Changes in food quality attributes of *Saccharina latissima* following pre-treatments, frozen storage and subsequent thawing

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ABSTRACT

Changes in relevant quality attributes for the food industry were monitored in *Saccharina latissima* following pre-treatment (PT), freezing and thawing. The evaluation included the monitoring of qualitative indicators (dry weight, drip loss, texture, colour) and microbial status over a 15-month storage period, as well as the characterization of the nutrient profile. Freezing without PT led to extensive drip loss upon thawing (15 to 24 % of the initial weight) as well as structural alterations reflected by textural changes. The resulting liquid fraction contains water-soluble compounds including minerals (predominantly K), carbohydrates (mainly mannitol) and some proteins and free amino acids. Among the tested PTs, steaming resulted in reduced drip loss and higher nutrient retention compared to freshwater blanching and salting. Stable levels of fucoxanthin pigment in steamed samples along with evidence from the characterization of liquid fractions (i.e., drip loss upon thawing) by nuclear magnetic resonance (NMR) indicate that this treatment inactivates enzymes responsible for the degradation of sensitive compounds. The iodine content of fresh *S. latissima* may limit its use as food ingredient. Steamed *S. latissima* was lower in iodine than the untreated control but higher than those of blanched and salted samples. The blanching and salting treatments also resulted in extensive losses of soluble compounds highlighting the compromise between iodine content reduction and nutrient retention in kelp processing. The presented data is directly relevant to kelp producers and food manufacturers in establishing processing methods in commercial production of the kelp *S. latissima*.

1. Introduction

Marine organisms and especially seaweeds are considered a promising alternative resource for the provision of food and feed ingredients as well as a raw material to be used in a variety of other industrial applications [1,2]. Seaweeds can be cultivated on a large scale in coastal areas without competing for fresh water or land area. In contrast to Asian countries, where this activity is well-established, the cultivation of seaweeds in Europe, mainly *Saccharina latissima* (sugar kelp) and *Alaria esculenta* (winged kelp) is emerging [3,4]. This activity is expected to grow following current trends promoting natural and sustainable

feedstocks for commercial applications including food and feed and international strategies for sustainable industrial development [1,5].

Seaweeds are a rich source of nutrients e.g., minerals, vitamins and trace elements, and health-promoting compounds providing benefits beyond basic nutrition [6–8]. Several edible species including the kelps *S. latissima* and *A. esculenta* are also prized for their flavours and physicochemical characteristics that can be used to enhance the palatability of the food to which they are added [9,10]. However, seaweeds may accumulate potentially toxic elements with negative effects on human health. Both non-essential metals (cadmium and inorganic arsenic) and the essential element iodine may be present in high amounts in kelps

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[11–13] hence, limiting their use as ingredient in large-scale food applications. Nevertheless, simple processing steps such as a short heat treatment in freshwater, are reported to reduce the level of these elements. However, this process is also associated with nutrient losses such as minerals, vitamin C, phenolic compounds, free amino acids, mannitol [11,14].

One of the main challenges in upscaling kelp biomass production is stabilizing and maintaining the quality of the biomass after harvesting, primarily due to its high water content (80 to 90 % of the wet weight (WW)) and rapid biodegradation. Post-harvest processing methods are critical steps affecting the quality of the final product [15] as well as the sustainability of the supply chain based on cultivated kelp biomass [16,17]. Conventional air-drying methods are energy-intensive, lowering the profitability and sustainability of the value chain [18], and limiting their applicability to the processing of large amounts of biomass in a short period [19].

Freezing is one of the most used methods for preserving foods since it limits the growth of microorganisms and slows down the rate of biochemical reactions leading to food deterioration. Large-scale freezing technology is available and well established in other industries e.g., the fishing industry. It can be operated on-board a vessel in combination with harvesting [19]. The loss of quality in frozen foods depends on technical aspects of the process including storage temperature and duration, as well as thawing procedures, but also on intrinsic factors to the raw material e.g., chemical composition [20]. Pre-freezing treatments such as applying heat (hot-water blanching, steaming) or cryoprotectants (like salt) may increase the stability of frozen foods by inactivating enzymes responsible for quality loss during storage. Many studies have focused on improving the quality of frozen biomaterials (such as vegetables, fruits, meat, fish) however, little efforts were committed to optimize the quality of frozen seaweeds [21–24]. Preliminary studies on freezing *S. latissima* reports significant loss of fluid (i.e., referred to as drip loss) ranging from 27 % to nearly 50 % WW upon thawing [15]. This water contains nutritional and bioactive compounds which may be lost during the process hence, lowering the value of the final product as food.

The objective of this study was to test and identify optimal pre-treatments (PTs) prior to freezing to optimize the quality of *S. latissima* and to assess the effect of frozen storage time on food quality attributes. The quality of the samples was characterized using qualitative indicators (drip loss, dry weight, colour and texture) and the quantitative analysis of nutrients, bioactive substances and potentially toxic elements of both solid and liquid fractions of frozen/thawed *S. latissima*. These results contribute to the current research efforts in understanding changes in quality of kelp biomaterial upon processing.

2. Material and methods

2.1. Kelp raw material

Biomass of *S. latissima* was harvested at Seaweed Solutions at Frøya (Trøndelag, Norway; latitude 63.7050°N, longitude 8.8707°E) on May 15, 2019, then transported by boat within one hour to the processing plant (HitraMat, Hitra, Norway) and stored in large tanks (2 m^3) provided with seawater circulation (8 °C).

2.2. Pre-treatments, freezing and thawing methods

The biomass was split into 4 batches of 12 kg receiving different PTs i.e., blanching, steaming and salting prior to freezing. Blanching was achieved by immersion of the kelp biomass in 1000 L hot freshwater at 60 °C for 2 min as described by Nielsen et al. [14]. Blanched kelp was then cooled in cold seawater (8 °C) for 1 min. A second batch was exposed to steam at 95 °C for 15 min, in a crab cooker (1000 L) in which the kelp biomass was placed in a mesh tray over boiling water. A third PT consisted of salting (Norsal Sea, GC Rieber AS, Norway) at a 2:3

weight ratio (salt:kelp) for 1 h. Thereafter the salted biomass was rinsed in seawater for 10 min. After treatments the biomass was gently centrifuged using an industrial salad spinner to remove excess water and vacuum-packed in individual bags of approximately 500 g. The weight of all samples was registered with one decimal accuracy. All PTs were performed in 3 replicates. The last untreated batch served as a control.

All samples were frozen using individual quick freezing (IQF) technology (Frigoscandia, Advantec) at −42 °C in 30 min then stored in a freezer warehouse at −20 ± 0.9 °C. Sample bags were taken after 11, 41, 105, 182 and 461 days of storage, then thawed by immersing the sealed bags in running tap water at 13 °C and 1 L min^{-1} for about 10 h. The experimental protocol employed during this study and sample handling is summarized in Fig. 1.

2.3. Quality indicators measurements

Dry weight (DW) The DW was determined gravimetrically as the residue remaining after drying in a laboratory oven at 105 °C for 24 h. Measurements was performed in triplicate for each sample bag, using randomly picked kelp blades.

Drip loss following freezing and thawing was determined gravimetrically. The kelp samples were transferred to a sieve and allowed to drip for 2 min. Drip losses were calculated as follows (eq. 1):

$$\frac{W_L}{W_i} \times 100 \qquad (1)$$

Where W_L is the weight of the liquid fraction from the thawed samples and W_i is the initial weight of the samples.

Texture The tensile strength of thawed *S. latissima* blades was measured using a TA.XTPlus texture analyser (Stable Micro Systems Ltd., Godalming, UK) following the method adapted from Choi et al. [22]. 10 cm-long sample fronds of *S. latissima* were cut ca. 3 cm over the meristematic zone (basal part of the frond) and fastened to a perforated plate mounted on the texture analyser. The peak load, i.e., the maximum force applied to the blade before rupture, was measured in tension mode (5.0 g trigger load, 1 mm s^{-1} crosshead speed) using a P5/S spherical probe (5-mm diameter) and expressed in gram. For each sample, texture measurements were conducted on 5 randomly picked kelp fronds.

Surface colour The surface colour of the samples was analysed by a computerized image technique known as computer vision system (CVS) as described by Stévant et al. [25]. The colour was analysed quantitatively using Photoshop (Photoshop CC 2022, Adobe Systems Inc.) and expressed in CIE L^* (whiteness or brightness), a^*(redness/greenness), and b^* (yellowness/blueness) coordinates. The green colour index was calculated as -a^*/b^*. Colour data from photographs of at least three kelp blades for each sample was collected and the results averaged.

2.4. Microbial load analyses

Approximately 5 g of fresh kelp fronds, cut 3 cm above the meristematic zone, were diluted in a ratio 1:10 using peptone water (pH 7.0 ± 0.2) and homogenized in a stomacher (Seward Ltd., Worthing, UK). Five serial dilutions were then plated onto different types of count plates, namely aerobic, coliform, and yeasts and mould count plates (3 M Petrifilm, Maplewood, MN, USA). The incubation time was 72 h at 30 °C for aerobes, 24 h at 37 °C for coliforms and 48 h at 25 °C for yeasts and moulds as validated by standard methods [26]. The total viable count (TVC) was enumerated following the manufacturer guidelines for each type of plate. The results were log-transformed and the microbial load of the samples expressed in colony forming units (CFU) per gram sample.

2.5. Chemical characterization of solid samples and liquid fractions

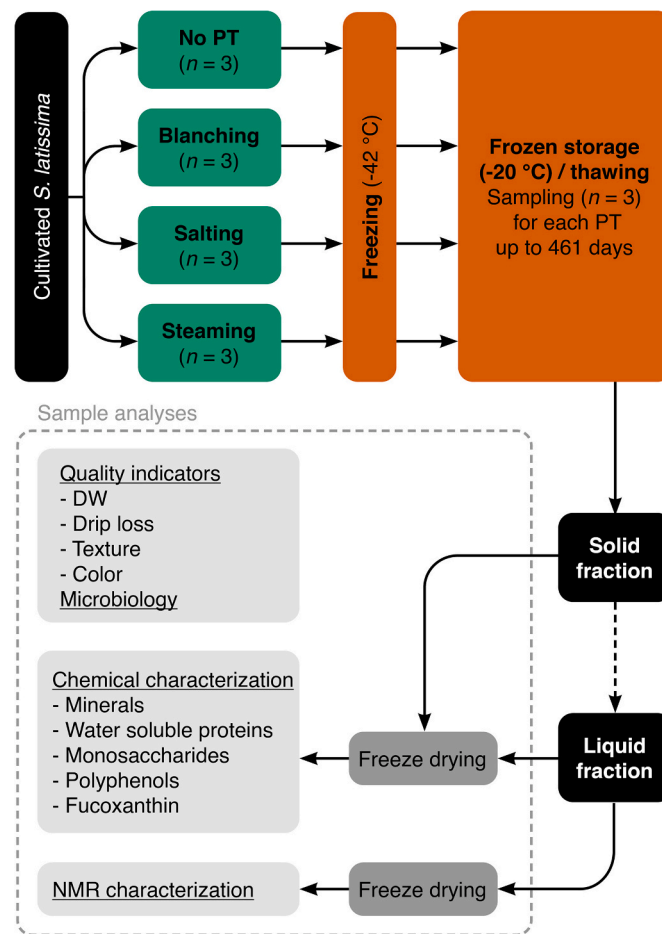All chemical analyses were conducted on freeze-dried samples

Fig. 1. Experimental design to study the effects of pre-treatments (PTs), freezing and frozen storage/thawing on the quality of *Saccharina latissima*.

following thawing. Analyses consisted of single extractions and measurements of replicate samples unless stated otherwise.

Minerals All solutions and dilutions were prepared using ultra-purified water 18.2 MΩ from a OmniaTap 10 UV system (Stakpure, Germany) and concentrated nitric acid (65 % HNO$_3$) purified by a Savillex DST-100 Acid Purification System (Savillex, USA) or 25 % tetramethylammonium hydroxide (TMAH). Standards for calibration curves were prepared from single element and mixed standard solutions from Inorganic Ventures (Christianburg, VA, USA). Sodium (Na), magnesium (Mg), phosphorus (P), sulphur (S), potassium (K), calcium (Ca), iron (Fe), copper (Cu), zink (Zn), arsenic (As), selenium (Se), cadmium (Cd), barium (Ba) and lead (Pb) were diluted in 5 % HNO$_3$ (v/v), while 1 % (v/v) TMAH were used for chlorine (Cl), bromine (Br) and iodine (I). Indium (In) and tellurium (Te) were used as internal standards. 200 mg freeze-dried samples were digested with 5 mL 50 % (v/v) nitric acid (HNO$_3$) at 250 °C in an UltraWAVE microwave oven (Milestone, Italy) and diluted to 5 % (v/v) upon analysis. Samples for Cl, Br and I analysis were extracted in parallel with 5 ml 20 % (v/v) TMAH at 80 °C in a bead bath overnight and diluted to 1 % (v/v) upon analysis. All elements were measured by an Agilent 8800 Triple Quadrupole inductively coupled plasma mass spectrometer (ICP-MS, Agilent Technologies, USA) with ISIS (Integrated Sample Introduction System), SPS4 autosampler (Agilent Technologies, USA) and a standard sample introduction system (Micro Mist glass concentric nebulizer, quartz double pass spray chamber, quartz torch with 2.5 mm id and standard nickel cones).

