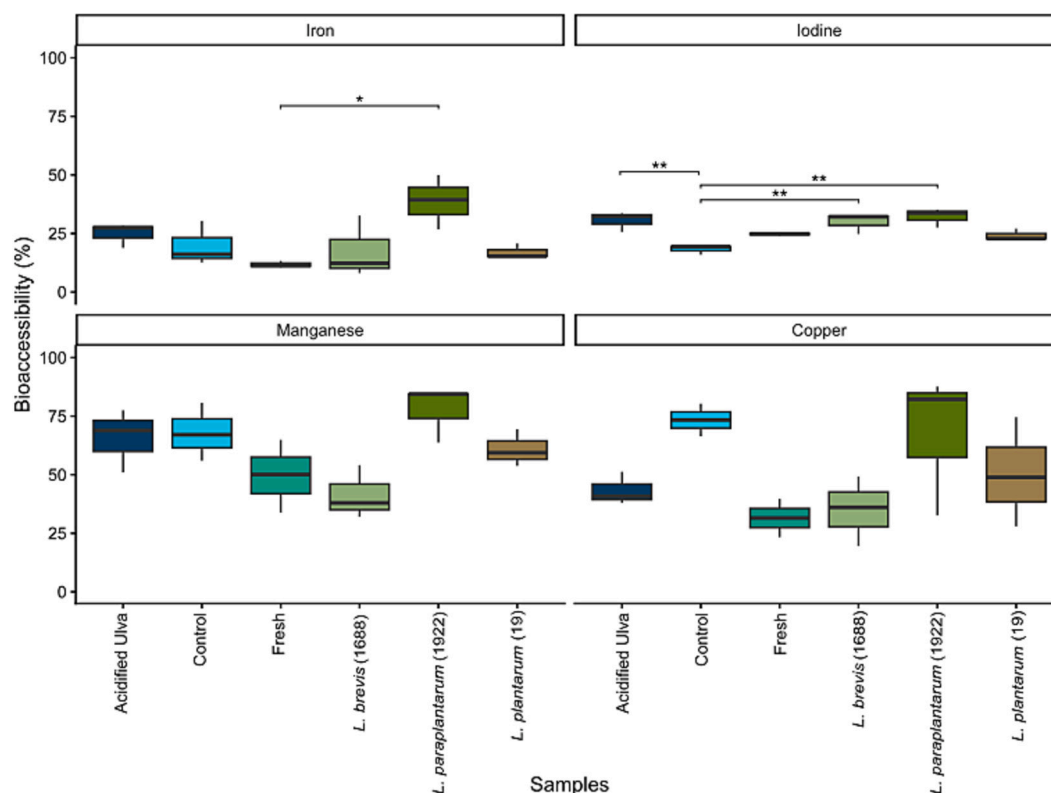


**Fig. 5.** Accessibility of four elements after in-vitro digestion of *Ulva* samples: biomass fermented with 3 LAB strains (*L. brevis* 1668, *L. paraplantarum* 1922, and *L. plantarum* 19), fresh biomass, the uninoculated biomass and incubated at 30 °C for 48 h (Control), and the acidified biomass (pH 4.5) incubated at 30 °C for 48 h (Acidified *Ulva*) ( $n = 3$ ). Significant differences are indicated in the figure as follows: (\*)  $p < 0.05$ , (\*\*)  $p < 0.01$ .

Table S4), corresponding to 87% of the daily recommended intake (DRI) by the Nordic Nutrition Recommendations (NNR) (Blomhoff et al., 2023), the same serving would only provide 0.226 mg of accessible iron, resulting in 2% of the DRI (~ 15 mg/day) (Blomhoff et al., 2023). Thus, the role of *Ulva* as a dietary iron source is, based on this study, limited.

#### 4. Conclusions

The present work contributes with new knowledge on the potential of LAB fermentation to improve the nutritional properties of seaweed. Particularly, it deepened the understanding of strain-specific fermentation effects on protein digestibility and mineral accessibility in green seaweed. The outcomes demonstrated that several lactic acid bacteria are capable of fermenting *U. fenestrata* biomass; specifically, 30 strains effectively acidified water extracts of *U. fenestrata*. Among them, *L. plantarum* NFICC19 showed the highest potential for biomass fermentation based on its rapid acidification, pronounced proteolytic activity and stable counts after 48 h of fermentation at both small (50 mL) and large scale (100 mL). *L. paraplantarum* NFICC1922 and *L. brevis* NFICC1668 showed similar trends as *L. plantarum* NFICC19, but with lower acidification capacity and proteolytic activity.

