



## Acid preservation of the brown seaweed *Saccharina latissima* for food applications

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### ABSTRACT

Acidification of the brown seaweed species *Saccharina latissima* using lactic or citric acid was investigated as a preservation method to increase the shelf life prior to further processing for use in food applications. Cut seaweed biomass was mixed with seawater or acid solution prepared using seawater and stored at ambient temperature for a period of up to 56 days. The samples were assessed through compositional analysis, recording of biomass loss and calculation of compound retentions. In addition, the sensory properties of selected samples were assessed through descriptive analysis. Acid addition resulted in a drop in pH to the range 3.6 to 4.1 for all treatments where the pH remained stable throughout the storage period. For the sample preserved in seawater only, pH dropped from 7.3 to 4.3 during the storage period due to production of lactic acid by naturally occurring bacteria. All treatments resulted in biomass and loss of minerals (mainly K) and carbohydrates, which correlated positively with acid concentration with the exception for mannitol where the highest loss was found in the seawater-preserved sample. Samples with low concentration of lactic or citric acid had the lowest total mass loss and highest retention of dry matter. Increasing concentrations of lactic acid led to lower total remaining dry matter and lower compound retention. Finally, the sensory evaluation showed that the intensity of sour taste significantly differed between treatments which were otherwise characterized by high saltiness. Higher acid dose resulted in a more sour taste and lactic acid was perceived as less sour than citric acid. In addition, lower sourness correlated with a more intense saltiness. The sensory profiles were not significantly affected by storage time.

### 1. Introduction

The interest in seaweed as a food source is increasing in Europe and several initiatives focus on the development of the seaweed cultivation industry and applications. The use of seaweed as food ingredient is at an early stage in Europe and to allow for increased commercial utilization more knowledge around production, processing and product development is needed [1]. Seaweed production is considered to be climate-friendly since they have the ability to capture carbon and act as carbon sinks (depending on end-use) and counteract local ocean eutrophication through the uptake of nitrogen and phosphorous [2]. In addition, the requirement for input factors such as fresh water and fertilizers are low. Furthermore, with a growth rate exceeding most terrestrial plants they produce large amounts of biomass in a short time

period [3]. With these properties and the wide range of potential applications, seaweed cultivation and utilization can play an important role in the European blue bioeconomy [4].

The Norwegian production is focused on the two brown seaweed species *Saccharina latissima* (sugar kelp) and *Alaria esculenta* (winged kelp). The production is carried out during the winter months with one harvest in late spring to ensure high quality and avoid biofouling. As of today, the volumes produced in Norway are small in a global perspective [5]. However, as the market demand for seaweed and seaweed-based products increase, the long Norwegian coastline with favourable conditions offers a potential to produce larger seaweed volumes [6,7]. Both *S. latissima* and *A. esculenta* are recognized for their contents of minerals, trace elements, dietary fibres, certain vitamins, and bioactive compounds with potential health benefits [8,9]. Their content of

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hydrocolloids and capacity to bind water and may improve the texture of food and together with the content of flavour-active compounds, i.e., umami-eliciting free amino acids, they have a large potential as ingredients in multiple food products.

Fresh seaweed has a high water content (70–90 % of fresh weight) and degrades rapidly after harvest. Therefore, post-harvest processing is required to stabilize the biomass and maintain quality for further use. Development of suitable stabilization methods has been highlighted as one of the main challenges for the growing industry [10]. Large volumes of harvested seaweeds put high demand on suitable processing facilities in close vicinity of the cultivation site and establishment of a processing line is coupled to large investments [11]. A preferred stabilization method should not only ensure high quality seaweed but also take energy input and operational costs into consideration [10]. Currently, the most common preservation methods are drying, freezing and lactic acid bacteria (LAB) fermentation or a combination of these [5]. For seaweed species high in iodine, such as *S. latissima*, low temperature blanching is typically employed prior to further processing to reduce the iodine content [12]. Drying facilities are rarely available in direct connection to the cultivation sites. Thus, using other stabilization methods, such as fermentation or freezing, can be required to increase shelf life of the wet biomass and allow intermediate storage prior to drying. Compared to LAB fermentation, freezing is more energy intensive especially as storage requires continuous energy input [13] and can lead to quality changes and large thawing losses [14]. Fermentation requires little energy input but is coupled to other challenges such as the risk for inadequate acidification which can lead to growth of unwanted bacteria and spoilage [15].

Ensiling, either through the addition of acids (i.e., acid preservation) or fermentation by natural microbiota under anaerobic conditions has been investigated as an alternative low-cost and energy-efficient stabilization method of *S. latissima* biomass to be used as animal feed [16,17], in biorefinery concepts [18–20] or production of biofuels [21,22]. During ensiling a low pH is either obtained through the conversion of water-soluble carbohydrates to organic acids by endogenous microorganisms or the direct addition of acids [21]. A rapid pH drop is essential to avoid growth of unwanted bacteria, e.g., *Clostridia* species, which utilizes lactic acid and water-soluble carbohydrates to produce butyric acid and CO<sub>2</sub>. Natural fermentation of seaweed has shown to be challenging due to the sometimes low and varying content of water-soluble carbohydrates, dry matter (DM) content and high buffering capacity [21,23]. On the other hand, addition of mineral and organic acids has been shown to maintain low pH during long storage periods, prevent spoilage and degradation of valuable compounds, such as alginate and cellulose [19,20,22]. In addition, apart from lowering pH, organic acids such as lactic and citric acid exhibit antimicrobial properties and are common in food preservation [24].

Using seawater instead of freshwater in seaweed processing have lately gained interest as it has been seen to better retain nutrients and lead to reduced mass losses due to lower osmotic pressure [25,26]. In addition, minimizing the use of freshwater is beneficial from an environmental and economical point of view. Seawater is naturally available at the harvest site and can be used directly if the post-harvest processing is performed on the harvesting vessel. Moreover, the use of seawater in processing of *S. latissima* has been seen to give a more intense saltiness and umami-flavour [26], which can be desirable sensory properties for the use food applications, than equal treatments using freshwater.