Water-soluble proteins (WSP) The proteins were extracted from ground samples by mixing 0.1 g of sample in 5 mL distilled water using a vortex mixer followed by resting for 10 to 15 min. The extracts were centrifuged at room temperature at 3600 *g* for 5 min and stored frozen until determination of the protein content. The WSP content in the thawed extracts was determined using the Lowry protein assay [27]. The extracts were diluted 1:4 with distilled water and added 2.5 mL alkaline copper reagent. They were allowed to rest for 10 min prior to adding 0.25 mL Folin-Ciocalteu reagent. After incubation for 30 min, the absorbance was read at 750 nm using a Pharmacia Biotech Ultrospec 2000 (GE Healthcare, USA). Bovine serum albumin was used as a standard. Measurements were performed in triplicates.

Monosaccharides and uronic acids Freeze-dry samples were ground into fine powder with a mortar and pestle and stored in exicator overnight prior analysis. 20–50 mg sample was transferred to 12 mL hydrolysis tubes with polytetrafluoroethylene (PTFE) phenolic screw cap. 0.5 mL 12 M sulfuric acid was added, vortexed and then incubated in a water bath at 30 °C for 1 h. Ultrapure water (UPW) was added to a final concentration of 2 M sulfuric acid, and the sample was hydrolysed at 100 °C for 4 h. After cooling down, 6 mL of UPW was added. Subsequently, 180 μL of the sample was combined with 750 μL of 0.15 M Ba (OH)$_2$ in a 1.5 ml Eppendorf tube. Following centrifugation, the supernatant was diluted tenfold with UPW and then transferred to a high-performance liquid chromatography (HPLC) vial. High-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) was performed on a Dionex ICS 5000+ system (Thermo Scientific) with a 4 × 250 mm CarboPac SA10 main column and 4 × 50 mm SA10 guard. A 25 μL sample was injected and eluted with 1 mM NaOH at a flow rate of 1.2 mL min^{-1}, with the temperature maintained at 28 °C. Elution conditions under HPAEC-PAD parameters are given as suppl. material (**S1**). Post column addition of 0.4 M NaOH, 0.3 ml min^{-1}

from a LC-20Ai pump was used to give a concentration of 80 mM NaOH during detection.

A monosaccharide standard mix (mannitol, fucose, arabinose, galactose, rhamnose, glucose, xylose, mannose) from 0.1 to 10.0 mg L^{-1} and uronic acids (mannuronic, glucuronic, guluronic, galacturonic acids) from 0.5 to 25 mg L^{-1} were analysed before and after the samples for quantification. Data was collected and processed with Chromeleon 7.2 software. Factors correcting for the degradation of released monosaccharides during hydrolysis were determined. These factors are calculated as the ratio of peak areas for a 5 mg L^{-1} standard before and after hydrolysis and are given in the suppl. material file (**S2**). An exception was the determination of mannuronic and guluronic acids where a standard alginate, FG = 0.504 measured with ^{1}H NMR was degraded. The water content was measured with a HC103 halogen moisture analyser (Mettler Toledo) and adjusted for the calculations. For calculation of dry weight (% w/w) of monosaccharides it was corrected for the addition of water when glycosidic linkages are hydrolysed.

Fucoxanthin Approximately 20 mg of freeze-dried sample was weighed and suspended in 200 μL of UPW in a 2 ml plastic tube. Ceramic beads were added to the tube, and samples were homogenized twice for 15 s each at maximum speed using a Precellys tissue homogenizer (Bertin Corp., USA). Homogenized samples were centrifuged at 2000 *g* for 5 min at 4 °C and supernatants were transferred to new microcentrifuge tubes then added 200 μL of liquid chromatography-mass spectrometry (LCMS)-grade ethanol (EtOH) and 500 μL of n-hexane containing 100 mg L^{-1} butylated hydroxytoluene (hexane + BHT). The mixture was vortexed for 60 s and then, centrifuged at 2000 *g* for 5 min at 4 °C. The top hexane-containing phase was transferred to a new tube. This extraction process was repeated, and the top phase from the second extraction was combined with the first in the same tube. The combined hexane phase was evaporated under nitrogen at room temperature. The residue was re-dissolved in 100 μL of LCMS-grade methanol. The solution was filtered using 0.45 μm PTFE filter (VWR, USA) then transferred to opaque glass tubes. The samples were analysed by HPLC coupled with ultraviolet at 450 nm on an Agilent 1260 Infinity HPLC with Agilent 1260 VWD detector.

Polyphenols Approximately 20 mg freeze dried and minced seaweed were added to 1.5 mL of 95 % methanol and homogenized 30 s with a Precellys homogenizer in a 2-mL tube containing 10 zirconium oxide beads. The samples were then incubated 48 h at room temperature in the dark and centrifuged at 13000 *g* for 5 min. 100 μL of the supernatant was transferred to tubes and 200 μL of 10 % Folin-Ciocalteu reagent was added. Finally, 800 μL of 700 mM sodium carbonate was added to the samples, which were vortexed and incubated for 2 h at room temperature in the dark. To generate a standard curve, 100 μL of gallic acid (50 μM- 2.5 mM) in 95 % methanol were used. Samples were transferred to a 96-well plate and UV absorption at 735 nm was measured using a BioTek Epoch Microplate Spectrophotometer and Gen5 – Microplate Data Collection & Analysis software (Agilent Technologies).

2.6. Characterization of liquid fractions by nuclear magnetic resonance (NMR) spectroscopy

9–12 mg lyophilized liquid fraction was dissolved in 600 μL D$_2$O (d-99.9 %) with 1 % w/v TSP (Trimethylsilylpropanoic acid, used for chemical shift reference 0 ppm) (Sigma-Aldrich, Norway) and transferred to a 5 mm Wilman WD-1000 NMR tubes (VWR, Norway). Not all samples completely dissolved thus, only allowing for qualitative assessment by NMR spectroscopy. All homo- and heteronuclear experiments were recorded at 25 °C on a BRUKER NEO 600 MHz equipped with 5 mm iProbe TBO (Bruker BioSpin AG) or Bruker AV-IIIHD 800 MHz spectrometer (Bruker BioSpin AG) equipped with a 5 mm cryogenic CP-TCI z-gradient probe. The spectra were recorded, processed and analysed using TopSpin 3.6pl7 and TopSpin 4.0.7 software (Bruker BioSpin AG).

1D spectrum (noesygppr1d), was recorded for each sample. For the assignment of relevant signals 2D ^{13}C HSQC (heteronuclear single quantum coherence) with multiplicity editing was collected for a representative sample of each treatment (sample #4, 5, 7, 10, 13, 16, 19, 22, suppl. material **S5-S13**). Selective 1D TOCSY spectrum (seldigpzs) with 70 ms mixing time and 2D COSY (homonuclear correlation spectroscopy) spectrum (cosydfph) collected on one steamed (sample #5) and one control sample (sample #10). The Biological Magnetic Resonance Data Bank (https://bmrb.io/) was employed to annotate the obtained chemical shifts and identify compounds.

2.7. Nutrient retention

The retention factors (RFs) of individual compounds in solid *S. latissima* samples following i) PT and ii) frozen storage/thawing were calculated following eq. 2, where *m* is the mass of a specific compound (in gram) in a processed sample at a time *t* and in the original sample i.e., untreated before PT and freezing (t_0). M_{tot} is the total mass (wet weight) of the sample measured at *t* and t_0.

$$RF = \frac{m(t) \times M_{tot}(t)}{m(t_0) \times M_{tot}(t_0)} \tag{2}$$

2.8. Data analysis

Raw data were pre-processed for descriptive statistics and the results expressed as mean ± standard deviation using R (version 4.1.2) [28]. A one-way analysis of variance (ANOVA, R function aov) was used to analyse differences among PTs in individual quality indicators and microbial counts prior to freezing (i.e., at t_0). A mixed model ANOVA (R function lmer) [29] was used to detect significant main effects ($p < 0.05$) of PTs and storage time on quality indicators, microbial counts and chemical content of *S. latissima* samples. Individual sample batches (i.e., PT replicates) were treated as random factor. The mixed model is summarized by the following formula:

$$Variable \sim PT + Storage + PT : Storage + (1|repl.) \tag{3}$$

The assumptions of homogeneity of variances and normal distribution were verified visually by inspecting residual and Q-Q plots respectively. The obtained *p*-values were adjusted using the Benjamini-Hochberg procedure to control for the false discovery under multiple testing. Post-hoc pairwise comparisons of least square means (R function lsmeans) [30] were performed following significant ANOVA results. A principal component analysis (PCA, R function prcomp) based on correlation matrix (i.e., scaling applied) was used to visualize differences in the chemical composition of liquid fractions obtained from frozen/thawed samples.

3. Results

3.1. Effects of pre-treatments, frozen storage periods and thawing methods on biomass quality indicators

The results from the measurement of quality indicators, including the dry matter content, drip loss, texture and colour of pre-treated and control (i.e., untreated) *S. latissima* samples before freezing (t_0 samples) and across a 15-month (461 days) frozen storage experiment are presented in Table 1 and Fig. 2 respectively. The combined effects of PTs and frozen storage, on these quality parameters is summarized in Table 2.

PTs significantly affected the dry matter content (i.e., DW) of the samples before freezing (ANOVA, $F_{3, 8} = 8.91$, $p < 0.010$). The salting treatment significantly increased the DW of *S. latissima* compared to the control (Table 1). A significant effect of PT was detected across frozen storage (Table 2) although post-hoc analysis of the results only reveal significant differences between salted and blanched samples at 11 and

461 days (LSmeans, $p = 0.020$).

Relatively large variations in drip loss were measured within samples (Fig. 2). The drip loss from steamed samples (mean values ranging from 11 to 16 %) across the experiment were generally lower than those from the untreated group (15 to 24 %) although this trend was not significant (LSmeans, $p = 0.050$). Storage time significantly affected the loss of fluid upon thawing (Table 2) with longer storage resulting in lower drip losses across PT groups, except for steamed samples in which drip loss did not significantly vary during the storage period.

The tensile strength of *S. latissima* i.e., the force required to disrupt the kelp blade, was significantly altered by PT methods (ANOVA $F_{3, 8} = 215$, $p < 0.01$, Table 1). Heat-treated samples (i.e., blanched and steamed) were less resistant to mechanical stress compared to the untreated control and salted samples prior to freezing. Frozen storage also had a significant impact on the tensile strength of the samples (Fig. 2, Table 2) although no distinct linear pattern could be observed across treatments. Blanched samples maintained a higher tensile strength compared to other samples throughout frozen storage except for the longest storage (i.e., 461 days) from which lower values, comparable to those of other groups, were measured.

Blanching and steam treatments produced significantly greener samples compared to untreated and salted samples (Table 1). Likewise, the greenness of untreated and salted samples increased following freezing and thawing compared to similar samples prior to freezing (Fig. 2). Overall, blanched and steamed samples maintained a greener colour throughout storage compared to the control and salted samples. Representative sample images taken at t_0 and after 105 days of frozen storage show a greater heterogeneity of the surface colour of untreated and salted *S. latissima* compared to blanched and steamed samples with visible brown patches across the fronds (suppl. material **S3**).

3.2. Chemical characterization of solid samples and nutrient retention

The differences in the chemical composition of *S. latissima* samples across PT methods and frozen storage time were investigated at day 105 and 182. The results expressed as part of the DW of the samples are presented in Table 3. Lower moisture contents were measured for salted and steamed samples before freezing (i.e., t_0) compared to untreated and blanched samples. Moisture variation among PT groups was lower after frozen storage and thawing. The moisture content of both untreated and blanched *S. latissima* diminished to levels closely resembling those quantified in the remaining groups. The nutrient composition expressed as part of the DW of the samples does not account for the loss of biomass which may occur upon processing of the kelp raw material. Hence, retention factors (RFs) of individual compounds were computed relatively to the WW of the samples. The RFs provide an overall estimate of the retention and losses of nutrients in *S. latissima* from PT as well as freezing/thawing (Fig. 3). RFs close to or above 1 indicate a high retention of the studied compounds whereas values close to 0 reflect high losses. Among PTs, steam exposure for 15 min provided the best

overall nutrient retention compared to blanching and salting.

As expected, high levels of Na (and Cl) were measured in salted samples compared to the other groups. Blanched samples showed a relatively high Na content compared to the untreated control samples which may be unexpected considering previous reports of Na reduction following freshwater blanching [11,14,31]. In the present study, a short cooling step (1 min) in seawater following freshwater blanching prior to freezing, can explain the increase of Na during the process. It should be noted that salting was also followed by a rinsing step (10 min) in seawater to remove the excess of salt crystals which may have affected the mineral balance of the samples. The initial K content of *S. latissima*, as measured in untreated samples, is more than twice as high as the Na content (Na/K ratio of 0.4, Table 3). K was particularly affected by blanching and salting as shown by considerably lower levels measured in these groups compared to the control, as well as low RFs for this element (Fig. 3). Steam exposure did not significantly affect the K levels of *S. latissima* compared to the untreated control (LSmeans, $p = 0.29$). The Ca and Mg levels of salted and steamed *S. latissima* were similar to those of control samples (Table 3). These macrominerals were significantly higher in blanched samples, indicating their accumulation in the biomass relatively to the losses of other compounds. Generally, the metal elements present as divalent cations (Ca, Mg, Zn, Fe, Cu, Ba, Cd, Pb) were relatively well retained from PTs in comparison to other mineral and metal elements (Fig. 3).