The relative abundance of glucose was reduced during fermentation, particularly by *L. plantarum* NFICC19 and *L. paraplantarum* NFICC1922, suggesting preferential utilisation of glucose. Despite the successful fermentation and higher initial degree of protein hydrolysis for both *L. plantarum* NFICC19 and *L. brevis* NFICC1668 compared to fresh biomass, only *L. brevis* NFICC1668 significantly improved the degree of protein hydrolysis after *in vitro* digestion of *U. fenestrata* compared to the control and the fresh biomass. Meanwhile, the accessibility of both iron and iodine was greatest after fermentation with *L. paraplantarum* NFICC1922, with iron accessibility increasing from 11% in the fresh

biomass to 38%, and iodine accessibility increasing from 18% in the control to 32%. Interestingly, acidification alone also raised protein digestibility, iodine accessibility and iron accessibility compared to the fresh *U. fenestrata*, which should be investigated further, and reveals strong potential for ensilaging.

Further investigations are also needed to unravel the microstructural and biochemical effects of direct acidification versus LAB-fermentation. For example, careful monitoring of the acidification curves provided by individual strains will clarify the roles of both acidification rate and acid type for protein digestibility. In addition, changes in the aroma profile during fermentation of *U. fenestrata* will be a key feature for consumers' acceptance of such products, and thus, should be unravelled and compared with effects from acidification alone.

This study demonstrates that selected LAB strains not only have the potential to ferment *U. fenestrata* and provide a pH level < 4.5, but also to improve the nutritional value by increasing both the total protein hydrolysis and accessibility of selected elements after *in vitro* digestion. Altogether, these findings provide a basis for further development of fermented products from green seaweeds.

#### CRediT authorship contribution statement

**Mar Vall-Iloera:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Evangelia Zioga:** Writing – review & editing, Resources, Methodology, Investigation. **Mathias Johansson:** Writing – review & editing, Resources, Investigation. **Sarah Verhoeven:** Writing – review & editing, Investigation. **Kristoffer Stedt:** Writing – review & editing, Resources. **Susan Løvstad Holdt:** Writing – review & editing, Resources. **Claus Heiner Bang-Berthelsen:** Writing – review & editing, Resources. **Anna Ström:** Writing – review & editing, Supervision, Resources. **Ingrid Undeland:** Writing – review & editing, Supervision,

Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2026.120668>.

## Data availability

Data will be made available on request.