With industrial applications in mind, direct acidification of fresh *S. latissima* biomass using citric and lactic acids in seawater was investigated as a post-harvest stabilization method in the context of food application. The effect of acid-preservation on pH, composition, nutrient retention and sensory profile was investigated during a storage period of 56 days (8 weeks).

## 2. Materials & methods

### 2.1. Seaweed biomass

Wild *S. latissima* biomass was harvested outside Rong, Norway (N60°50.807' E4°89.531') in November 2022. The biomass was placed in mesh bags submerged in seawater until sample preparation (<24 h). The holdfast and a few cm of the stem was removed and discarded. The remaining blade and stem were cut into pieces of approximately 5 × 5 cm and then transferred to a barrel providing a representative mixture of biomass to be used in the sample preparation. A representative portion of the biomass (approximately 3 kg) was vacuum packed and stored cold (4 °C) until the next day when it was oven dried at 50 °C for 16 h. This biomass is referred to as the control biomass and used as a reference for the chemical characterisation.

### 2.2. Acid preservation and sampling

Three different concentrations of lactic acid were used (3, 6 and 9 g lactic acid per kg fresh seaweed weight (FW)) and one concentration of citric acid (3 g citric acid per kg FW). Table 1 provides an overview of the different samples, acid type and concentration. Lactic acid was provided as an 80 % solution and citric acid as monohydrate in solid form. The lactic acid dose is reported as pure lactic acid (g kg<sup>-1</sup> FW). The acid concentrations were chosen based on initial analysis of the seaweeds buffering capacity, defined as the ability to resist pH changes upon addition of acid. To determine the buffer capacity, fresh wet *S. latissima* biomass was mixed with seawater (1:1) and lactic acid solution (0.1 M) was added until the mixture reached a pH of 4. The mixture was left to equilibrate for approximately 2 h and pH was again adjusted to 4 using lactic acid and the total amount acid added was recorded. Buffering capacity was determined as g acid per kg FW needed to decrease pH to 4. Acid additions were based around the determined buffering capacity. Acid solutions were prepared using seawater. 1 kg of cut biomass was thoroughly mixed with 1 kg of acid solution/seawater into vacuum bags. Initial pH was measured in the liquid within a few minutes after preparation using a hand-held pH-meter (Mettler Toledo Seven2go S2, Columbus, Ohio, USA). The bags were folded to minimize the headspace and sealed using a vacuum sealer (Audion 1020 MV-2, Weesp, Netherlands). The sealed bags were placed in a second, opaque vacuum bag and vacuum packed. Eight bags were prepared for each treatment. All samples were stored indoors at ambient temperature (18–22 °C) until sampling for up to 56 days. The wet storage time of 56 days was selected based on information regarding industrial drying capacity. This capacity is limited and typically harvested seaweed volumes therefore need to be preserved many weeks before drying can take place.

At sampling, the liquid was drained from the solid seaweed using a sieve with a hole size of 2 mm until no liquid was dripping. Both fractions were weighed on a scale with two decimals accuracy and evaluated for off-odour and other signs of spoilage. pH was measured (pH 213 Microprocessor pH meter, Hanna Instruments, Smithfield, Rhode Island, USA) in the liquid fraction and the solid fraction was dried using a hot air oven at 50 °C for 7 h prior to further analysis. The final weight of the dried seaweed was recorded. One bag from each treatment was sampled at day 7, 14, 28 and 42 ( $n = 1$ ), while 3 bags were samples at day 56 ( $n = 3$ ).

**Table 1**  
Sample and treatment overview.

Sample name	Preservation media	Acid concentration (g acid kg <sup>-1</sup> FW)
Control	None	0
SW	Seawater	0
LA 9	Lactic acid solution	9
LA 6	Lactic acid solution	6
LA 3	Lactic acid solution	3
CA 3	Citric acid solution	3

### 2.3. Determination of moisture, ash and organic content

Moisture content in the dried seaweed samples was determined gravimetrically at 103 °C for 24 h. The moisture content was used to determine chemical composition based on dry weight. Ash content was determined by incineration of samples (0.5–0.7 g) in a muffle furnace at 550 °C for 8 h.

### 2.4. Analysis of minerals and heavy metals

Macro minerals (Na, Mg, K, P and Ca), trace elements (I) and heavy metals (total As and Cd) were determined at the Institute of Marine Research (IMR) by ISO accredited methods according to Reksten et al. [27]. Macro minerals and heavy metals were determined by inductively coupled plasma-mass spectrometry (ICP-MS, Thermofisher Scientific, MA, USA) equipped with an autosampler (FAST SC-4Q DX, Elemental Scientific, NE, USA) after acid wet digestion in a microwave oven (UltraWave, Milstone, Italy) using nitric acid (65 %) according to the method described by Julshamn et al. [28]. Elements were quantified using external calibration curves of Na, Mg, K, P, Ca, As and Cd. I content was determined using ICP-MS according to the method described by Dahl et al. [29]. One mL of tetramethylammonium hydroxide (TMAH) and 5 mL deionized water before extraction at 90 ± 3 °C for 3 h. The samples were thereafter diluted and centrifuged. Prior to quantification, samples were filtered through a 0.45 µm syringe filter. Tellurium was used as an internal standard in order to correct for instrumental drift.

### 2.5. Monosaccharide analysis

The monosaccharide composition was determined using the method described by Manns et al. [30]. 150 mg dry sample was mixed with 1 mL 72 % w/w H<sub>2</sub>SO<sub>4</sub> and hydrolysed in a water bath at 30 °C for 1 h. Thereafter, 42 mL deionized water was added to dilute the mixture to 4 % w/w H<sub>2</sub>SO<sub>4</sub>, and samples were incubated at 120 °C for 40 min using an autoclave. The acid hydrolysates and seaweed residues were separated through centrifugation and supernatants were filtered through a 0.22 µm disposable syringe filter into 2 mL Eppendorf tubes. Prior to analysis samples were diluted appropriately in deionized water. Monosaccharide composition was determined by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using an ICS5000 system (Dionex, Sunnyvale, USA) equipped with a CarboPac™ PA1 column. Neutral sugars were eluted isocratically with 15 mM NaOH and uronic acids by linear gradient of NaOAc from 0 to 150 mM. Quantification of monosaccharides was performed using Chromeleon software (Version 7.2.9, Thermo Fisher Scientific, Waltham, Massachusetts, USA) using standards of mannitol, glucose, fucose, galactose, mannose, xylose, guluronic acid, glucuronic acid and manuronic acid, hydrolysed in the same manner as the seaweed samples.