The iodine content of blanched *S. latissima* samples was 91 % lower than those measured in untreated samples and this is reflected by a very low RF (0.07 ± 0.01) for this element (Fig. 3). Both salting and steaming also resulted in significant decreases in iodine compared to untreated samples (LSmeans, $p < 0.01$) although to a lesser extent compared to blanching (Table 3). Lower iodine levels were measured in untreated samples after frozen storage for 105 days and thawing compared to initial levels prior to freezing (LSmeans, $p < 0.01$), suggesting that part of the iodine leaches in the drip water. This is supported by the relatively low RF of iodine in untreated samples (0.73 ± 0.14) from frozen storage compared to the RFs of other compounds in these samples (Fig. 3). The Cd content of steamed *S. latissima* was slightly higher than the control although not significantly different (LSmeans, $p = 0.07$). Likewise, the RF of Cd was higher in this group compared to the others. The lowest Cd levels were measured in salted material (Table 3). Blanching and salting significantly reduced the As levels of *S. latissima* (LSmeans, $p < 0.01$) whereas concentrations measured in steamed samples were similar to those of untreated samples (Table 3). This is reflected by relatively low RFs for As in the two former sample groups (0.40 ± 0.03 and 0.37 ± 0.07) compared to 0.94 ± 0.20 for As in steamed samples (Fig. 3). A significantly lower As content was measured in untreated samples following frozen storage then thawing compared to levels measured at t_0 (LSmeans, $p < 0.01$). The blanching and steam treatments did not significantly alter the Pb levels of the samples whereas higher levels were measured in salted samples (Table 3). This is illustrated in Fig. 3 by a markedly high RF for this element (11.21 ± 1.59) in these samples.

All tested PTs substantially reduced the WSP content of *S. latissima* (LSmeans, $p < 0.01$). The retention of soluble proteins was highest in salted samples (RF $= 0.61 \pm 0.07$) and lowest in blanched samples (RF $= 0.36 \pm 0.04$, Fig. 3). Besides, a significantly lower protein content was observed in untreated samples after 182 days of frozen storage compared to initial levels (LSmeans, $p = 0.010$; Table 3). It should be noted that the RFs for WSPs from frozen/thawed pre-treated samples were relatively low compared to other analysed compounds (Fig. 3) indicating a greater loss of the remaining proteins (i.e., after PT) compared to other nutrients in these samples.

The total monosaccharide and uronic acid (TMUA) content of untreated biomass after harvest was 38.5 ± 8.1 g 100 g^{-1} DW (Table 3). The monosaccharides of these samples consisted primarily of mannitol (31.5 % of TMUA) followed by glucose (10.7 % of TMUA). Fucose only represented 4.2 % of the TMUA. Small amounts of mannose, galactose and xylose were detected in all samples as well as trace amounts of
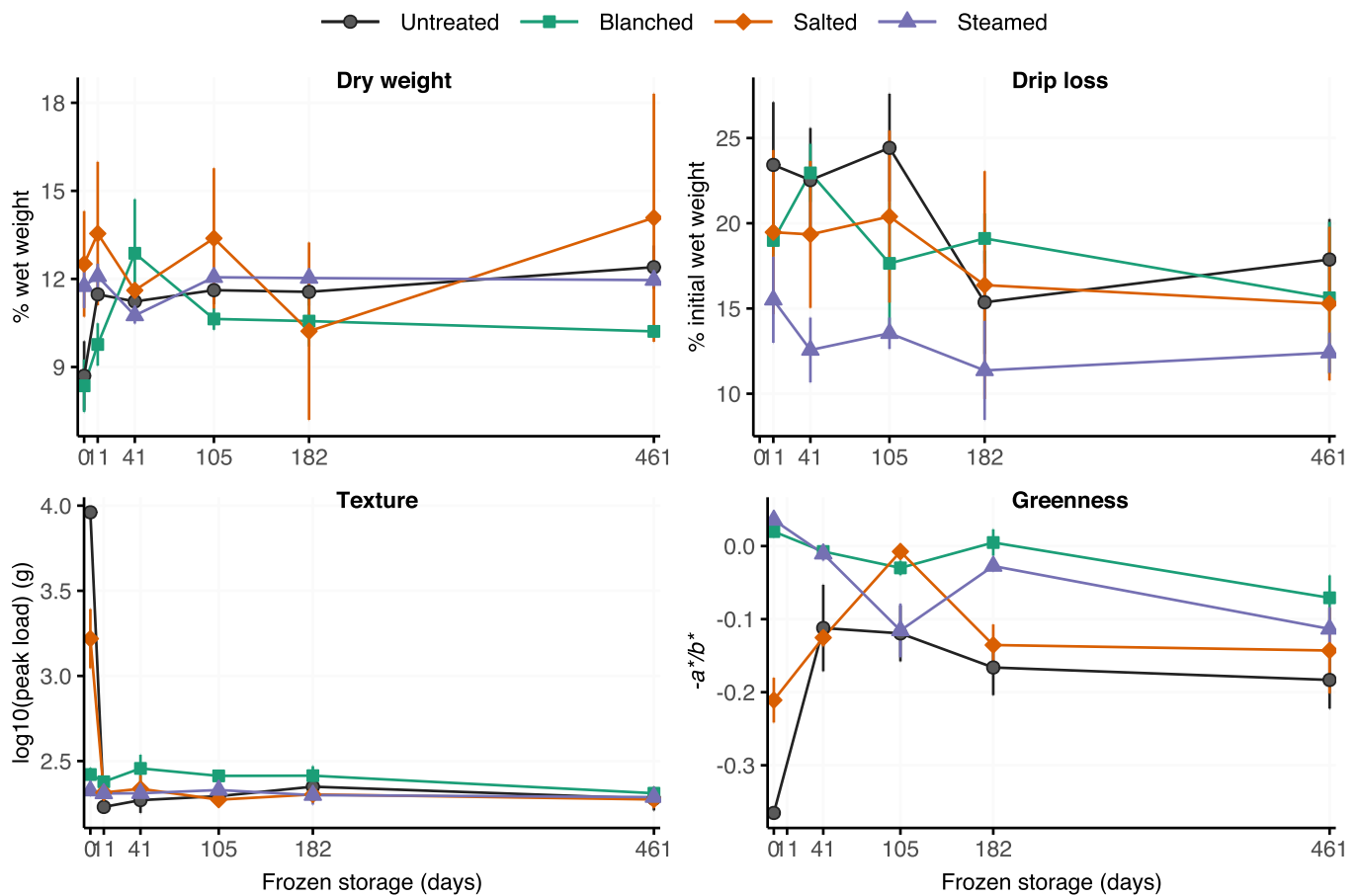
Table 1

Quality indicators of *Saccharina latissima* samples (t_0 samples) following pre-treatments (PTs) prior to freezing.

	Untreated	Blanched	Salted	Steamed	*p*-value
DW (% WW)	8.7 (1.2) [ab]	8.4 (0.9) [a]	12.5 (1.8) [c]	11.7 (0.7) [bc]	**0.006**
Tensile strength (log(peak load) (g))	4.0 (0.0) [c]	2.4 (0.0) [a]	3.2 (0.2) [b]	2.3 (0.0) [a]	**0.000**
Greenness (dimensionless)	−0.37 (0.00) [a]	0.02 (0.01) [c]	−0.21 (0.03) [b]	0.04 (0.01) [c]	**0.000**

Values are given as mean ($n = 3$, standard deviation in parenthesis). Different superscript letters in the same row indicate significant differences among samples (LSmeans, $p < 0.05$).

Fig. 2. Dry weight, drip loss, texture and greenness of *Saccharina latissima* following pre-treatments (PTs) and frozen storage. Values are given as mean ± standard deviation ($n = 3$).

Table 2

Summary of the analysis of variance (ANOVA) from the linear mixed effect model analysing the effect of frozen storage duration and pre-treatment (PT) method on individual quality indicators of *Saccharina latissima*.

Quality indicator	Main effects	Df	Mean Sq.	F-value	p-value
DW	Storage	4	1.921	0.852	0.501
	PT	3	7.763	3.441	**0.034**
	Storage: PT	12	3.918	1.737	0.103
Drip loss	Storage	4	52.57	11.87	**0.000**
	PT	3	16.88	3.811	0.058
	Storage: PT	12	12.30	2.776	**0.021**
Tensile strength	Storage	4	0.007	3.187	**0.031**
	PT	3	0.037	17.46	**0.000**
	Storage: PT	12	0.003	1.702	0.103
Greenness[1]	Storage	3	0.010	10.13	**0.000**
	PT	3	0.031	30.82	**0.000**
	Storage: PT	9	0.006	6.014	**0.000**

The studied main effects include storage (excluding data from t_0 samples, 5 levels, except in [1] 4 levels), and PT (4 levels). Significant results ($p < 0.05$) are indicated in bold.

rhamnose and arabinose. Mannuronic and guluronic acids were the main uronic acids of untreated samples (21.4 and 20.3 % of TMUA respectively) while lower amounts of glucuronic acid were measured (5.9 % of TMUA). Losses of carbohydrates following PTs were mainly due to extensive losses of mannitol, particularly in blanched and salted samples (Table 3, Fig. 3). Other monosaccharides and uronic acids were associated with RFs close to 1.0. Lower retention of glucuronic and

mannuronic acids were observed in pre-treated compared to untreated samples following frozen storage and thawing.

No significant differences in fucoxanthin levels were observed across PT groups before freezing (Table 3). The fucoxanthin of untreated and blanched samples decreased during frozen storage compared to their initial levels while salted and steamed samples maintained or increased initial levels (likely as a result of loss of other compounds), reflecting the higher RFs in these groups compared to the former PT groups for this compound (Fig. 3). Highly significant differences in polyphenol content were detected among PTs at t_0, i.e., higher levels were measured in untreated and blanched compared to salted and steamed *S. latissima* (LSmeans, $p < 0.010$). The polyphenol content of the samples remained stable across the storage period except in untreated samples in which lower levels were measured after 182 days of storage compared to 105 days, although this trend is weak (LSmeans, $p = 0.040$).

3.3. Characterization of liquid fractions

The composition of liquid fractions (drip loss) of pre-treated *S. latissima* samples stored for 3 (105 days) and 6 month (182 days) was characterized by NMR spectroscopy, using 1D proton and 2D ^{13}C HSQC spectra. PTs clearly affected the composition of the liquid fraction upon thawing. A qualitative assessment of the components observed in the liquid fractions is summarized in Fig. 4. Spectra with annotation are given in supplementary data (S5–12). Free amino acids, namely Ala, Glu, Gln, and Asp, were detected in the liquid fraction of untreated and steam-treated kelp but not found in the liquid fraction of blanched kelp. Only Ala and Asp are detected from salt-treated *S. latissima*. The sugar

Table 3
Nutrient content of solid samples of *Saccharina latissima* after pre- treatments (PT) before freezing (t_0) and after 105 and 182 days of frozen storage.