## References

- Admantin, C. Y., Turner, M., Lo, R., Nguyen, V. H., & Paul, N. (2023). *Fermentation of green seaweed Ulva fasciata using six different strain of lactic acid bacteria (LAB)* (pp. 611–624). [https://doi.org/10.2991/978-94-6463-122-7\\_58](https://doi.org/10.2991/978-94-6463-122-7_58)
- Afonso, C., Cardoso, C., Ripol, A., Varela, J., Quental-Ferreira, H., Pousão-Ferreira, P., ... Bandarra, N. M. (2018). Composition and bioaccessibility of elements in green seaweeds from fish pond aquaculture. *Food Research International*, 105(November 2017), 271–277. <https://doi.org/10.1016/j.foodres.2017.11.015>
- Akomea-Frempong, S., Skonberg, D. I., Camire, M. E., & Perry, J. J. (2021). Impact of blanching, freezing, and fermentation on physicochemical, microbial, and sensory quality of sugar kelp (*Saccharina latissima*). *Foods*, 10(10). <https://doi.org/10.3390/foods10102258>
- Allahgholi, L., Jönsson, M., Christensen, M. D., Jasilionis, A., Nouri, M., Lavasani, S., ... Karlsson, E. N. (2023). Fermentation of the Brown seaweed *Alaria esculenta* by a lactic acid Bacteria consortium able to utilize mannitol and Laminari-oligosaccharides. *Fermentation*, 9(6). <https://doi.org/10.3390/fermentation9060499>
- Anekella, K., & Pérez-Díaz, I. M. (2020). Characterization of robust *Lactobacillus plantarum* and *Lactobacillus pentosus* starter cultures for environmentally friendly low-salt cucumber fermentations. *Journal of Food Science*, 85(10), 3487–3497. <https://doi.org/10.1111/1750-3841.15416>
- AOAC. (2023a). Official method 938.08 Ash of seafood. In *Official Methods of Analysis of AOAC INTERNATIONAL*. Oxford University Press. <https://doi.org/10.1093/9780197610145.003.3246>
- AOAC. (2023b). Official Method 978.18 Water Activity of Canned Vegetables. In *Official Methods of Analysis of AOAC INTERNATIONAL*. Oxford University Press. <https://doi.org/10.1093/9780197610145.003.3562>
- Blomhoff, R., Andersen, R., Arnesen, E. K., Christensen, J. J., Eneroth, H., Erkkola, M., ... Trolle, E. (2023). *Nordic nutrition recommendations 2023*. Nordic Council of Ministers. <https://doi.org/10.6027/nord2023-003>
- Brandão, M., Marques, D. J., Sousa, S., Mateus, M., Pinheiro, H. M., da Fonseca, M. M. R., ... Cesário, M. T. (2025). Lactic acid Bacteria and yeast fermentation to improve the nutritional value of *Ulva rigida*. *Marine Drugs*, 23(3), 1–29. <https://doi.org/10.3390/md23030106>
- Brodtkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., ... Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols*, 14(4), 991–1014. <https://doi.org/10.1038/s41596-018-0119-1>
- Bruhn, A., Brynning, G., Johansen, A., Lindegaard, M. S., Sveigaard, H. H., Aarup, B., ... Børsting, M. E. (2019). Fermentation of sugar kelp (*Saccharina latissima*)—effects on sensory properties, and content of minerals and metals. *Journal of Applied Phycology*, 31(5), 3175–3187. <https://doi.org/10.1007/s10811-019-01827-4>
- Cavonius, L. R., Albers, E., & Undeland, I. (2016). In vitro bioaccessibility of proteins and lipids of pH-shift processed *Nannochloropsis oculata* microalgae. *Food and Function*, 7(4), 2016–2024. <https://doi.org/10.1039/c5fo01144b>
- Cherry, P., O'hara, C., Magee, P. J., Mccorley, E. M., & Allsopp, P. J. (2019). Risks and benefits of consuming edible seaweeds. *Nutrition Reviews*, 77(5), 307–329. <https://doi.org/10.1093/nutrit/nuy066>
- Christensen, L. F., García-Béjar, B., Bang-Berthelsen, C. H., & Hansen, E. B. (2022). Extracellular microbial proteases with specificity for plant proteins in food fermentation. *International Journal of Food Microbiology*, 381(July). <https://doi.org/10.1016/j.ijfoodmicro.2022.109889>
- Cook, M. K., Dial, A. R., & Hendy, I. L. (2022). Iodine stability as a function of pH and its implications for simultaneous multi-element ICP-MS analysis of marine carbonates for paleoenvironmental reconstructions. *Marine Chemistry*, 245(July), Article 104148. <https://doi.org/10.1016/j.marchem.2022.104148>
- De Souza Celente, G., Sui, Y., & Acharya, P. (2023). Seaweed as an alternative protein source: Prospective protein extraction technologies. *Innovative Food Science & Emerging Technologies*, 86, Article 103374. <https://doi.org/10.1016/j.ifset.2023.103374>
- Flores, S. R. L., Dobbs, J., & Dunn, M. A. (2015). Mineral nutrient content and iron bioavailability in common and Hawaiian seaweeds assessed by an in vitro digestion/Caco-2 cell model. *Journal of Food Composition and Analysis*, 43, 185–193. <https://doi.org/10.1016/j.jfca.2015.06.008>