### 2.6. Analysis of organic acids in the liquid fraction

Organic acids and mannitol was analysed using high performance liquid chromatography, HPLC, according to the method described by Grønnevik et al. [31]. One gram of sample, 2.5 mL deionized water, 0.2 mL 0.5 M H<sub>2</sub>SO<sub>4</sub> and 8.0 mL acetonitrile (Merck, Germany) was mixed for 30 min and centrifuged at 3000 ×g for 15 min. Thereafter the samples were filtered through a 0.2 µm syringe filter into vials. Analysis was performed on an Aminex HPX-87H column (Bio-Rad Laboratories, CA, USA), held at 32 °C, connected to a HPLC (1260 Infinity, Agilent Technologies, Singapore) equipped with a DAD-UV- and RI-detector. The mobile phase was 5 mM H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.4 mL min<sup>-1</sup>. Standards solutions of citric-, α-ketoglutaric-, pyruvic-, succinic-, lactic-, formic-, acetic-, propionic- and pyro-glutamic acid (Merck, Germany) were prepared in the same manner as samples.

### 2.7. Analysis of free amino acids

To determine the amount of free amino acids in the solid seaweed fraction, 200 mg of dried milled sample was mixed with 10 mL deionized water and incubated under agitation for 1 h. Thereafter the samples were sonicated for 30 min and centrifuged at 3000 ×g for 20 min. The supernatant was used for further analysis according to Moe et al. [32] with some modifications. Free amino acids were analysed by adding 5.0 g of internal standard (0.1 M HCl; 0.4 µmol mL<sup>-1</sup> L-norvalin; Sigma-Aldrich, St. Louis, MO, USA) to 5.0 g of sample (supernatant). The samples were mixed in a Grant Bio-mixer and placed in ice for 30 min. After centrifugation at 15,600 ×g for 5 min at 4 °C, the samples were filtered (0.2 µm cellulose acetate filter, Advantec, Dublin, CA) and stored at -20 °C until analysis. Separation of amino acids was performed using an Agilent 1200 series pump, autosampler, column oven, thermostat, and fluorescence detector (Agilent Technologies, Singapore) on a Xterra RP 18 column (150 × 4.6 mm, Waters, Milford, MA). Prior to separation, 350 µL of borate buffer (0.4 M, pH 10.2, Agilent Technologies) was added to 50 µL of sample. Derivatization was done by addition of o-phthalaldehyde/3-mercaptopropionic (OPA/MPA, Agilent Technologies) according to Bütikofer and Ardö [33]. At 5 °C, 5 µL OPA/MPA solution was added to 5 µL of sample, mixed 6 times and incubated for 0.15 min prior to injection. The derivatized amino acids were separated by a stepwise linear gradient of 100 mM NaOAc (pH 7.20) with 80 % acetonitrile and 0.1 M titriplex III (3.3 to 20.7 % over 13 min, 20.7 to 30 % over 12 min and 30 to 100 % over 4 min) at a flow rate of 0.7 mL min<sup>-1</sup> at 45 °C. The detector parameters were set to detect the derivatives at excitation 340 nm and emission 455 nm.

### 2.8. Mass balance and nutrient retention

The weight of both liquid and solid fraction was recorded at sampling. To determine the proportion of a compound remaining in the solid biomass post treatment, true retention (TR) was calculated according to the formula described by Murphy et al. [34]:

$$\%TR = \frac{\text{g retained nutrient} \times \text{g total product post treatment}}{\text{g original nutrient} \times \text{g total product prior to treatment}} \times 100$$

### 2.9. Sensory evaluation

A generic descriptive analysis (GDA) [35] was used to characterize and compare the sensory profile of SW, LA9, LA3 and CA3 (see Table 2 for description of attributes) after 7 and 56 days of storage. LA6 was excluded from the analysis due to capacity limitations. The sensory panel consisted of 8 judges selected and trained according to the guidelines in ISO:8586:1 [36]. All assessors had some experience with sensory evaluation of seaweeds. The panellists were all members of the staff at Møreforskning and gave their consent to participate in the sensory evaluation. All samples were assessed in dried powdered form. During the first training phase, the assessors developed a vocabulary describing the samples' odour and flavour across 11 attributes listed in Table 2. The panel members evaluated the samples using a scale from 0 to 9 (lowest to highest intensity) in a sensory test facility equipped with individual booths. Four samples, coded with three-digit numbers, were evaluated in two replicate sessions. The sensory evaluation program RedJade (Tragon Corp., Palo Alto, CA, USA) was used to collect the data. The sensory data was processed according to the General Data Protection Regulation (GDPR).

### 2.10. Data analysis

Statistical analysis of chemical data was performed using R (version 4.1.0, R Development Core Team 2021). The results from analysis of replicate samples are described as mean ± standard deviation. A one-way ANOVA analysis was used to detect significant differences ( $p <$

**Table 2**

Sensory attributes and their definitions associated to the *Saccharina latissima* samples assessed.