PT	Untreated			Blanched			Salted			Steamed		
Storage (days)	0	105	182	0	105	182	0	105	182	0	105	182
Moisture [1]	91.3 (1.0)[bB]	88.5 (0.3)[aAB]	88.6 (0.4)[aA]	91.6 (0.8)[bB]	89.5 (0.3)[aB]	89.6 (0.7)[aA]	87.5 (1.6)[aA]	87.3 (1.8)[aA]	88.3 (2.9)[aA]	88.2 (0.7)[aA]	88.0 (0.2)[aAB]	88.2 (0.4)[aA]
Tot. minerals [2]	38.0 (2.3)[aAB]	30.8 (2.3)[aAB]	n.a.	30.6 (0.6)[aA]	22.9 (1.7)[aA]	n.a.	53.7 (17.5)[bB]	43.2 (8.3)[aB]	n.a.	31.9 (1.1)[aA]	29.3 (0.6)[aAB]	n.a.
Na [2]	5.2 (0.4)[aA]	4.3 (0.4)[aA]	n.a.	9.8 (0.3)[aA]	7.9 (0.4)[aAB]	n.a.	18.1 (7.4)[bB]	13.8 (3.5)[aB]	n.a.	4.7 (0.1)[aA]	4.2 (0.2)[aA]	n.a.
K [2]	12.3 (0.9)[bB]	10.3 (0.2)[aC]	n.a.	0.8 (0.1)[aA]	0.6 (0.1)[aA]	n.a.	2.4 (1.2)[aA]	2.5 (1.2)[aB]	n.a.	11.2 (0.5)[bB]	10.2 (0.4)[aC]	n.a.
Mg [2]	0.8 (0.0)[aA]	0.7 (0.1)[aA]	n.a.	1.5 (0.0)[aB]	1.4 (0.0)[aB]	n.a.	1.0 (0.0)[aA]	1.0 (0.2)[aAB]	n.a.	0.8 (0.0)[aA]	0.7 (0.0)[aA]	n.a.
Ca [2]	0.7 (0.0)[aA]	0.8 (0.0)[aA]	n.a.	1.2 (0.0)[aB]	1.2 (0.0)[aB]	n.a.	0.8 (0.2)[aA]	0.9 (0.1)[bA]	n.a.	0.8 (0.0)[aA]	0.8 (0.0)[aA]	n.a.
P [2]	0.3 (0.01)[bC]	0.3 (0.0)[aBC]	n.a.	0.2 (0.0)[aB]	0.2 (0.0)[aB]	n.a.	0.2 (0.0)[aA]	0.2 (0.0)[aA]	n.a.	0.3 (0.0)[aC]	0.3 (0.0)[aC]	n.a.
S [2]	1.0 (0.0)[aA]	1.0 (0.0)[aA]	n.a.	1.6 (0.0)[bB]	1.4 (0.0)[aC]	n.a.	1.2 (0.3)[aA]	1.3 (0.1)[aBC]	n.a.	1.2 (0.0)[atA]	1.1 (0.0)[aAB]	n.a.
Cl [2]	17.2 (1.9)[aA]	13.0 (1.7)[aAB]	n.a.	15.3 (0.5)[aA]	10.2 (1.3)[aA]	n.a.	29.8 (12.3)[aB]	23.3 (6.2)[aB]	n.a.	12.6 (1.0)[aA]	11.5 (0.3)[aAB]	n.a.
Fe [3]	41.0 (1.0)[aA]	63.7 (4.0)[bA]	n.a.	84.0 (10.4)[aB]	103.3 (13.1)[bB]	n.a.	59.3 (16.0)[aAB]	73.0 (12.5)[bA]	n.a.	65.0 (11.8)[aAB]	71.3 (3.1)[aA]	n.a.
Cu [3]	1.1 (0.2)[aA]	1.2 (0.1)[bA]	n.a.	1.0 (0.0)[aA]	1.2 (0.1)[bA]	n.a.	0.8 (0.2)[aA]	1.0 (0.1)[bA]	n.a.	1.1 (0.1)[aA]	1.2 (0.0)[aA]	n.a.
Zn [3]	26.0 (1.0)[aAB]	33.3 (1.5)[bAB]	n.a.	40.0 (3.0)[aC]	47.3 (1.2)[bC]	n.a.	22.0 (6.9)[aA]	27.3 (3.8)[bA]	n.a.	35.7 (4.5)[aBC]	43.0 (8.7)[bBC]	n.a.
As [3]	69.7 (8.1)[bC]	58.7 (3.2)[aB]	n.a.	36.0 (1.0)[bB]	28.7 (1.5)[aA]	n.a.	24.3 (8.1)[aA]	25.7 (5.1)[aA]	n.a.	66.3 (1.5)[aC]	62.0 (0.0)[aB]	n.a.
Se [3]	0.3 (0.1)[aA]	0.5 (0.1)[aA]	n.a.	0.4 (0.1)[aA]	0.4 (0.1)[aA]	n.a.	0.3 [aA]	0.4 (0.0)[aA]	n.a.	0.5 (0.1)[aA]	0.5 (0.0)[aA]	n.a.
Br [3]	1315 (102)[bB]	1066 (146)[aA]	n.a.	867 (64)[bA]	704 (37)[aA]	n.a.	936 (259)[aAB]	996 (298)[aA]	n.a.	1104 (65)[aAB]	1053 (43)[aA]	n.a.
Cd [3]	1.2 (0.1)[aBC]	1.1 (0.1)[aB]	n.a.	1.1 (0.1)[aB]	1.1 (0.1)[aB]	n.a.	0.7 (0.3)[aA]	0.8 (0.1)[aA]	n.a.	1.4 (0.1)[aC]	1.4 (0.1)[aC]	n.a.
I [3]	4012 (349)[bC]	3457 (169)[aC]	n.a.	342 (24)[aA]	331 (13)[aA]	n.a.	957 (447)[aA]	1151 (566)[aB]	n.a.	2827 (229)[aB]	2712 (192)[aC]	n.a.
Ba [3]	6.8 (0.3)[aA]	8.1 (0.5)[bA]	n.a.	10.7 (0.6)[aB]	13.0 (0.0)[bB]	n.a.	6.9 (2.4)[aA]	8.2 (1.0)[bA]	n.a.	9.4 (0.5)[aB]	9.8 (0.3)[aA]	n.a.
Pb [3]	0.1 (0.0)[aA]	0.1 (0.0)[aA]	n.a.	0.1 (0.0)[aA]	0.1 (0.0)[aA]	n.a.	0.6 (0.2)[aB]	0.7 (0.1)[aB]	n.a.	0.1 (0.0)[aA]	0.1 (0.1)[aA]	n.a.
WSP [2]	3.0 (0.4)[bB]	2.6 (0.1)[abC]	2.4 (0.0)[aC]	1.4 (0.1)[aA]	1.4 (0.0)[aA]	1.1 (0.1)[aA]	1.6 (0.1)[abA]	1.9 (0.0)[bBC]	1.5 (0.1)[aAB]	1.7 (0.3)[aA]	1.5 (0.2)[aAB]	1.6 (0.4)[aB]
TMUA [2]	38.5 (8.1)[aB]	41.4 (2.8)[aB]	41.3 (5.1)[aA]	34.1 (0.8)[aAB]	35.7 (5.3)[aAB]	35.8 (5.2)[aA]	26.3 (4.6)[aA]	32.1 (3.2)[aA]	33.2 (1.8)[aA]	32.8 (0.9)[aAB]	35.4 (1.2)[aAB]	39.6 (2.0)[aA]
Mannitol [2]	12.3 (5.1)[bC]	8.1 (1)[aB]	7.8 (0.6)[aC]	0.3 (0.1)[aA]	0.2 (0.1)[aA]	0.2 (0.1)[aA]	1.9 (1.2)[aAB]	1.3 (0.5)[aA]	1.5 (0.7)[aAB]	4.2 (0.6)[aB]	5.1 (0.7)[aB]	4.8 (0.3)[aBC]
Glucose [2]	4.2 (1.3)[aA]	5.2 (0.8)[aA]	5.6 (0.5)[aA]	5.3 (0.4)[aA]	5.4 (1.0)[aA]	5.3 (0.7)[aA]	3.9 (1.2)[aA]	4.8 (0.4)[aA]	5.1 (0.5)[aA]	4.2 (0.6)[aA]	5.5 (0.0)[aA]	5.3 (0.2)[aA]
Fucose [2]	1.6 (0.3)[aA]	1.5 (0.1)[aA]	1.8 (0.2)[aA]	1.7 (0.3)[aA]	1.8 (0.3)[aA]	1.9 (0.4)[aA]	1.3 (0.2)[aA]	1.5 (0.2)[aA]	1.7 (0.1)[aA]	1.7 (0.2)[aA]	1.8 (0.1)[aA]	1.8 (0.2)[aA]
GulA [2]	7.8 (3.0)[aA]	12.0 (0.7)[bAB]	10.9 (2.4)[abA]	10.5 (2.0)[aA]	14.8 (2.2)[bB]	12.5 (1.8)[aA]	7.4 (1.5)[aA]	13.1 (1.1)[bAB]	11.9 (0.9)[aA]	8.7 (0.9)[aA]	10.7 (1.1)[bA]	12.1 (0.9)[abA]
GlcA [2]	2.2 (0.2)[aA]	2.5 (0.7)[aA]	2.6 (0.2)[aA]	2.8 (0.8)[aB]	2.1 (0.2)[aA]	2.8 (0.3)[aA]	2.2 (0.7)[aA]	2.2 (0.7)[aA]	2.1 (0.2)[aA]	2.9 (0.6)[aA]	2.0 (0.3)[aA]	2.3 (0.4)[aA]
ManA [2]	8.2 (2.2)[aA]	10.1 (0.8)[aA]	10.4 (2.4)[aA]	11.3 (1.2)[bB]	9.1 (1.3)[aA]	10.8 (1.8)[abA]	7.7 (0.6)[aA]	7.4 (1.2)[aA]	8.9 (0.8)[aA]	8.8 (0.5)[abAB]	7.8 (0.7)[aA]	10.9 (0.9)[bA]
Fucoxanthin [3]	612 (35)[bA]	282 (65)[aA]	325 (3)[aA]	693 (84)[cA]	381 (12)[aA]	550 (168)[bB]	663 (134)[aA]	683 (71)[aB]	735 (70)[aC]	598 (20)[aA]	572 (90)[aB]	783 (12)[bC]
Polyphenols [2]	0.6 (0.1)[abB]	0.7 (0.0)[bB]	0.5 (0.1)[aAB]	0.7 (0.0)[aB]	0.7 (0.1)[aB]	0.7 (0.2)[aB]	0.4 (0.1)[aA]	0.4 (0.0)[aA]	0.4 (0.0)[aA]	0.4 (0.0)[aA]	0.4 (0.0)[aA]	0.3 (0.0)[aA]

Values are given as mean ($n = 3$, standard deviation in parenthesis) expressed in [1] % WW, [2] g $(100\ g)^{-1}$ DW, [3] mg kg^{-1} DW. Different lowercase superscript letters within individual PT indicate significant differences among sampling times. Different uppercase superscript letters indicate significant differences between PT at each individual sampling time (LSmeans, $p < 0.05$). WSP: water soluble proteins; TMUA: total monosaccharides and uronic acids; GulA: guluronic acid; GlcA: glucuronic acid; ManA: mannuronic acid; n.a.: not analysed. The measured selenium concentration in salted samples at t_0 were below limit of quantification in 2 out of 3 replicate samples.

alcohol mannitol remains detectable for all treatment conditions; however, its presence is clearly diminished in the blanched kelp. An intriguing observation is the occurrence of laminarin in liquid fraction from steam-treated kelp samples only. Moreover, free glucose and signals of α- and β-glycosidic linkages are observed in liquid fractions from control and salted kelp, likely indicating enzymatic activity. The β-glycosidic linkages in the liquid fraction indicate the release of polysaccharides from the kelp. Furthermore, both glycerol and choline are present in the liquid fraction of blanched kelp. This is a typical sign of cell-membrane disruption since choline is associated with the lipid membrane of the cell.

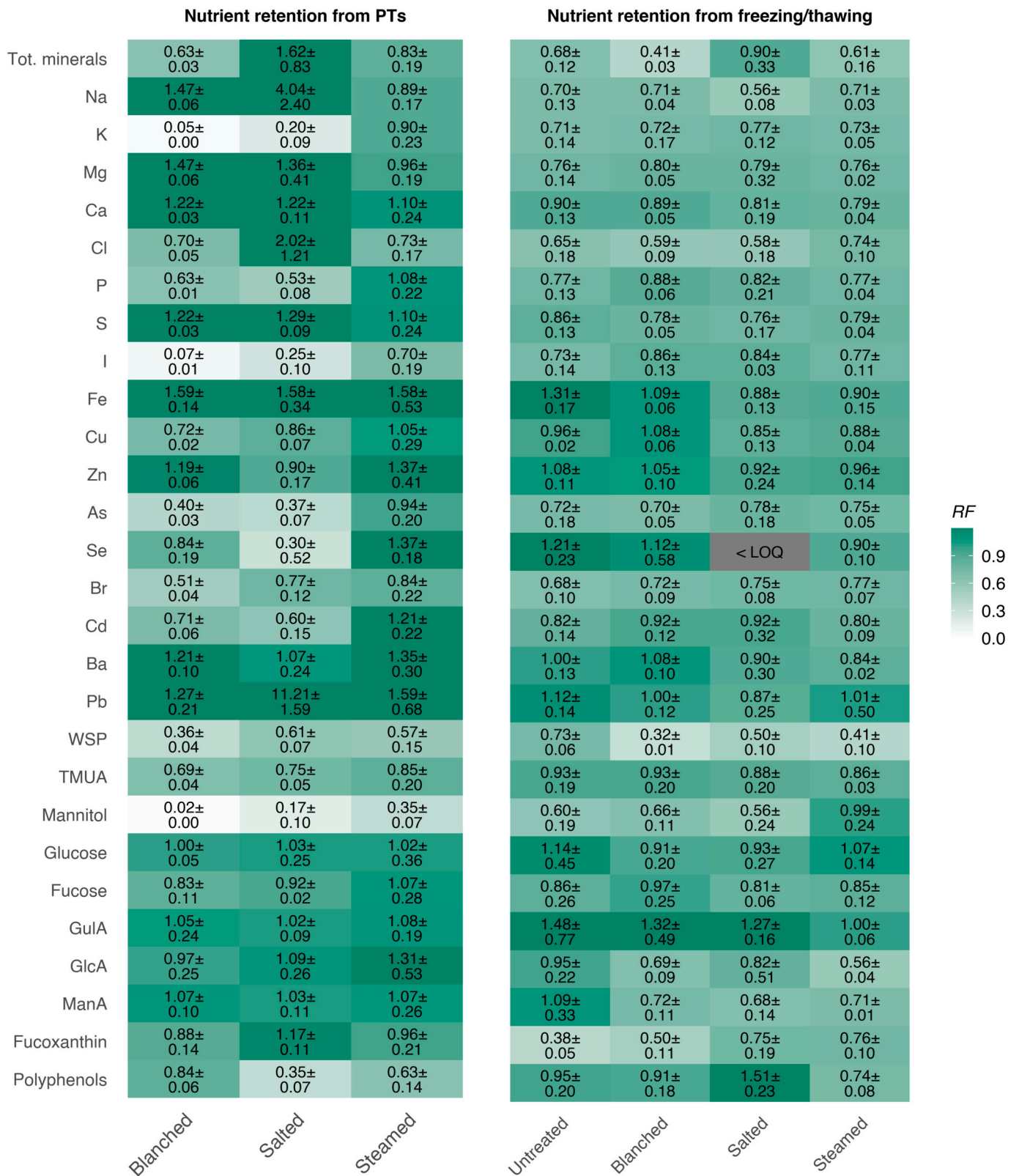Liquid fractions of *S. latissima* samples stored frozen for 3 month

Fig. 3. Retention factors (RFs) of nutritional compounds from pre-treatments (PTs) of compared to untreated control samples (left pane) and from frozen storage/thawing relatively to pre-treated samples at t_0 prior to freezing (right pane). The RFs were computed using measured values expressed relatively to the wet weight (WW) of the samples. Values are given as mean ($n = 3 \pm$ st. dev.). WSP: water soluble proteins; TMUA: total monosaccharides and uronic acids; GulA: guluronic acid; GlcA: glucuronic acid; ManA: mannuronic acid; LOQ: limit of quantification.