- Fu, W., & Mathews, A. P. (1999). Lactic acid production from lactose by *Lactobacillus plantarum*: Kinetic model and effects of pH, substrate, and oxygen. *Biochemical Engineering Journal*, 3(3), 163–170. [https://doi.org/10.1016/S1369-703X\(99\)00014-5](https://doi.org/10.1016/S1369-703X(99)00014-5)
- García-Sartal, C., Barciela-Alonso, M. d. C., Moreda-Piñeiro, A., & Bermejo-Barrera, P. (2013). Study of cooking on the bioavailability of As, Co, Cr, Cu, Fe, Ni, Se and Zn from edible seaweed. *Microchemical Journal*, 108, 92–99. <https://doi.org/10.1016/j.microc.2012.10.003>
- Ghelichi, S., Sørensen, A. D. M., Náthia-Neves, G., & Jacobsen, C. (2024). pH-dependent extraction of antioxidant peptides from red seaweed *Palmaria palmata*: a sequential approach. *Marine Drugs*, 22(9). <https://doi.org/10.3390/md22090413>
- Gupta, S., Abu-Ghannam, N., & Rajauria, G. (2012). Effect of heating and probiotic fermentation on the phytochemical content and antioxidant potential of edible Irish brown seaweeds. *Botanica Marina*, 55(5), 527–537. <https://doi.org/10.1515/bot-2011-0052>
- Gupta, S., Abu-Ghannam, N., & Scannell, A. G. M. (2011). Growth and kinetics of *Lactobacillus plantarum* in the fermentation of edible Irish brown seaweeds. *Food and Bioprocess Processing*, 89(4), 346–355. <https://doi.org/10.1016/j.fbp.2010.10.001>
- Healy, L., Zhu, X., Dong, G., Selli, S., Kelebek, H., Sullivan, C., ... Tiwari, B. K. (2024). Investigation into the use of novel pretreatments in the fermentation of *Alaria esculenta* by *Lactiplantibacillus plantarum* and kombucha SCOBY. *Food Chemistry*, 442. <https://doi.org/10.1016/j.foodchem.2023.138335>
- Hou, X., Yan, X., & Chai, C. (2000). Chemical species of iodine in some seaweeds II. Iodine-bound biological macromolecules. *Journal of Radioanalytical and Nuclear Chemistry*, 245(3), 461–467. <https://doi.org/10.1023/A:1006728420096>
- Hurd, C. L., Harrison, P. J., Bischof, K., & Lobban, C. S. (2014). Light and photosynthesis. In *Seaweed ecology and physiology* (2nd ed., p. 551). Cambridge University Press.
- Johansson, M., Johansson, D., Ström, A., Rydén, J., Nilsson, K., Karlsson, J., Moriana, R., & Langton, M. (2022). Effect of starch and fibre on faba bean protein gel characteristics. *Food Hydrocolloids*, 131. <https://doi.org/10.1016/j.foodhyd.2022.107741>
- Johansson, M., Karlsson, J., van den Berg, F. W. J., Ström, A., Ahrne, L., Sandström, C., & Langton, M. (2024). Effect of cellulose-rich fibres on faba bean protein gels is determined by the gel microstructure. *Food Hydrocolloids*, 156. <https://doi.org/10.1016/j.foodhyd.2024.110295>
- Jönsson, M., Merkel, A., Fredriksson, C., Karlsson, E. N., & Wendin, K. (2023). Nutritional, physicochemical, and sensory characterization of four common northern European seaweed species intended for food. *Algal Research*, 75(August), Article 103258. <https://doi.org/10.1016/j.algal.2023.103258>
- Juul, L., Stødkilde, L., Ingerslev, A. K., Bruhn, A., Jensen, S. K., & Dalsgaard, T. K. (2022). Digestibility of seaweed protein from *Ulva* sp. and *Saccharina latissima* in rats. *Algal Research*, 63, Article 102644. <https://doi.org/10.1016/j.algal.2022.102644>
- Kidgell, J. T., Magnusson, M., de Nys, R., & Glasson, C. R. K. (2019). Ulvan: A systematic review of extraction, composition and function. *Algal Research*, 39, Article 101422. <https://doi.org/10.1016/j.algal.2019.101422>
- Li, M., Zou, L., Zhang, L., Ren, G., Liu, Y., Zhao, X., & Qin, P. (2025). Plant-based proteins: Advances in their sources, digestive profiles *in vitro* and potential health benefits. *Critical Reviews in Food Science and Nutrition*, 65(10), 1929–1949. Taylor and Francis Ltd <https://doi.org/10.1080/10408398.2024.2315448>
- Luo, J., Ye, C., Wen, Y., Bao, Y., & Quek, S. Y. (2025). Investigation of solid-state fermentation of red seaweed (*Pyropia* spp.) with lactic acid Bacteria: Effects on protein profile and *in vitro* digestibility. *Food Chemistry*, 469. <https://doi.org/10.1016/j.foodchem.2024.142667>
- Maiorano, G., Ramirez, F. A., Durante, M., Palamà, I. E., Blando, F., De Rinaldis, G., ... Bleve, G. (2022). The controlled semi-solid fermentation of seaweeds as a strategy for their stabilization and new food applications. *Foods*, 11(18). <https://doi.org/10.3390/foods11182811>