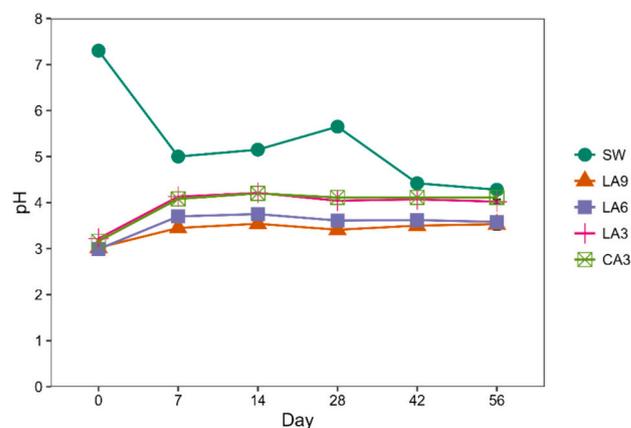
Sensory attribute	Label	Scale anchors	Description
Odour			
Hay	O-Hay	None   much	Freshly dried grass or straw
Iodine	O-Iodine	None   much	Chemical, medicinal aroma
Flavour			
Fresh sea	F-Fresh sea	None   much	Fresh sea, marine odour and flavour
Fermented	F-Fermented	None   much	Matured flavour, opposite to fresh
Sour	F-Sour	None   much	Sour taste
Salty	F-Salty	None   much	Salty taste
Umami	F-Umami	None   much	Umami taste e.g., meat stock, brown crabmeat
Bitter	F-Bitter	None   much	Bitter taste
Sickening	F-Sickening	None   much	Sweet, nauseating as in well-ripened fruits and vegetables
Metal	F-Metal	None   much	Rust, metal, blood
Astringent	F-Astringent	None   much	Tingling sensation of dryness in the mouth after tasting

0.05) between samples with different lactic acid concentrations (0, 3, 6 and 9 g acid kg<sup>-1</sup> FW). Tukey HSD was used for pairwise comparison of samples (R function glht). A two-sample *t*-test was used to detect significant differences ( $p < 0.05$ ) between citric acid and lactic acid at the same dose (i.e., 3 g acid kg<sup>-1</sup> FW). A mixed model analysis of variance (ANOVA) (R function lmer [37]) was used to detect differences in sensory properties between samples. In this model, individual panellists and replicate sessions were treated as random factors. The Benjamini-Hochberg procedure was applied to control for the false discovery rate under multiple testing. Tukey HSD was computed for the pairwise comparison of sample groups (R function glht). A principal component analysis (PCA, R function prcomp) based on covariance matrix (no scaling) was applied to visualize differences in sensory- and free amino acid profiles among samples.

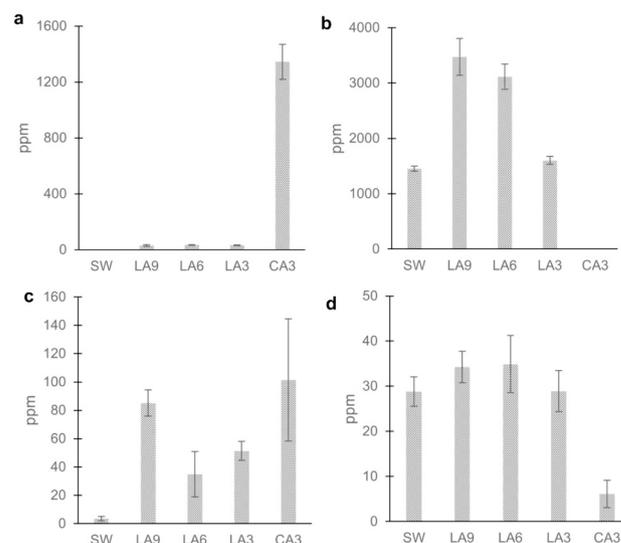
### 3. Results

#### 3.1. pH development and content of organic acids

The results from the pH-measurements during sampling of acid- and the seawater-preserved samples are presented in Fig. 1. On the day of sample preparation, the buffering capacity (defined as the amount of acid needed to lower the pH to 4) of fresh *S. latissima* was determined to 4.7 g acid per kg FW. This amount gave an indication of the range of acid addition needed to lower the pH of the biomass. The initial pH of acid-preserved samples (measured within 30 min of sample preparation) ranged between 3.0 (LA9) and 3.2 (LA3 and CA3). For these samples, the pH increased slightly during the first days of storage and at the first sampling point (after 7 days) pH was highest in LA3 and CA3 at 4.1 in both samples. Simultaneously, the pH of the seawater-preserved sample (SW) dropped from 7.3 to 5.0 and continued to decrease throughout the storage period of 56 days to a final pH of 4.2 which was close to the final pH of LA3 and CA3 of 4.0 and 4.1, respectively. After 56 days LA9 and LA6 had similar pH of 3.5 and 3.6, respectively. The content of lactic-, citric-, acetic- and DL-pyroglutamic acid analysed in the liquid fractions resulting from the draining of samples after 56 days of storage, is shown in Fig. 2. Liquid fractions from SW, LA9, LA6 and LA3 mainly contained lactic acid, while CA3 mainly contained citric acid. Small amounts of



**Fig. 1.** pH in seawater- and acid-preserved seaweed samples after 0, 7, 14, 28, 42 ( $n = 1$ ) and 56 days ( $n = 3$ ) of storage measured in the liquid fraction. LA9, LA6 and LA3 denote samples with 9, 6 and 3 g lactic acid kg<sup>-1</sup> FW, respectively, and CA3 denotes sample with 3 g citric acid kg<sup>-1</sup> FW. The control sample was preserved in seawater (SW) without acid. Data for 56 days is presented as mean  $\pm$  standard deviation.



**Fig. 2.** Content of a) citric acid, b) lactic acid, c) acetic acid, and d) DL-pyroglutamic acid in liquid fractions after 56 days of storage. LA9, LA6 and LA3 was preserved with 9, 6 and 3 g lactic acid kg<sup>-1</sup> FW ( $n = 3$ ), respectively, CA3 was preserved with 3 g citric acid kg<sup>-1</sup> FW ( $n = 2$ ) and SW was preserved with seawater only ( $n = 3$ ). Results are given in ppm and bars represent mean  $\pm$  standard deviation. Note the different scales on the y-axis.

acetic and DL-pyroglutamic acid were detected in all liquid fractions.