(105 days) were analysed for their nutritional content (i.e., minerals, WSP, carbohydrates, polyphenols and fucoxanthin). Due to frothing of some liquid samples during freeze-drying, the determination of their dry matter content and mass balance calculations from solid to liquid fractions was not possible. The profile of the liquid fractions reflecting the relative proportions of the compounds analysed (expressed in g 100 g^{-1} dry sample) is presented in suppl. material (**S13**). Despite variations among PT groups, the solid residue of liquid fractions consisted mainly of minerals (in average 76 % of total solids among groups). The remaining part was dominated by carbohydrates (of which mainly mannitol) and some WSP. Small amounts of fucoxanthin and polyphenols were measured i.e., ranging from 0.3 to 2.5 mg kg^{-1} and 1.4 to 5.5 g (100 g)$^{-1}$ dry sample respectively. The variation in the nutrient composition of the samples among PTs was analysed by PCA. The first two components of the PCA explains 87.8 % of the variance (70.0 % and 17.8 % by PC1 and PC2 respectively, Fig. 5) in the composition of liquid samples obtained from freezing/thawing pre-treated *S. latissima*. The first component (PC1) clearly discriminates salted and blanched samples on the left-hand side of the plot against untreated and steamed samples on the right-hand side. Liquid fractions from salted and blanched samples are correlated with a high level of Na, Cl, and S whereas those from untreated and steamed samples are correlated with a higher proportion of K, I, P, WSP, mannitol and fucose compared to the two former groups. PC2 discriminates the thawing liquid from steamed samples and to a lesser extent blanched samples on the top of the plot to those of salted and untreated samples on the bottom. Liquid samples from steamed *S. latissima* correlate with higher fucoxanthin levels compared to those of salted and untreated material.

3.4. Microbial status

The initial aerobic bacteria count of untreated fresh *S. latissima* samples was 4.2 ± 0.8 log(CFU g^{-1}) (Fig. 6). A significantly lower bacterial load was measured from salted samples before freezing compared to untreated samples (LSmeans, $p = 0.02$). The results from the mixed model ANOVA of aerobic bacteria counts across the experiment revealed the significant effects of frozen storage (ANOVA, $F_{2, 23} =$ 16.71, $p < 0.01$) and the interaction between frozen storage and PT (ANOVA, $F_{6, 23} = 6.40$, $p < 0.01$). This is explained by the non-detection of aerobes in any replicate of untreated samples taken at 11 days of frozen storage while similar levels to those of pre-treated samples were found at 457 days (Fig. 6). No significant effect of PT ($F_{3, 23} = 0.44$, $p = 0.72$) on the total viable count of aerobes was detected. Neither yeasts and moulds nor coliforms were detected in any of the samples.

4. Discussion

4.1. Freezing and thawing: changes in S. latissima based on quality indicators

Typical alterations of frozen foods include physical alterations (e.g., drip loss and texture) as well as chemical changes including colour, flavour and aroma, and nutrient loss due to biochemical reactions upon freezing and thawing. During the freezing process of plant tissue, ice generally forms in the extracellular matrix because cell walls constitute a physical barrier to crystal growth. This leads to an increase in solute concentration in the unfrozen portion of the matrix [20,32]. Due to the osmotic potential between the intra- and extracellular spaces, water migrates out of the cell contributing to the growth of extracellular ice, resulting in cell dehydration and shrinkage, and subsequent membrane damage. This water does not return to the cell upon thawing, resulting in drip loss and impairment of textural properties (e.g., product firmness). In the present study, this is reflected by considerable drip loss measured in untreated *S. latissima* frozen samples (mean values ranging from 15 to 24 % across the experiment) and a decrease in tensile strength following freezing and thawing. Comparable drip loss were reported by Akomea-Frempong et al. [24] for untreated *S. latissima* following freezing/thawing whereas higher values (40 to 50 %) have also been reported [15] suggesting that biomass origin and the physiological state of the thallus (influencing the chemical composition) greatly affect the final results.

Rapid freezing, as the IQF technology employed in this study, generally produces small ice crystals, and limits the osmotic transfer of water due to intracellular formation of ice compared to slower methods.

Fig. 4. Summary of the compounds observed in liquid fractions (drip loss) of frozen/thawed *Saccharina latissima* samples after pre-treatments (PTs) using both ^{13}C HSQC spectra and proton spectra. Spectra with annotations are given as suppl. material (**S5-S12**).
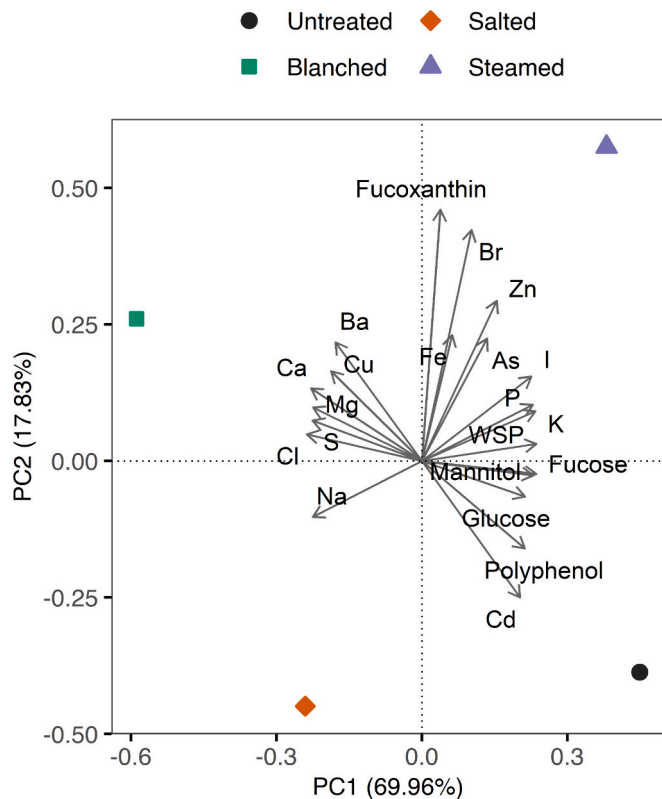
Fig. 5. Biplot (1st and 2nd principal component axes) from the principal component analysis (PCA) of the chemical composition of the liquid fraction (drip loss) obtained from freezing/thawing *Saccharina latissima* samples (105 days of frozen storage) after pre-treatments. The PCA was conducted on average values ($n = 3$) for each group. Vectors indicate loadings representing the variation in the content of individual compounds among sample groups.

However, the high solute concentration in the unfrozen matrix, especially high salt as in the case of *S. latissima*, can cause damage to structural polymers (e.g., alginate) irrespectively of the freezing rate of the material [20]. Quick thawing at low temperature is often preferred in food production to limit temperature increase in the product and preserve food quality attributes [20]. Choi et al. [22] reported considerable changes in colour and texture of the edible brown seaweed *Undaria pinnatifida* following thawing times longer than 6 h. The thawing rate of a frozen biomaterial highly depends on sample size (small vs large frozen blocks). Block size is therefore an important factor to consider in commercial production as thawing large blocks may affect drip loss and overall product quality.

4.2. Effects of PTs on quality indicators

PTs including exposure to heat (e.g., water blanching, steaming) and the use of cryoprotectants (e.g., inorganic salts) can limit quality deterioration during frozen storage and are commonly applied to industrial processes of foods. The tested PTs in the present study are typically applied to the preservation of fruits and vegetables (blanching, steaming) as well as meat and fish (salting). All PTs generally affected the measured quality indicators compared to untreated *S. latissima*. The average drip loss measured in pre-treated samples across the experiment were relatively similar to those of the untreated control except in steamed samples from which relatively lower drip loss were obtained. Lower drip loss was expected in salted samples since this pre-treatment resulted in the removal of a large amount of water from the biomass prior to freezing. A significant amount of water was likely absorbed again during the rinsing step in seawater and was later released upon thawing. Lower drip loss was also measured after long storage time i.e.,
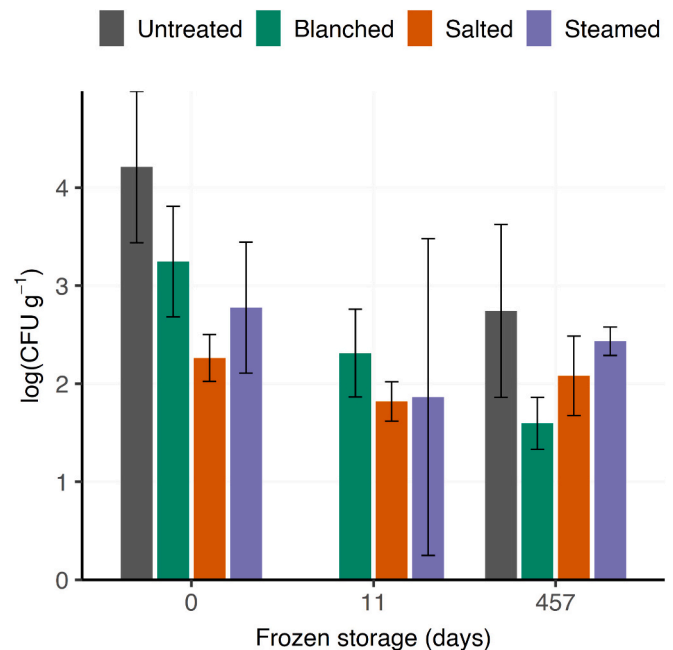

Fig. 6. Total aerobes enumerated from samples of *Saccharina latissima* following pre-treatments and frozen storage. Values are given as mean $\pm$ standard deviation ($n = 3$).

6 and 15 months (182 and 461 days) compared to shorter storage. Structural changes in the kelp during frozen storage may increase the water binding capacity of the frozen kelp matrix resulting in decreased drip loss. The evolution of drip loss as a function of storage time varies widely across food types. Increasing losses are generally observed with time in pork meat [33] whereas storage time does not seem to affect drip loss in salmon filets [34].

Heat treatments produced greener samples compared to untreated material as reported in previous studies [11,23]. This colour change is attributed to the dissociation of fucoxanthin pigment from the light harvesting complexes, leaving the chlorophylls exposed and giving the kelp material the aspect of green vegetable. Both the salting PT and freezing without any PT also produced greener samples relatively to the unprocessed raw material at t_0 but to a lower extent compared to heat-treated kelp. According to Akomea-Frempong et al. [35], the greener colour of blanched *S. latissima* ingredient in a kelp salad was associated with higher hedonic scores from a sensory panel suggesting that a bright vegetable-like colour is more attractive to Western consumers and may lower the threshold for including kelp in the diet. Similar results were obtained by Perry et al. [36] from consumer testing of a kelp salad prepared with dry salted *A. esculenta* where greener salted material (180 and 200 mg kg^{-1}) was associated with higher hedonic scores compared to less green (from less salted) material.

All PTs considerably affected the texture of *S. latissima* compared to the original raw material. The tensile strength was lowest in heat-treated samples prior to freezing. However, blanched samples maintained a higher resistance to mechanical stress throughout frozen storage up to 6 months compared to the other treatments. These observations partly contradict the results of Akomea-Frempong et al. [24] who reports no effects of various blanching methods on texture attributes of *S. latissima* but who nevertheless measured a progressive decrease in hardness, chewiness and resilience of kelp blades during 12-month frozen storage. A lower firmness of blanched *S. latissima* fronds is reported by Wirenfeldt et al. [37] compared to untreated material. Alginates and cellulose are the main skeletal compound of the intercellular matrix in kelps, giving the algae stiffness, mechanical strength et flexibility. The loss of tensile strength of *S. latissima* blades during frozen storage may be the result of the depolymerization of the cellulose and alginate matrix. These

changes will inevitably affect the mouthfeel of kelp ingredients and warrant further optimization of their textural properties tailored to specific food applications.