- Marmon, S. K., & Undeland, I. (2013). Effect of alkaline pH-shift processing on in vitro gastrointestinal digestion of herring (*Clupea harengus*) fillets. *Food Chemistry*, 138(1), 214–219. <https://doi.org/10.1016/j.foodchem.2012.10.035>
- Marrion, O., Schwartz, A., Fleurence, J., Guéant, J. L., & Villaume, C. (2003). Improvement of the digestibility of the proteins of the red alga *Palmaria palmata* by physical processes and fermentation. *Nahrung – Food*, 47(5), 339–344. <https://doi.org/10.1002/food.200390078>
- Melse-Boonstra, A., Bossink, R., Sollie, V., van den Berg, K. B., & Verhagen, N. (2026). Content, bio-accessibility and intake of iodine from seaweed-containing food products available in the Netherlands. *European Journal of Nutrition*, 65(1). <https://doi.org/10.1007/s00394-025-03835-2>
- Moreda-Piñeiro, J., Moreda-Piñeiro, A., Romarís-Hortas, V., Domínguez-González, R., Alonso-Rodríguez, E., López-Mahía, P., Muniategui-Lorenzo, S., Prada-Rodríguez, D., & Bermejo-Barrera, P. (2012). Trace metals in marine foodstuff: Bioavailability estimation and effect of major food constituents. *Food Chemistry*, 134(1), 339–345. <https://doi.org/10.1016/j.foodchem.2012.02.165>
- Nagarajan, D., Oktarina, N., Chen, P. T., Chen, C. Y., Lee, D. J., & Chang, J. S. (2022). Fermentative lactic acid production from seaweed hydrolysate using *Lactobacillus* sp. and *Weissella* sp. *Bioresource Technology*, 344(Pt A), Article 126166. <https://doi.org/10.1016/j.biortech.2021.126166>
- Nielsen, P. M., Petersen, D., & Dambmann, C. (2001). Improved method for determining food protein degree of hydrolysis. *Journal of Food Science*, 66(5), 642–646. <https://doi.org/10.1111/j.1365-2621.2001.tb04614.x>
- Olsson, J., Toth, G. B., & Albers, E. (2020). Biochemical composition of red, green and brown seaweeds on the Swedish west coast. *Journal of Applied Phycology*, 32, 3305–3317. <https://doi.org/10.1007/s10811-020-02145-w/Published>
- Panel, E. B., Allende, A., Alvarez-Ordóñez, A., Bortolaia, V., Bover-Cid, S., De Cesare, A., ... Barizzone, F. (2026). Updated list of QPS-recommended microorganisms for safety risk assessments carried out by EFSA. Zenodo. <https://doi.org/10.5281/zenodo.18329226>
- Rauf Dahlstedt, S., Trigo, J. P., Stedt, K., Varmanen, P., Chamlagain, B., Rosqvist, F., ... Sandvik, P. (2026). Cultivation conditions, harvest, and hot water treatment affect the sensory profile, safety, and microelement composition of edible seaweeds *Ulva fenestrata* and *Saccharina latissima*. *Future Foods*, 13(January), Article 100995. <https://doi.org/10.1016/j.fufo.2026.100995>
- Reboleira, J., Silva, S., Chatzifragkou, A., Niranjan, K., & Lemos, M. F. L. (2021). Seaweed fermentation within the fields of food and natural products. *Trends in Food Science & Technology*, 116, 1056–1073. Elsevier Ltd <https://doi.org/10.1016/j.tifs.2021.08.018>
- Reisky, L., Préchoux, A., Zühlke, M. K., Bäumgen, M., Robb, C. S., Gerlach, N., ... Hehemann, J. H. (2019). A marine bacterial enzymatic cascade degrades the algal polysaccharide ulvan. *Nature Chemical Biology*, 15(8), 803–812. <https://doi.org/10.1038/s41589-019-0311-9>
- Rioux, L. E., & Turgeon, S. L. (2015). Seaweed carbohydrates. In *Seaweed Sustainability: Food and Non-Food Applications*. Elsevier Inc.. <https://doi.org/10.1016/B978-0-12-18697-2.00007-6>