#### 3.2. Effect of preservation on composition of *S. latissima* biomass

Compositional analysis was used to assess the effect of preservation on *S. latissima* constituents. The composition of dried biomass (control) can be seen in Table 3 together with the composition of seawater- (SW) and acid-preserved samples (LA9, LA6, LA3 and CA3) after 56 days of storage. The total dry matter (DM) was lower in the preserved samples compared to the control. The samples with high and medium concentration of lactic acid (LA9 and LA6, respectively) had significantly higher DM content than samples with lowest lactic acid concentration (LA3) and the seawater-preserved sample (SW). No significant difference in total DM was found between LA3, and the sample preserved with citric acid at the same concentration (CA3). Among the mono-saccharides, mannitol was highly affected by preservation, and was

**Table 3**

Composition of dried *Saccharina latissima* (Control) and preserved samples after 56 days. Data for preserved samples are shown as mean  $\pm$  standard deviation ( $n = 3$ ). SW was preserved with seawater without acid. LA9, LA6 and LA3 denotes samples with 9, 6 and 3 g lactic acid  $\text{kg}^{-1}$  FW, respectively, and CA3 denotes sample with 3 g citric acid  $\text{kg}^{-1}$  FW. Different letters in the same row indicate significant differences ( $p < 0.05$ ) between different levels of lactic acid (0, 3, 6, 9 g  $\text{kg}^{-1}$  FW). An asterisk indicates significant difference ( $p < 0.05$ ) between different acids at the same level (3 g  $\text{kg}^{-1}$  FW).

	Control	SW	LA9	LA6	LA3	CA3
Days of storage	0	56				
DM (% of WW)	11.2	9.6 $\pm$ 0.2 <sup>a</sup>	10.3 $\pm$ 0.0 <sup>b</sup>	10.1 $\pm$ 0.1 <sup>b</sup>	9.6 $\pm$ 0.1 <sup>a</sup>	9.5 $\pm$ 0.1
Ash (% of DW)	41.9	37.0 $\pm$ 0.3 <sup>a</sup>	32.7 $\pm$ 0.5 <sup>b</sup>	34.5 $\pm$ 0.7 <sup>c</sup>	36.2 $\pm$ 0.4 <sup>d</sup>	36.7 $\pm$ 0.1 <sup>*</sup>
Carbon (% of DW)	28.5	29.6 $\pm$ 0.1 <sup>a</sup>	31.8 $\pm$ 0.2 <sup>b</sup>	31.3 $\pm$ 0.3 <sup>b</sup>	30.0 $\pm$ 0.4 <sup>a</sup>	29.7 $\pm$ 0.3
Nitrogen (% of DW)	1.9	1.9 $\pm$ 0.0 <sup>ab</sup>	2.0 $\pm$ 0.0 <sup>c</sup>	2.0 $\pm$ 0.0 <sup>ac</sup>	1.9 $\pm$ 0.0 <sup>b</sup>	1.9 $\pm$ 0.0
Monosaccharides and uronic acids (% of DW)						
Total <sup>1</sup>	41.1	30.7 $\pm$ 1.1 <sup>a</sup>	32.8 $\pm$ 1.2 <sup>ab</sup>	33.6 $\pm$ 1.7 <sup>ab</sup>	32.7 $\pm$ 0.6 <sup>b</sup>	36.8 $\pm$ 2.8
Mannitol	8.9	0.5 $\pm$ 0.1 <sup>a</sup>	1.5 $\pm$ 0.2 <sup>b</sup>	2.0 $\pm$ 0.2 <sup>bc</sup>	1.9 $\pm$ 0.2 <sup>c</sup>	3.9 $\pm$ 0.4 <sup>*</sup>
Fucose <sup>2</sup>	3.3	2.4 $\pm$ 0.1 <sup>a</sup>	2.1 $\pm$ 0.1 <sup>b</sup>	2.1 $\pm$ 0.1 <sup>b</sup>	2.0 $\pm$ 0.0 <sup>ab</sup>	2.7 $\pm$ 0.2 <sup>*</sup>
Glucose <sup>2</sup>	9.2	9.7 $\pm$ 0.4 <sup>a</sup>	10.2 $\pm$ 0.5 <sup>a</sup>	10.1 $\pm$ 0.5 <sup>a</sup>	9.9 $\pm$ 0.2 <sup>a</sup>	11.0 $\pm$ 1.0
Guluronic acid	4.7	5.8 $\pm$ 0.3 <sup>a</sup>	4.3 $\pm$ 0.2 <sup>b</sup>	4.3 $\pm$ 0.2 <sup>b</sup>	4.0 $\pm$ 0.1 <sup>b</sup>	4.4 $\pm$ 0.4
Mannuronic acid	12.1	10.0 $\pm$ 0.4 <sup>a</sup>	12.2 $\pm$ 0.4 <sup>b</sup>	12.3 $\pm$ 0.2 <sup>b</sup>	12.4 $\pm$ 0.4 <sup>b</sup>	11.9 $\pm$ 0.8 <sup>*</sup>
Minerals and trace elements (g $\text{kg}^{-1}$ DW)						
Na	51.3	80.3 $\pm$ 2.6 <sup>a</sup>	78.8 $\pm$ 1.5 <sup>a</sup>	76.8 $\pm$ 1.0 <sup>a</sup>	82.0 $\pm$ 2.9 <sup>a</sup>	80.9 $\pm$ 1.2
K	131.0	72.6 $\pm$ 2.7 <sup>a</sup>	58.0 $\pm$ 0.8 <sup>b</sup>	61.5 $\pm$ 1.0 <sup>bc</sup>	65.9 $\pm$ 0.8 <sup>c</sup>	72.2 $\pm$ 1.1 <sup>*</sup>
Ca	19.7	26.4 $\pm$ 0.2 <sup>a</sup>	24.4 $\pm$ 0.5 <sup>b</sup>	23.4 $\pm$ 0.5 <sup>b</sup>	24.4 $\pm$ 0.2 <sup>b</sup>	24.9 $\pm$ 0.7
Mg	8.6	11.7 $\pm$ 0.6 <sup>a</sup>	11.3 $\pm$ 0.5 <sup>a</sup>	11.0 $\pm$ 0.0 <sup>a</sup>	12.0 $\pm$ 0.1 <sup>a</sup>	12.1 $\pm$ 0.1
I	5.1	2.9 $\pm$ 0.2 <sup>a</sup>	2.5 $\pm$ 0.1 <sup>b</sup>	2.6 $\pm$ 0.0 <sup>ab</sup>	2.8 $\pm$ 0.1 <sup>a</sup>	2.9 $\pm$ 0.1
Total As <sup>3</sup>	83.0	43.3 $\pm$ 2.1 <sup>a</sup>	52.5 $\pm$ 0.1 <sup>b</sup>	52.7 $\pm$ 4.0 <sup>b</sup>	48.1 $\pm$ 0.8 <sup>ab</sup>	46.9 $\pm$ 1.2
Cd <sup>3</sup>	0.54	0.53 $\pm$ 0.02 <sup>a</sup>	0.46 $\pm$ 0.00 <sup>a</sup>	0.44 $\pm$ 0.06 <sup>a</sup>	0.44 $\pm$ 0.01 <sup>a</sup>	0.45 $\pm$ 0.01