4.3. Nutrient content and food safety of S. latissima following pretreatment, freezing and thawing

4.3.1. Nutrient retention

The kelp *S. latissima* is recognized as a rich source of nutrients e.g., minerals and trace elements, and other bioactive compounds providing health benefits beyond basic nutrition [8]. The samples produced during this experiment following PT and freezing/thawing were characterized for their phytochemical content to assess the nutrient retention from the tested processing methods. Lower moisture contents (i.e., higher dry matter contents) in salted and steamed samples compared to untreated and blanched samples suggest a greater release of intracellular water combined with limited losses of phytochemical compounds in the two former groups. Uptake of salt, as reflected by elevated Na content in salted samples compared to other groups, is a consequence of the salting process affecting the moisture/dry matter content of the product. A higher moisture content was expected in blanched samples compared to the untreated control as reported in previous studies [11,14,38]. This can be explained by the Na uptake following the short cooling step in seawater. The frozen storage and subsequent thawing steps resulted in the release of water (i.e., drip loss) containing some level of nutrients adding to the losses from PT when applied.

As part of studying the effects of freshwater blanching on the nutrient content of *S. latissima*, Nielsen et al. [14] computed RFs of individual compounds based on the variation of the total fresh weight ($M_{tot}(t\text{-}t_0)$) during processing of relatively small samples (150 g). In the present study, estimating fresh weigh variations of large, processed batches (4 kg) in an accurate way was challenging. Therefore, $M_{tot}(t_0)$ was measured from smaller sub-samples and $M_{tot}(t)$ was calculated based on measured drip loss. This calculation method carries uncertainty of the measures explaining some RFs >1. Although the present RFs do not reflect the absolute mass balance of specific compounds, they provide an overall estimate of the retention and losses of nutrients in *S. latissima* samples from i) PTs and ii) frozen storage/thawing.

Among the tested PTs, steaming resulted in the best nutrient retention in *S. latissima*. Freshwater blanching and, to a lesser extent salting, resulted in extensive losses of soluble compounds mainly K, iodine, WSP and the sugar alcohol mannitol. As reported in the literature *S. latissima* is a rich source of minerals [25,31,39]. *Saccharina latissima* contains high levels of K compared to Na (Na/K ratio of 0.4 in untreated samples) making this species an interesting ingredient as salt replacer in the food industry. Processed food is often characterized by a high Na/K ratio (> 5.0) with negative implications on public health [40]. Edible seaweeds may contribute to healthier mineral profiles of food formulations (e.g., meat products) although species and dosage must be carefully considered to prevent excessive Na intakes [41,42]. Na and K are present in macroalgal cell as monovalent cations involved in osmotic acclimation [43]. Hence their levels were greatly affected by osmotic stress during blanching and salting as reported in previous studies [11,38]. These PTs largely compromise the value of the final products as salt replacing ingredients to the food industry. In comparison, the metals present as divalent cations (e.g., Ca, Mg, Cd) were well retained which is supported by previous experiments of kelp processing cited above as well as metabolic observations of seaweeds in response to salinity changes [43]. The affinity of alginates for divalent cations and their metal chelation properties have been demonstrated [44–46] and is likely an important factor explaining the retention of these elements in *S. latissima* during processing.

Blanching, salting and, to a lesser extent steam treatments, resulted in losses of soluble carbohydrates, mainly mannitol, as well as WSP. Mannitol is a storage carbohydrate particularly abundant within the intracellular matrix of kelps during late spring and summer [47]. It is

also involved in osmotic adjustments in algal cells in response to salinity changes [48]. Mannitol is a sweet compound which, in combination with other flavour compounds present in a Japanese kelp broth (*kombu-dashi*) such as monosodium glutamate (MSG) and potassium salt (KCl), elicits a pleasant *kokumi* flavour [49]. It is described as a long-lasting flavour richness and mouthfullness resulting from the enhanced perception of basic tastes like sweetness, saltiness and umami [50]. The WSP fraction of *S. latissima* includes free amino acids, which were found to consist mainly of glutamate and aspartate (i.e., umami flavour compounds), as well as alanine [51]. The positive correlation between the levels of these compounds and the flavour potential of edible seaweeds (e.g., *S. latissima*) have been demonstrated [52,53]. Although the characterization conducted in this study did not include the sensory evaluation of the samples, the low retention of mannitol and WSP following blanching and salting suggest that these treatments reduce the flavour potential of the final product. This is supported by the descriptive sensory analysis of *S. latissima* by Krook et al. [38] reporting lower umami from blanched samples compared to steam-treated samples. Le Pape et al. [21] reports on the effects of freezing/thawing on the sensory profile of the red seaweed *Palmaria palmata*, producing green aromas ("hay", "cut grass") compared to the marine flavours of fresh material. To our knowledge, no similar studies have been conducted on *S. latissima*.

Soluble glucose is found in kelps in the form of laminarin, a storage glucan accumulating in the biomass during summer and autumn [47]. Fucose is mainly present in sulphated form as the major constituent of fucoidans. In a nutritional perspective, neither laminarin or fucoidans are digested in the human gut and are therefore regarded as dietary fibres [8]. Findings both from in vitro and in vivo experiments suggest the bioactivity of these compounds including a role in immunoregulation [54], prebiotic [55] and anti-inflammatory [56] activities. Losses of glucose and fucose were observed in blanched and salted *S. latissima* samples but not in steamed samples. Of note, NMR shows laminarin still present in the liquid fraction of steam-treated samples and not in the control, in which glucose and α/β-glycosidic bonds were observed. This suggests that this treatment inactivates enzymes (endogenous and/or from microorganisms) responsible for the degradation of this compound.

Polyphenolic compounds and the carotenoid pigment fucoxanthin also exerts a range of bioactivities linked to their antioxidant potential [25,57,58], although these compounds are less abundant in *S. latissima* than in other kelp species (e.g., *A. esculenta*). These molecules and associated properties contribute to the increasing interest for using kelp as health-promoting ingredient in food and animal feed applications [59,60]. Blanching provided a better retention of polyphenols compared to other PTs, as reported earlier [14,61]. Similarly to the results of Wirenfeldt et al. [37] from blanching at 76 °C for 2 min in fresh water, the fucoxanthin remained relatively unaffected by the tested PTs in this study. However, the levels of fucoxanthin in blanched and untreated *S. latissima* after freezing/thawing were lower compared to initial levels in these samples at t_0. This is opposed to stable levels in steamed and salted samples during frozen storage (105 and 182 days) and a higher retention of this compound. The stability of fucoxanthin extracts is known to be affected by light, pH, temperature and oxygen [62]. Although the storage conditions of the samples in this study (frozen storage in the dark in vacuum-sealed pouches) would limit the negative effects of these factors, oxidative enzymes present in the raw material may also affect fucoxanthin stability. Salting and steaming treatments may inactivate such enzymes, hence increase fucoxanthin stability in *S. latissima* throughout frozen storage.

According to the present results, steaming appears a superior alternative to blanching and salting as PT to freezing of *S. latissima*, due to relatively lower drip loss from thawing, a greater nutrient retention, and a higher flavour and bioactive potential. This contradicts the results of Lafeuille et al. [63] reporting extensive nutrient loss (mostly minerals) following steam treatments although their treatment was followed by a

cooling step (2 min) in iced freshwater, which is likely the cause of nutrient leaching rather than steam exposure. The conditions tested under salting of *S. latissima* may also be optimized to limit nutrient losses from the process. Besides, steam PT is relevant to prevent the unwanted turnover of easily accessible nutrients in the kelp due to enzymatic degradation.

In a commercial production setting, nutrient losses upon blanching and salting may hardly be recovered as they would occur in a relatively dilute form. However, the liquid fraction obtained upon thawing of untreated and steamed material contains minerals (predominantly K), mannitol and WSP including free amino acids. These results support the recent characterization of drip loss from frozen and thawed *S. latissima* and *A. esculenta* (no PT applied) by Sund et al. [64] consisting of over 90 % moisture. The remaining dry matter content of these liquid fractions consisted mainly of minerals (70 %, dominated by K and Na), mannitol, small peptides and free amino acids, and some phenolic compounds associated with antioxidant activity. Potential uses of these fractions such as flavour or functional ingredient (e.g., for iodine fortification) were suggested by the authors, although this requires further investigation.

4.3.2. Levels of potentially toxic elements

The high iodine content of commercially cultivated kelp species, particularly *S. latissima*, and the uncertainty regarding potential health risks are a major challenge for a broad use of kelps in food applications. Iodine is an essential element for the production of thyroid hormones i. e., triiodothyronine (T3) and thyroxine (T4) which are involved in cell metabolism, growth and reproduction, and in the development of the central nervous system [65,66]. Excessive iodine intakes can affect the thyroid function with potentially negative health effects primarily in susceptible individuals e.g., those with pre-existing thyroid disease, the elderly, foetuses and neonates [67]. There are currently no EU regulations for levels of iodine in seaweed used as food.

The initial iodine content of the samples was 4012 ± 349 mg kg^{-1} DW. All tested PTs in this study significantly reduced the iodine content of *S. latissima*. However, only blanching and salting reduced levels to below the recommended limit of 2000 mg kg^{-1} by the French food authority for iodine in edible seaweeds [68]. This supports earlier reports of extensive iodine content reduction in kelps following soaking treatments at high temperatures [11,14,38,69]. Freezing/thawing of untreated *S. latissima* also reduces its iodine content, due to iodine leaching into the liquid fraction upon thawing, but not below the above-mentioned limit. Considering the adequate iodine intake (AI) of 150 µg day^{-1} for adults [70] and the levels measured in untreated *S. latissima* samples (prior to freezing) in this study, only a small amount (i.e., 0.04 g) of this kelp may be ingested to cover the AI, as reported in recent publications [38,69,71]. Freshwater blanching increases this amount substantially to 0.44 g. A higher amount of kelp ingredient, i.e., 0.15 g and 1.75 g of untreated and blanched *S. latissima* respectively, will provide the tolerable upper intake level (UL) of 600 µg iodine day^{-1} for adults.

Recent reports have raised concern over the frequent consumption of food products containing *S. latissima*, based on estimated iodine exposure per servings and potentially adverse health effects associated to long-term excessive iodine intakes [13,69,72–74]. It is therefore recommended that the iodine content in kelp-containing foods remains within tolerable limits and that appropriate information is available on the product packaging. The health consequences of excess iodine intakes are not well understood, and these reports also warrant further research. Mild to moderate iodine deficiency is a global issue and is particularly widespread in Europe [75,76] impairing cognition and growth development during early life stages. Besides, plant-based alternative to traditional dietary iodine sources (milk, dairy products, fish) are not iodine fortified and may put certain groups at risk of iodine deficiency [77]. In this context, using *S. latissima*, as food ingredients or other kelp species with a lower iodine content (e.g., *A. esculenta*) also represents an opportunity to provide a natural, plant-based source of iodine to deficient population groups.

Other potentially toxic elements which are known to accumulate in kelps include As and Cd. The levels of total As measured in untreated *S. latissima* samples was 69.7 ± 8.1 mg kg^{-1} DW, in the range of reported As concentrations in this species [39,71]. These levels were significantly reduced by blanching and salting but not by steam treatment. As levels may be further reduced from freezing and thawing (RFs comprised between 0.70 and 0.78) although significant difference before and after freezing/thawing were detected only in untreated and blanched samples. The fraction of inorganic As (i.e., the most toxic form of As) was not analysed in the present study but is generally low i.e., below 1 % of total As in this species [39]. The toxicity of organic As forms in kelps (arsenosugars and methyl derivates) is not fully characterized [78]. The Cd content of original fresh raw material (1.2 ± 0.1 mg kg^{-1} DW) was not significantly reduced by blanching and was increased by steaming to 1.4 ± 0.1 mg kg^{-1} DW. Significantly lower levels (0.7 ± 0.3 mg kg^{-1} DW) were measured after salting, in line with previous results showing Cd reduction in brown seaweeds following hypersaline soaking treatments [11,79]. On the other hand, Pb levels were unexpectedly higher in salted samples (0.6 ± 0.2 mg kg^{-1} DW) compared to the other PT groups (0.1 ± 0.0 mg kg^{-1} DW). However, this level remains low and under the maximum level allowed in food supplements i.e., 3.0 mg kg^{-1} DW (European commission regulation 1881/2006 [80]) and in the low range of values reported in seaweeds in the literature [12,13]. Models of an increased consumption of kelp-containing foods (made from *S. latissima*) showed that dietary exposure to iAs, Cd and Pb would remain at levels within the range of prior exposure (considering a reference diet) [73,74]. However, the exposure to total As may be higher, warranting further research on public health consequences [73].

4.3.3. Microbial load of pre-treated, frozen and thawed S. latissima

Samples of *S. latissima* were analysed for their microbial load, including the total number of aerobes, yeasts and moulds, and coliforms, using standard culture-based techniques in food control. The load of aerobic bacteria in fresh untreated samples was similar to the levels measured on *S. latissima* cultivated in Denmark [37]. However, heat treatments did not reduce the bacterial load to the level reported by Wirenfeldt et al. [37] following blanching in potable water (0.9–1.8 log (CFU g^{-1})). This may be due to the lower temperature used in the present experiment (60 °C) compared to the aforementioned study (76 °C). Only the salting PT significantly lowered the bacterial load of the samples compared to the untreated fresh material. Freezing greatly reduced the total aerobic bacteria initially present in *S. latissima*. It is established that both crystallization and osmotic stress upon freezing causes the apparent death of 10 to 60 % of the viable microbiota of vegetables, a percentage that gradually increases with time of frozen storage [20].