- Rubak, Y. T., Lalel, H. J. D., Ngurah, B. I. G. M., Turupadang, W. L., Mahara, F. A., & Yusuf, D. (2026). Ulvan Polysaccharide of <i>Ulva lactuca</i> From coastal indonesia: functional prebiotic properties via enzymatic and nonenzymatic extraction. *International Journal of Food Science*, 1. <https://doi.org/10.1155/ijfo/4529168>
- Sabeena Farvin, K. H., & Jacobsen, C. (2013). Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food Chemistry*, 138 (2–3), 1670–1681. <https://doi.org/10.1016/j.foodchem.2012.10.078>
- Sandbakken, I. S., Sæther, M., Funderud, J., & Aasen, I. M. (2018). Acid preservation of *Saccharina latissima* for application as a carbon source for fermentation to biofuels and chemicals. *Journal of Applied Phycology*, 30(6), 3581–3588. <https://doi.org/10.1007/s10811-018-1489-z>
- Sedó Molina, G. E., Shetty, R., Xiao, H., Wätjen, A. P., Tovar, M., & Bang-Berthelsen, C. H. (2022). Development of a novel lactic acid bacteria starter culture approach: From insect microbiome to plant-based fermentations. *LWT*, 167(August). <https://doi.org/10.1016/j.lwt.2022.113797>
- Sørensen, J. S., Madsen, S. K., Bang-Berthelsen, C. H., & Hansen, L. T. (2021). Quality and safety aspects in fermentation of winged kelp (*Alaria esculenta*) and sugar kelp (*Saccharina latissima*) by the natural microbiota with or without addition of a *Lactiplantibacillus plantarum* starter culture. *Food Research International*, 150. <https://doi.org/10.1016/j.foodres.2021.110800>
- Sousa, R., Portmann, R., Dubois, S., Recio, I., & Egger, L. (2020). Protein digestion of different protein sources using the INFOGEST static digestion model. *Food Research International*, 130(September 2019), Article 108996. <https://doi.org/10.1016/j.foodres.2020.108996>
- Stedt, K., Steinhagen, S., Trigo, J. P., Kollander, B., Undeland, I., Toth, G. B., ... Pavia, H. (2022). Post-harvest cultivation with seafood process waters improves protein levels of *Ulva fenestrata* while retaining important food sensory attributes. *Frontiers in Marine Science*, 9(September), 1–12. <https://doi.org/10.3389/fmars.2022.991359>
- Stedt, K., Trigo, J. P., Steinhagen, S., Nylund, G. M., Forghani, B., Pavia, H., & Undeland, I. (2022). Cultivation of seaweeds in food production process waters: evaluation of growth and crude protein content. *Algal Research*, 63, Article 102647. <https://doi.org/10.1016/j.algal.2022.102647>
- Steinhagen, S., Enge, S., Larsson, K., Olsson, J., Nylund, G. M., Albers, E., ... Toth, G. B. (2021). Sustainable large-scale aquaculture of the northern hemisphere sea lettuce, *Ulva fenestrata*, in an off-shore seafarm. *Journal of Marine Science and Engineering*, 9 (6), 1–19. <https://doi.org/10.3390/jmse9060615>
- Stévant, P., Zioga, E., Steenholdt Sørensen, J., Heiner Bang-Berthelsen, C., Dahl, T. H., Barnung, T., ... Løvdal, T. (2024). Fermentation of sugar kelp (*Saccharina latissima*): Exploring the potential of the kelp's native microbiota as starter culture and the microbiological food safety of fermented products. *Frontiers in Food Science and Technology*, 4(February), 1–14. <https://doi.org/10.3389/frfst.2024.1490547>