<sup>1</sup> Xylose/mannose, galactose and glucuronic acid are included in total monosaccharides.

<sup>2</sup> The content of fucose reflects the content of fucoidan and the content of glucose reflects the content of laminarin and cellulose.

<sup>3</sup> mg  $\text{kg}^{-1}$  DW.

significantly lower in the seawater-preserved sample compared to LA-samples after 56 days of storage. The mannitol content was significantly lower in LA9 than LA3, which was significantly lower than in CA3. The content of guluronic and mannuronic acid was also significantly lower in the control compared to in LA-samples. Additionally, the fucose content was lower in preserved samples compared to the control and significantly lower in LA9 and LA6 compared to the control and LA3. CA3 had a significantly higher fucose content than LA3. The content of K was found to be reduced with around 50 % in the preserved samples. Again, the content was lowest in LA9 ( $58.0 \pm 0.8$  g  $\text{kg}^{-1}$  DW) and increased with decreasing LA concentration. The difference in K-content was the only significant found among the analysed minerals between LA3 and CA3. All preserved samples had a higher content of Na and divalent ions Ca and Mg than the control. Preservation reduced the I and As content to close to half of the level found in the control while Cd-content remained relatively similar. In general, between LA-samples, the

content of the individual minerals was significantly affected by increasing acid concentration, which corresponds well to the analysed ash content.

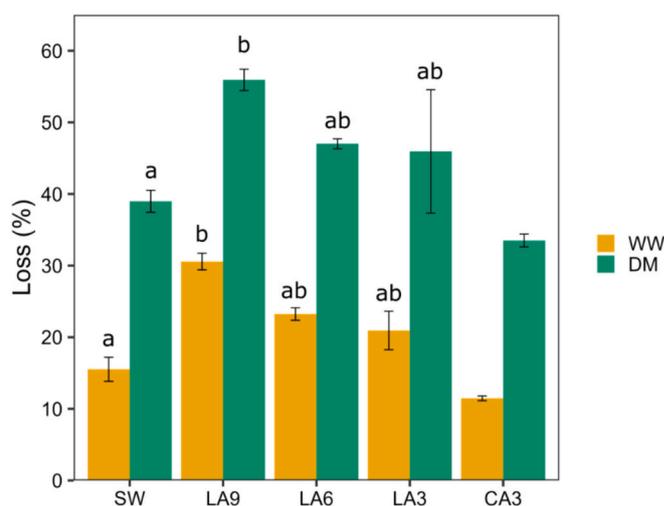
### 3.3. Mass balance and compound retention in preserved samples

To assess biomass losses during storage and subsequent storage, the solid and liquid fraction of the samples were separated and weighed at sampling. The total loss of wet weight (WW) and DM after 56 days of storage is shown in Fig. 3. LA9 had the highest total biomass loss, i.e., wet weight, with  $30.6 \pm 1.2$  %, which was not significantly different from LA6 and LA3 which lost  $23.2 \pm 0.9$  and  $20.9 \pm 2.7$  % of their initial weight, respectively. There was no significant difference in either loss of WW or DM between LA3 and CA3. On the other hand, for the LA-samples and SW, the same significant differences were seen for DM loss as for WW loss. CA3 had the lowest DM loss with  $33.5 \pm 0.9$  %.

The recorded biomass loss and composition of control and preserved biomass was used to calculate the true retention (TR) of nutrients for preserved samples after 56 days of storage (Table 4). A TR of 100 implies that a compound is fully retained, i.e., nothing is lost, during processing. Apart from Na in CA3, TR were below 100 % for all nutrients in all samples. In general, LA9 had the lowest retention and CA3 together with SW had the highest. With the exception for mannitol, glucose, mannuronic acid and total As, significant differences were found between SW (no added acid) and LA9 (added 9 g lactic acid per kg FW). No significant differences were found between the samples with added lactic acid. The retention of mannitol and K was significantly higher in CA3 compared to LA3.

### 3.4. Sensory properties and free amino acids

A general descriptive analysis was used to evaluate the effect of preservation on the flavour and odour of *S. latissima*. In order to detect any changes during storage, samples stored for 7 days were included in the analysis. The chemical composition can be found in the Supplementary Material (Table S1). To visualize differences in sensory profiles, the mean scores from the panellists were used to conduct a PCA. The 1st and 2nd principal component (PC) accounted for a total of 69.83 % of the variation in the data, as can be seen in the biplot in Fig. 4. The