There is currently little information available on the microbial safety of frozen kelp to commercial food applications. Frequent sampling of *S. latissima* from commercial cultivation sites in densely populated areas repeatedly detected foodborne pathogens present at low levels including *Escherichia coli*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and *Salmonella enterica* [81]. Stabilization of the biomass immediately after harvest using e.g., drying or freezing methods will reduce the surface pathogen load and limit food safety risks [23,82]. However, spore-formers such as *Bacillus* spp. have been identified as a potential threat in kelp production as these microorganisms may survive the freezing step and further grow upon thawing [83]. Colonies of *Bacillus pumilus* and *Bacillus licheniformis* were isolated from raw, frozen and heat-treated samples of *A. esculenta* and *S. latissima* [23], most likely from a contamination during handling, highlighting the importance of management practices during kelp production. Heat treatment at temperatures >90 °C (e.g., steam treatment) effectively inactivate foodborne viruses and microbial pathogens and may therefore increase the safety of kelp-based food ingredients.

4.4. Considerations and perspectives for commercial processing of kelp

In a commercial setting, the selection of stabilization and processing methods relevant to food applications is driven by quality requirements of the end-product including nutritional quality, product safety and sensory properties. The results from this study support earlier reports of extensive biomass loss from freezing/thawing kelp [15,24,64] which can represent an economic loss. While optimal freezing protocols of foods aim at maintaining tissue integrity, textural and sensory attributes of the biomaterial, radical alteration such as extensive cell rupture may also facilitate further processing of the biomass such as dewatering and extraction of compounds. The liquid fraction of untreated *S. latissima* may also represent a valuable by-product to be used as e.g., flavour-active, functional ingredient.

Other criteria including available technology, processing costs and sustainability must also be considered when developing processing strategies for industrial kelp production. Large-scale freezing technology is available as it is an established process in the fishing industry and can be fitted to a vessel for on-board stabilization of the biomass after harvesting. However, a life-cycle assessment (LCA) reported freezing (in a shipping container) and frozen storage to be energy-intensive, hence, contributing with greater environmental impacts compared to other preservation methods of kelp biomass such as fermentation [16]. The IQF technology used in this study is highly energy-efficient compared to other freezing techniques (e.g., blast freezing) with a marginal energy requirement compared to the demand of warehouse storage (9 month) [84]. Improving the energy efficiency of the process and the insulation of storage spaces as well as shortening storage time of the biomass will improve the environmental performance of the process. Additional PT steps e.g., blanching, salting, steaming, will likely affect the environmental performance of the system although LCA data from applying these processes to kelp production is not currently available in the literature. These issues highlight important trade-offs between environmental sustainability, product quality and food safety to be considered by kelp producers and food manufacturers producing or using kelp ingredients in commercial applications.

5. Conclusions

This study demonstrates that freezing and subsequent thawing leads to drastic quality changes in *S. latissima* including extensive drip loss containing mainly minerals (dominated by K and Na), mannitol and some WSP (including free amino acids), as well as structural alterations reflected by textural changes from the process. Among the tested PTs in this study, steaming prior to freezing resulted in somehow lower drip loss compared to untreated *S. latissima* and a better overall nutrient retention compared to freshwater blanching and salting. The latter treatments resulted in significant losses of water-soluble compounds (minerals, mannitol, WSP). These results suggest a higher potential of steamed kelp prior to freezing to be used as flavour and bioactive ingredient compared to blanched and salted *S. latissima*. On the other hand, steaming does not reduce the iodine content to below the recommended limit, as opposed to blanching and salting. Using ingredients from *S. latissima* with high iodine levels in mass food products may lead to excessive iodine exposure to the consumer and associated negative health effects. This highlights the compromise between iodine reduction and retention of nutritional, bioactive and flavour compounds during kelp processing to food applications.

The present study provides new data related to food quality attributes of the kelp *S. latissima* from relevant processing and conservation methods in commercial production. The applicability of these methods both at a large scale as well as alternative smaller production scales should be investigated further in techno-economic and environmental impact studies. These results contribute to further the understanding of changes in edible kelp following processing as a key to maximizing the quality of kelp-based products and create value from the kelp biomass

production.

Statement of informed consent, human/animal rights

No conflicts related to informed consent and human/animal rights are applicable to this study.

CRediT authorship contribution statement

Pierrick Stévant: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Finn Lillelund Aachmann:** Writing – review & editing, Resources, Investigation. **Øystein Arlov:** Writing – review & editing, Investigation. **Tom Ståle Nordtvedt:** Writing – review & editing, Investigation. **Antonio Sarno:** Investigation. **Olav Andreas Aarstad:** Writing – review & editing, Investigation. **Leesa Jane Klau:** Writing – review & editing, Investigation. **Turid Rustad:** Writing – review & editing, Investigation. **Maren Sæther:** Writing – review & editing, Investigation. **Céline Rebours:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, 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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2024.103612.

References

[1] M. Barbier, R. Araújo, C. Rebours, B. Jacquemin, S.L. Holdt, B. Charrier, Development and objectives of the PHYCOMORPH European guidelines for the sustainable aquaculture of seaweeds (PEGASUS), Bot. Mar. 63 (2020) 5.

[2] A. Rotter, A. Bacu, M. Barbier, F. Bertoni, A.M. Bones, M.L. Cancela, J. Carlsson, M. F. Carvalho, M. Cegłowska, M.C. Dalay, T. Dailianis, I. Deniz, D. Drakulovic, A. Dubnika, H. Einarsson, A. Erdoğan, O.T. Eroldoğan, D. Ezra, S. Fazi, R. J. FitzGerald, L.M. Gargan, S.P. Gaudêncio, N. Ivošević DeNardis, D. Joksimovic, M. Katarżytė, J. Kotta, M. Mandalakis, I. Matijošytė, H. Mazur-Marzec, A. Massa-Gallucci, M. Mehiri, S.L. Nielsen, L. Novoveská, D. Overlingė, M.E. Portman, K. Pyrc, C. Rebours, T. Reinsch, F. Reyes, B. Rinkevich, J. Robbens, V. Rudovica, J. Sabotič, I. Safarik, S. Talve, D. Tasdemir, X.T. Schneider, O.P. Thomas, A. Toruńska-Sitarz, G.C. Varese, M.I. Vasquez, A New Network for the Advancement of Marine Biotechnology in Europe and Beyond, Front. Mar. Sci. 7 (2020).

[3] P. Stévant, C. Rebours, A. Chapman, Seaweed aquaculture in Norway: recent industrial developments and future perspectives, Aquac. Int. 25 (2017) 1373–1390.

[4] J.K. Kim, C. Yarish, E.K. Hwang, M. Park, Y. Kim, Seaweed aquaculture: cultivation technologies, challenges and its ecosystem services, Algae 32 (2017) 1–13.

[5] R. Araújo, F. Vázquez Calderón, J. Sánchez López, I.C. Azevedo, A. Bruhn, S. Fluch, M. Garcia Tasende, F. Ghaderiardakani, T. Ilmjärv, M. Laurans, M. Mac Monagail, S. Mangini, C. Peteiro, C. Rebours, T. Stefansson, J. Ullmann, Current status of the algae production industry in Europe: An emerging sector of the blue bioeconomy, Frontiers in marine, Science 7 (2021) 626389.

[6] P. MacArtain, C.I.R. Gill, M. Brooks, R. Campbell, I.R. Rowland, Nutritional value of edible seaweeds, Nutr. Rev. 65 (2007) 535–543.

[7] S.L. Holdt, S. Kraan, Bioactive compounds in seaweed: functional food applications and legislation, J. Appl. Phycol. 23 (2011) 543–597.

[8] M.L. Wells, P. Potin, J.S. Craigie, J.A. Raven, S.S. Merchant, K.E. Helliwell, A. G. Smith, M.E. Camire, S.H. Brawley, Algae as nutritional and functional food sources: revisiting our understanding, J. Appl. Phycol. 29 (2017) 949–982.

[9] O.G. Mouritsen, L. Williams, R. Bjerregaard, L. Duelund, Seaweeds for umami flavour in the new Nordic cuisine, Flavour 1 (2012) 4.

[10] A.S. Chapman, P. Stévant, W.E. Larssen, Food or fad? Challenges and opportunities for including seaweeds in a Nordic diet, Bot. Mar. 58 (2015) 423–433.

[11] P. Stévant, H. Marfaing, A. Duinker, J. Fleurence, T. Rustad, I. Sandbakken, A. Chapman, Biomass soaking treatments to reduce potentially undesirable compounds in the edible seaweeds sugar kelp (*Saccharina latissima*) and winged kelp (*Alaria esculenta*) and health risk estimation for human consumption, J. Appl. Phycol. 30 (2017) 2047–2060.

[12] A. Duinker, M. Kleppe, E. Fjære, I. Biancarosa, H.E. Heldal, L. Dahl, B.T. Lunestad, Knowledge Update on Macroalgae Food and Feed Safety - Based on Data Generated in the Period 2014–2019 by the Institute of Marine Research, IMR reports, Institute of Marine Research, Bergen, Norway, Norway, 2020.

[13] J.L. Banach, E.F. Hoek-van den Hil, H.J. van der Fels-Klerx, Food safety hazards in the European seaweed chain, Compr. Rev. Food Sci. Food Saf. 19 (2020) 332–364.

[14] C.W. Nielsen, S.L. Holdt, J.J. Sloth, G.S. Marinho, M. Sæther, J. Funderud, T. Rustad, Reducing the high iodine content of *Saccharina latissima* and improving the profile of other valuable compounds by water blanching, Foods 9 (2020) 569.

[15] P. Stévant, Seaweeds in Food Applications: Effects of Processing on Product Quality, Department of Biotechnology and Food Sciences, Norwegian University of Science and Technology (NTNU), Trondheim, Norway, 2019.

[16] J.-B.E. Thomas, M. Sodré Ribeiro, J. Potting, G. Cervin, G.M. Nylund, J. Olsson, E. Albers, I. Undeland, H. Pavia, F. Gröndahl, A comparative environmental life cycle assessment of hatchery, cultivation, and preservation of the kelp *Saccharina latissima*, ICES J. Mar. Sci. 78 (2020) 451–467.

[17] L. Hasselström, J.-B.E. Thomas, A critical review of the life cycle climate impact in seaweed value chains to support carbon accounting and blue carbon financing, Clean. Environ. Syst. 6 (2022) 100093.

[18] G. Philis, E.O. Gracey, L.C. Gansel, A.M. Fet, C. Rebours, Comparing the primary energy and phosphorus consumption of soybean and seaweed-based aquafeed proteins – a material and substance flow analysis, J. Clean. Prod. 200 (2018) 1142–1153.

[19] P. Stévant, C. Rebours, Landing facilities for processing of cultivated seaweed biomass: a Norwegian perspective with strategic considerations for the European seaweed industry, J. Appl. Phycol. 33 (2021) 3199–3214.

[20] M.S. Rahman, J.F. Velez-Ruiz, Food preservation by freezing, in: M.S. Rahman (Ed.), Handbook of Food Preservation, Second edition, CRC Press, 2007, pp. 635–665.

[21] M.A. Le Pape, J. Grua-Priol, M. Demaimay, Effect of two storage conditions on the odor of an edible seaweed, *Palmaria palmata*, and optimization of an extraction procedure preserving its odor characteristics, J. Food Sci. 67 (2002) 3135–3139.

[22] J.-S. Choi, B.-B. Lee, S. An, J. Sohn, K. Cho, I. Choi, Simple freezing and thawing protocol for long-term storage of harvested fresh *Undaria pinnatifida*, Fish. Sci. 78 (2012) 1117–1123.

[23] M.J. Blikra, T. Løvdal, M.R. Vaka, I.S. Roiha, B.T. Lunestad, C. Lindseth, D. Skipnes, Assessment of food quality and microbial safety of brown macroalgae (*Alaria esculenta* and *Saccharina latissima*), J. Sci. Food Agric. 99 (2018) 1198–1206.

[24] S. Akomea-Frempong, J.J. Perry, D.I. Skonberg, Effects of pre-freezing blanching procedures on the physicochemical properties and microbial quality of frozen sugar kelp, J. Appl. Phycol. 34 (2021) 609–624.

[25] P. Stévant, H. Marfaing, T. Rustad, I. Sandbakken, J. Fleurence, A. Chapman, Nutritional value of the kelps *Alaria esculenta* and *Saccharina latissima* and effects of short-term storage on biomass quality, J. Appl. Phycol. 29 (2017) 2417–2426.

[26] ISO 07.100.30, Food microbiology including microbiology of animal feeding stuffs (ICS 07.100.30), International Organization for Standardization, Geneva, Switzerland.

[27] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[28] R Development Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2022.

[29] D. Bates, M. Mächler, B. Bolker, S. Walker, Fitting linear mixed-effects models using lme4, J. Stat. Softw. 67 (2015) 1–48.

[30] R.V. Lenth, Least-squares means: the R package lsmeans, J. Stat. Softw. 69 (2016) 1–33.