- Trigo, J. P., Chamlagain, B., Thorén, J., Strand, R., Vall-IloeraJuanola, M., Steinhagen, S., ... Undeland, I. (2025). Sea lettuce (*Ulva fenestrata*) as a rich source of cobalamin (vitamin B12) – Both as processed whole biomass and as an extracted protein ingredient. *Food Chemistry*, 483(April). <https://doi.org/10.1016/j.foodchem.2025.144302>
- Trigo, J. P., Engström, N., Steinhagen, S., Juul, L., Harrysson, H., Toth, G. B., ... Undeland, I. (2021). In vitro digestibility and Caco-2 cell bioavailability of sea lettuce (*Ulva fenestrata*) proteins extracted using pH-shift processing. *Food Chemistry*, 356. <https://doi.org/10.1016/j.foodchem.2021.129683>
- Trigo, J. P., Steinhagen, S., Stedt, K., Krona, A., Verhagen, S., Pavia, H., ... Undeland, I. (2025). A new method for protein extraction from sea lettuce (*Ulva fenestrata*) via surfactants and alkaline aqueous solutions. *Food Chemistry*, 464(October 2024). <https://doi.org/10.1016/j.foodchem.2024.141839>
- Vall-Iloera, M., Steinhagen, S., Pavia, H., & Undeland, I. (2024). Brining as an effective method to stabilise sea lettuce (*Ulva fenestrata*) - impact on colour, texture, chemical characteristics and microbial dynamics. *Algal Research*, 83(September), Article 103700. <https://doi.org/10.1016/j.algal.2024.103700>
- Wei, R., Jiang, B., Chen, J., Xiang, L., & Liu, X. (2024). Removal of fishy flavor in kelp (*Laminaria japonica*) by natural antioxidant soaking combined with microbial fermentation. *Food Bioscience*, 60(February). <https://doi.org/10.1016/j.fbio.2024.104212>
- Winger, R. J., Koenig, J., Lee, S. J., & Wham, C. (2005). Technological issues with iodine fortification of foods. In *New Zealand Food Safety Authority*. Number May.
- Wirenfeldt, C. B., Hermund, D. B., Feyissa, A. H., Hyldig, G., & Holdt, S. L. (2024). Nutritional value, bioactive composition, physico-chemical and sensory properties of *Ulva* sp. and *Fucus vesiculosus* depending on post-harvest processing: a drying comparison study. *Journal of Applied Phycology*. <https://doi.org/10.1007/s10811-024-03210-4>
- Xu, L., Cao, J., & Chen, W. (2015). Structural characterization of a broccoli polysaccharide and evaluation of anti-cancer cell proliferation effects. *Carbohydrate Polymers*, 126, 179–184. <https://doi.org/10.1016/j.carbpol.2015.03.011>
- Yang, Y., Lin, H., & Fu, X. (2024). Fermentation of *Pyropia* spp. seaweed: A comprehensive review on processing conditions, biological activities and potential applications in the food industry. *Critical Reviews in Food Science and Nutrition*. <https://doi.org/10.1080/10408398.2024.2400233>. Taylor and Francis Ltd.
- Yen, Y., Weisbjerg, M. R., Rautenberger, R., Fečkaninová, A., & Novoa-Garrido, M. (2022). Improving fermentation of *Saccharina latissima* and *Alaria esculenta* silages with additives for preserving biomass and antioxidants. *Journal of Applied Phycology*, 34(1), 625–636. <https://doi.org/10.1007/s10811-021-02628-4>
- Zhu, W., Jiang, B., Zhong, F., Chen, J., & Zhang, T. (2021). Effect of microbial fermentation on the fishy-odor compounds in kelp (*Laminaria japonica*). *Foods*, 10 (11), 2532. <https://doi.org/10.3390/foods10112532>
- Zioga, E., Holdt, S. L., Gröndahl, F., & Bang-Berthelsen, C. H. (2025). Screening approaches and potential of isolated lactic acid bacteria for improving fermentation of *Saccharina latissima*. *BMC Biotechnology*, 25(1). <https://doi.org/10.1186/s12896-024-00926-6>