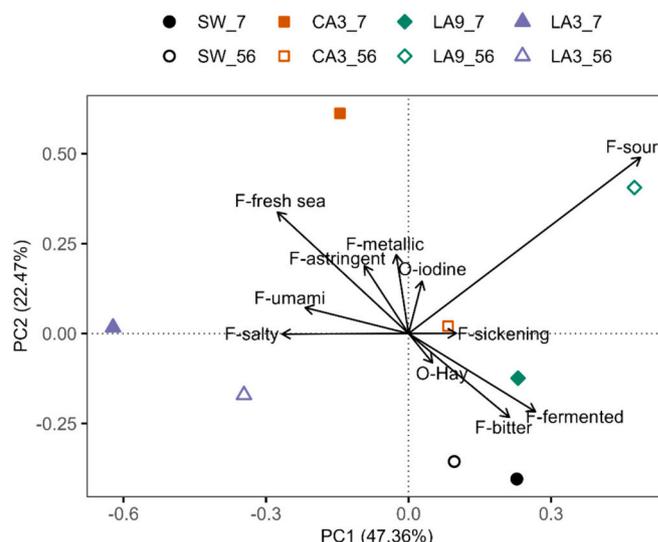
**Fig. 3.** Loss of wet weight (WW) and dry matter (DM) of seawater- and acid-preserved seaweed samples (solid fraction) after 56 days of storage ( $n = 3$ ). Results are given as mean  $\pm$  standard deviation. LA9, LA6 and LA3 denote samples with 9, 6 and 3 g lactic acid  $\text{kg}^{-1}$  FW, respectively, and CA3 denotes sample with 3 g citric acid  $\text{kg}^{-1}$  FW. SW was preserved in seawater without acid. Different letters above bars denote significant differences between different lactic acid concentrations (0, 9, 6 and 3 g  $\text{kg}^{-1}$  FW).

**Table 4**

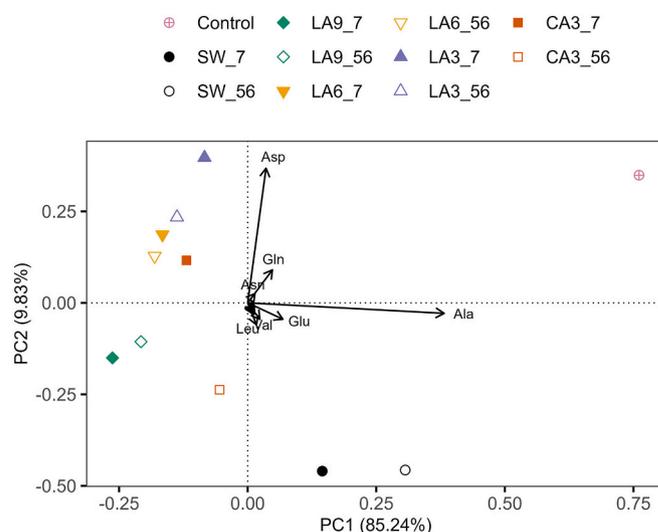
True retention (%) of compounds post-treatment in preserved *Saccharina latissima* after 56 days of storage relative to dried *Saccharina latissima* presented as mean  $\pm$  standard deviation ( $n = 3$ ). SW was preserved in seawater without acid. LA9, LA6 and LA3 denote samples with 9, 6 and 3 g lactic acid  $\text{kg}^{-1}$  FW, respectively, and CA3 denotes sample with 3 g citric acid  $\text{kg}^{-1}$  FW. Different letters in the same row indicate significant differences ( $p < 0.05$ ) between different levels of lactic acid (0, 3, 6, 9  $\text{g kg}^{-1}$  FW). An asterisk indicates significant difference ( $p < 0.05$ ) between different acids at the same level (3  $\text{g kg}^{-1}$  FW).

Compound	SW	LA9	LA6	LA3	CA3
Moisture	72.7 $\pm$ 3.0 <sup>a</sup>	48.8 $\pm$ 1.6 <sup>b</sup>	59.7 $\pm$ 1.4 <sup>ab</sup>	64.0 $\pm$ 9.6 <sup>ab</sup>	79.9 $\pm$ 0.6
DM	61.0 $\pm$ 1.0 <sup>a</sup>	44.1 $\pm$ 1.5 <sup>b</sup>	53.0 $\pm$ 0.7 <sup>ab</sup>	54.1 $\pm$ 8.6 <sup>ab</sup>	66.5 $\pm$ 0.9
Ash	59.3 $\pm$ 2.2 <sup>a</sup>	37.8 $\pm$ 1.8 <sup>b</sup>	47.6 $\pm$ 0.9 <sup>ab</sup>	51.0 $\pm$ 8.2 <sup>ab</sup>	64.1 $\pm$ 0.4
C	63.3 $\pm$ 1.5 <sup>a</sup>	49.2 $\pm$ 1.4 <sup>b</sup>	58.1 $\pm$ 1.0 <sup>ab</sup>	56.7 $\pm$ 8.3 <sup>ab</sup>	69.3 $\pm$ 0.6
N	62.8 $\pm$ 2.6 <sup>a</sup>	47.7 $\pm$ 1.2 <sup>b</sup>	56.8 $\pm$ 1.3 <sup>ab</sup>	55.3 $\pm$ 8.7 <sup>ab</sup>	68.3 $\pm$ 0.7
<b>Monosaccharides</b>					
Total	45.7 $\pm$ 2.5 <sup>a</sup>	35.3 $\pm$ 1.5 <sup>a</sup>	43.4 $\pm$ 0.3 <sup>a</sup>	47.4 $\pm$ 13.5 <sup>a</sup>	59.5 $\pm$ 2.0
Mannitol	3.6 $\pm$ 2.1 <sup>a</sup>	7.3 $\pm$ 2.0 <sup>a</sup>	11.7 $\pm$ 3.0 <sup>a</sup>	14.3 $\pm$ 6.0 <sup>a</sup>	29.1 $\pm$ 2.1 <sup>*</sup>
Fucose	43.9 $\pm$ 3.8 <sup>a</sup>	27.4 $\pm$ 1.6 <sup>b</sup>	33.0 $\pm$ 0.6 <sup>ab</sup>	29.0 $\pm$ 2.8 <sup>ab</sup>	54.1 $\pm$ 1.5
Glucose	64.3 $\pm$ 3.9 <sup>a</sup>	48.5 $\pm$ 1.5 <sup>a</sup>	57.8 $\pm$ 1.1 <sup>a</sup>	52.3 $\pm$ 6.5 <sup>a</sup>	79.2 $\pm$ 6.7
Guluronic acid	74.2 $\pm$ 5.0 <sup>a</sup>	39.8 $\pm$ 2.2 <sup>b</sup>	47.9 $\pm$ 2.7 <sup>ab</sup>	41.6 $\pm$ 4.3 <sup>ab</sup>	61.6 $\pm$ 2.2
Mannuronic acid	50.6 $\pm$ 4.3 <sup>a</sup>	44.7 $\pm$ 2.3 <sup>a</sup>	54.0 $\pm$ 4.4 <sup>a</sup>	49.5 $\pm$ 5.3 <sup>a</sup>	65.5 $\pm$ 2.8
<b>Minerals and trace elements</b>					
Na	95.5 $\pm$ 5.6 <sup>a</sup>	67.6 $\pm$ 3.1 <sup>b</sup>	79.3 $\pm$ 2.1 <sup>ab</sup>	85.9 $\pm$ 10.7 <sup>ab</sup>	104.8 $\pm$ 1.0
K	33.8 $\pm$ 2.1 <sup>a</sup>	19.5 $\pm$ 0.9 <sup>b</sup>	24.9 $\pm$ 0.7 <sup>b</sup>	27.2 $\pm$ 4.7 <sup>ab</sup>	36.6 $\pm$ 0.3 <sup>*</sup>
Ca	81.9 $\pm$ 2.4 <sup>a</sup>	54.8 $\pm$ 2.9 <sup>b</sup>	63.1 $\pm$ 0.5 <sup>b</sup>	66.0 $\pm$ 10.0 <sup>ab</sup>	84.2 $\pm$ 1.8
Mg	83.0 $\pm$ 5.5 <sup>a</sup>	57.8 $\pm$ 4.5 <sup>b</sup>	67.4 $\pm$ 1.0 <sup>ab</sup>	75.2 $\pm$ 11.3 <sup>ab</sup>	93.1 $\pm$ 0.7
I	34.9 $\pm$ 2.5 <sup>a</sup>	21.3 $\pm$ 1.0 <sup>b</sup>	27.2 $\pm$ 0.4 <sup>ab</sup>	30.1 $\pm$ 6.2 <sup>ab</sup>	37.5 $\pm$ 0.9
Total As	31.8 $\pm$ 1.9 <sup>a</sup>	27.9 $\pm$ 0.9 <sup>a</sup>	33.7 $\pm$ 3.0 <sup>a</sup>	31.3 $\pm$ 5.2 <sup>a</sup>	37.6 $\pm$ 1.1
Cd	60.6 $\pm$ 3.3 <sup>a</sup>	37.8 $\pm$ 1.2 <sup>b</sup>	43.2 $\pm$ 6.6 <sup>b</sup>	44.5 $\pm$ 6.9 <sup>ab</sup>	56.9 $\pm$ 2.1