[31] H. Correia, C. Soares, S. Morais, E. Pinto, A. Marques, M.L. Nunes, A. Almeida, C. Delerue-Matos, Seaweeds rehydration and boiling: impact on iodine, sodium, potassium, selenium, and total arsenic contents and health benefits for consumption, Food Chem. Toxicol. 155 (2021) 112385.

[32] D.S. Reid, D.M. Barett, Fruit freezing, in: L. Somogyi, D.M. Barett, H. S. Ramaswamy (Eds.), Processing Fruits: Science and Technology, Second edition, CRC Press, Boca Raton, 2004, pp. 161–172.

[33] T.M. Ngapo, I.H. Babare, J. Reynolds, R.F. Mawson, Freezing and thawing rate effects on drip loss from samples of pork, Meat Sci. 53 (1999) 149–158.

[34] B.T. Rotabakk, L.H. Stien, T. Skåra, Thaw rigor in Atlantic salmon (*Salmo salar*) fillets, as affected by thawing rate and frozen storage time, LWT 167 (2022) 113793.

[35] S. Akomea-Frempong, D.I. Skonberg, M.E. Camire, J.J. Perry, Impact of blanching, freezing, and fermentation on physicochemical, microbial, and sensory quality of sugar kelp (*Saccharina latissima*), Foods 10 (2021) 2258.

[36] J.J. Perry, A. Brodt, D.I. Skonberg, Influence of dry salting on quality attributes of farmed kelp (*Alaria esculenta*) during long-term refrigerated storage, LWT Food Sci. Technol. 114 (2019) 108362.

[37] C.B. Wirenfeldt, J.S. Sørensen, K.J. Kreissig, G. Hyldig, S.L. Holdt, L.T. Hansen, Post-harvest quality changes and shelf-life determination of washed and blanched sugar kelp (*Saccharina latissima*), Front. Food Sci. Technol. 2 (2022).

[38] J.L. Krook, A. Duinker, W.E. Larssen, I.M. Birkeland, S. Skeie, S.J. Horn, P. Stévant, Approaches for reducing the iodine content of the brown seaweed *Saccharina latissima*—effects on sensory properties, J. Appl. Phycol. 36 (2023) 783–796.

[39] I. Biancarosa, I. Belghit, C.G. Bruckner, N.S. Liland, R. Waagbo, H. Amlund, S. Heesch, E.J. Lock, Chemical characterization of 21 species of marine macroalgae common in Norwegian waters: benefits of and limitations to their potential use in food and feed, J. Sci. Food Agric. 98 (2018) 2035–2042.

[40] WHO, Guideline: Potassium Intake for Adults and Children, Geneva, 2012.

[41] P. Cherry, P. O'Hara, P.J. Magee, E.M. McSorley, P.J. Allsopp, Risks and benefits of consuming edible seaweeds, Nutr. Rev. 77 (2019) 307–329.

[42] P. Gullón, G. Astray, B. Gullón, D. Franco, P.C.B. Campagnol, J.M. Lorenzo, Inclusion of seaweeds as healthy approach to formulate new low-salt meat products, current opinion in food, Science 40 (2021) 20–25.

[43] G.O. Kirst, Osmotic adjustment in phytoplankton and MacroAlgae, in: R.P. Kiene, P.T. Visscher, M.D. Keller, G.O. Kirst (Eds.), Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds, Springer, US, Boston, MA, 1996, pp. 121–129.

[44] T.A. Davis, B. Volesky, A. Mucci, A review of the biochemistry of heavy metal biosorption by brown algae, Water Res. 37 (2003) 4311–4330.

[45] A. Haug, O.A. Smidsrod, The effect of divalent metals on the properties of alginate solutions. II. Comparison of different metal ions, Acta Chem. Scand. 19 (1965) 341–351.

[46] A. Haug, O.A. Smidsrod, Selectivity of some anionic polymers for divalent metal ions, Acta Chem. Scand. 24 (1970) 843–854.

[47] P. Schiener, K.D. Black, M.S. Stanley, D.H. Green, The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*, J. Appl. Phycol. 27 (2015) 363–373.

[48] R.H. Reed, I.R. Davison, J.A. Chudek, R. Foster, The osmotic role of mannitol in the Phaeophyta: an appraisal, Phycologia 24 (1985) 35–47.

[49] V. Figueroa, M. Farfán, J.M. Aguilera, Seaweeds as novel foods and source of culinary flavors, Food Rev. Int. 39 (2021) 1–26.

[50] R. Lindsay, Flavors, in: S. Demodaran, K.L. Parkin, O.R. Fennema (Eds.), Fennema's Food Chemistry, 4th Edition, CRC Press, Taylor & Francis Group, Boca Raton, 2008, pp. 639–687.

[51] P. Stévant, E. Indergård, A. Ólafsdóttir, H. Marfaing, W.E. Larssen, J. Fleurence, M. Y. Roleda, T. Rustad, R. Slizyte, T.S. Nordtvedt, Effects of drying on the nutrient content and physico-chemical and sensory characteristics of the edible kelp *Saccharina latissima*, J. Appl. Phycol. 30 (2018) 2587–2599.

[52] P. Stévant, A. Ólafsdóttir, P. Déléris, J. Dumay, J. Fleurence, B. Ingadóttir, R. Jónsdóttir, É. Ragueneau, C. Rebours, T. Rustad, Semi-dry storage as a maturation process for improving the sensory characteristics of the edible red seaweed dulse (*Palmaria palmata*), Algal Res. 51 (2020) 102048.

[53] S. Jensen, A. Ólafsdóttir, B. Einarsdóttir, G.Ó. Hreggviðsson, H. Guðmundsson, L. B. Jónsdóttir, Ó.H. Friðjónsson, R. Jónsdóttir, New wave of flavours – on new ways of developing and processing seaweed flavours, Int. J. Gastron. Food Sci. 29 (2022) 100566.

[54] A.M. Neyrinck, A. Mouson, N.M. Delzenne, Dietary supplementation with laminarin, a fermentable marine β (1–3) glucan, protects against hepatotoxicity induced by LPS in rat by modulating immune response in the hepatic tissue, Int. Immunopharmacol. 7 (2007) 1497–1506.

[55] L. O'Sullivan, B. Murphy, P. McLoughlin, P. Duggan, P.G. Lawlor, H. Hughes, G. E. Gardiner, Prebiotics from marine macroalgae for human and animal health applications, Mar. Drugs 8 (2010) 2038–2064.

[56] M.T. Ale, A.S. Meyer, Fucoidans from brown seaweeds: an update on structures, extraction techniques and use of enzymes as tools for structural elucidation, RSC Adv. 3 (2013) 8131–8141.

[57] A. Fung, N. Hamid, J. Lu, Fucoxanthin content and antioxidant properties of *Undaria pinnatifida*, Food Chem. 136 (2013) 1055–1062.

[58] J. Mildenberger, J.K. Stangeland, C. Rebours, Antioxidative activities, phenolic compounds and marine food allergens in the macroalgae *Saccharina latissima* produced in integrated multi-trophic aquaculture systems, Aquaculture 546 (2022) 737386.

[59] L.-E. Rioux, L. Beaulieu, S.L. Turgeon, Seaweeds: a traditional ingredients for new gastronomic sensation, Food Hydrocoll. 68 (2017) 255–265.

[60] L. Michalak, B. Morales-Lange, R. Montero, S.J. Horn, L.T. Mydland, M. Øverland, Impact of biorefinery processing conditions on the bioactive properties of fucoidan extracts from *Saccharina latissima* on SHK-1 cells, Algal Res. 75 (2023) 103221.

[61] G. Rajauria, A.K. Jaiswal, N. Abu-Ghannam, S. Gupta, Effect of hydrothermal processing on colour, antioxidant and free radical scavenging capacities of edible Irish brown seaweeds, Int. J. Food Sci. Tech. 45 (2010) 2485–2493.

[62] C. Lourenço-Lopes, M. Fraga-Corral, C. Jimenez-Lopez, M. Carpena, A.G. Pereira, P. Garcia-Oliveira, M.A. Prieto, J. Simal-Gandara, Biological action mechanisms of fucoxanthin extracted from algae for application in food and cosmetic industries, Trends Food Sci. Technol. 117 (2021) 163–181.

[63] B. Lafeuille, É. Tamigneaux, K. Berger, V. Provencher, L. Beaulieu, Variation of the nutritional composition and bioactive potential in edible macroalga *Saccharina latissima* cultivated from Atlantic Canada subjected to different growth and processing conditions, Foods 12 (2023) 1736.

[64] R. Sund, T. Rustad, A. Duinker, D. Skipnes, The effects of freezing and thawing on *Alaria esculenta*, J. Appl. Phycol. (2024).

[65] M. Andersson, B. De Benoist, I. Darnton-Hill, F. Delange, Iodine Deficiency in Europe: A Continuing Public Health Problem, World Health Organization, Geneva, 2007.

[66] B. de Benoist, E. McLean, M. Andersson, L. Rogers, Iodine deficiency in 2007: global progress since 2003, Food Nutr. Bull. 29 (2008) 195–202.

[67] A.M. Leung, L.E. Braverman, Consequences of excess iodine, nature reviews, Endocrinology 10 (2014) 136–142.

[68] ANSES, Opinion of the French Agency for Food, Environmental and Occupational Health & Safety on the Risk of Excess Iodine Intake from the Consumption of Seaweed in Foodstuffs, Maison-Alfort, France, 2018, p. 24.

[69] M.J. Blikra, S. Henjum, I. Aakre, Iodine from brown algae in human nutrition, with an emphasis on bioaccessibility, bioavailability, chemistry, and effects of processing: a systematic review, Compr. Rev. Food Sci. Food Saf. 21 (2022) 1517–1536.

[70] EFSA, Scientific opinion on dietary reference values for iodine, EFSA J. 12 (2014) 3660.

[71] M.J. Blikra, X. Wang, P. James, D. Skipnes, *Saccharina latissima* cultivated in northern Norway: reduction of potentially toxic elements during processing in relation to cultivation depth, Foods 10 (2021) 1290.

[72] I. Aakre, D. Doblaug Solli, M. Wik Markhus, H.K. Mæhre, L. Dahl, S. Henjum, J. Alexander, P.-A. Korneliussen, L. Madsen, M. Kjellevold, Commercially available kelp and seaweed products – valuable iodine source or risk of excess intake? Food Nutr. Res. 65 (2021).

[73] R.E. Vellinga, M. Sam, H. Verhagen, L.S. Jakobsen, G. Ravn-Haren, M. Sugimoto, D. Torres, R. Katagiri, B.J. Thu, K. Granby, J. Hoekstra, E.H.M. Temme, Increasing seaweed consumption in the Netherlands and Portugal and the consequences for the intake of iodine, sodium, and exposure to chemical contaminants: a risk-benefit study, Front. Nutr. 8 (2022).

[74] EFSA, B. Dujardin, R. Ferreira de Sousa, J.Á. Gómez Ruiz, Dietary exposure to heavy metals and iodine intake via consumption of seaweeds and halophytes in the European population, EFSA J. 21 (2023) e07798.

[75] J.H. Lazarus, Iodine status in Europe in 2014, Eur. Thyroid J. 3 (2014) 3–6.

[76] A.L. Brantsæter, H.K. Knutsen, N.C. Johansen, K.A. Nyheim, I. Erlund, H. M. Meltzer, S. Henjum, Inadequate iodine intake in population groups defined by age, life stage and vegetarian dietary practice in a Norwegian convenience sample, Nutrients 10 (2018) 230.

[77] K. Nicol, E.-L. Thomas, A.P. Nugent, J.V. Woodside, K.H. Hart, S.C. Bath, Iodine fortification of plant-based dairy and fish alternatives: the effect of substitution on iodine intake based on a market survey in the UK, Br. J. Nutr. 129 (2022) 832–842.

[78] F. Cubadda, B.P. Jackson, K.L. Cottingham, Y.O. Van Horne, M. Kurzius-Spencer, Human exposure to dietary inorganic arsenic and other arsenic species: state of knowledge, gaps and uncertainties, Sci. Total Environ. 579 (2017) 1228–1239.

[79] W.A. Stirk, J. van Staden, Desorption of cadmium and the reuse of brown seaweed derived products as biosorbents, Bot. Mar. 45 (2002) 9.

[80] EU, in: E. Commission (Ed.), Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, 2006.

[81] O.N. Barberi, C.J. Byron, K.M. Burkholder, A.T. St, A.K. Williams Gelais, Assessment of bacterial pathogens on edible macroalgae in coastal waters, J. Appl. Phycol. 32 (2020) 683–696.

[82] J.G. Vorse, C.T. Moody, L.C. Massoia, J.J. Perry, K.M. Burkholder, C.J. Byron, Effect of post-harvest processing methods on the microbial safety of edible seaweed, J. Appl. Phycol. 35 (2023) 1331–1346.

[83] T. Løvdal, B.T. Lunestad, M. Myrmel, J.T. Rosnes, D. Skipnes, Microbiological food safety of seaweeds, Foods 10 (2021) 2719.

[84] K. Shen, P. Logozzo, M. Sawant, B. Yuan, N. Bolis, Y. Kim, B. Li, Life-cycle assessment based energy consumption analysis for cold food storage facilities, Procedia CIRP 116 (2023) 624–629.