treatments significantly affected sour and salty flavour. While the scores for saltiness were in the upper half of the 9-point scale used (between 6.0  $\pm$  1.3 to 7.2  $\pm$  0.9), the sour flavour was scored in the mid- to lower half of the scale (between 2.8  $\pm$  1.0 to 4.9  $\pm$  2.3; See Supplementary Material, Table S2). Pairwise comparison of samples revealed significantly higher salty flavour of LA3 and CA3 compared to LA9 after 7 days of storage. There was no significant difference in saltiness after 56 days of storage. LA9 was perceived more sour than the SW and LA3. The mean scores for LA3 and CA3 (same acid dose of 3 g acid  $\text{kg}^{-1}$  FW) indicated a difference in the perception of sour taste between the samples with different type of acid, albeit not significant. CA3 had higher mean scores for sour taste than LA3 with 4.0  $\pm$  2.1 and 3.0  $\pm$  1.9, respectively, after 7 days of storage and 3.7  $\pm$  1.6 and 2.8  $\pm$  1.0, respectively, after 56 days of storage. Overall, the effect of treatment was not significant for umami. However, the umami flavour of LA3 at 7 days was significantly higher than in SW sample at both sampling points and CA3 at 56 days. Free amino acids (FAA) were analysed to investigate if differences in sensory profile could be coupled to the FAA profile. The FAA profile of the control sample was dominated by alanine (Ala), aspartic acid (Asp) and



**Fig. 4.** Biplot (1st and 2nd principal component) obtained from the principal component analysis (PCA) of the sensory scores of seawater- (SW) and acid-preserved seaweed samples (LA9, LA3, CA3) stored for 7 and 56 days. Average scores over panellists ( $n = 8$ ) and replicate sessions ( $n = 2$ ) were used for the PCA. Vectors indicate loadings representing the variation in intensity for individual sensory attributes including odour (O) and flavours (F) among all samples. The number at the end of the sample name (7 and 56) indicates days of storage.



**Fig. 5.** Biplot (1st and 2nd principal component) obtained from the principal component analysis (PCA) of the content of free amino acids in control (dried *S. latissima* biomass), seawater- and acid-preserved samples after 7 and 56 days of storage. The number at the end of sample names of preserved samples indicates number of days of storage.

glutamic acid (Glu). Fig. 5 shows a biplot resulting from the PCA conducted to visualize differences between the FAA profiles of the samples. The control and LA6 was included in the analysis despite being excluded from the sensory analysis. The FAA profile of LA3, LA6 sample and CA3\_7 had a higher contribution from Asp, Gln and Asn than the SW samples and CA3\_56. The control was well separated from the other samples mainly by PC1 and from SW, LA9 samples and CA3\_56 by PC2 which show a large change in FAA content due to preservation and storage. The differences between the acid preserved samples and SW samples were mainly attributed to a higher Ala and Glu content in SW.

## 4. Discussion

### 4.1. Acid preservation and pH stability

Direct acidification using lactic or citric acid efficiently reduced the pH of *S. latissima* biomass. After a small increase during the first days of storage of acid-preserved samples, the pH remained stable throughout the storage period of 56 days. The ability of *S. latissima* biomass to resist pH changes, i.e., the buffering capacity, is mainly attributed to the content of the anionic polysaccharide alginate, as well as the content on anions which is reflected in the high ash content [22,38]. Variations in composition as well as level of biofouling (growth of other marine organisms on the biomass), especially the occurrence of calcifying organisms can affect the buffer capacity and thus the acid addition needed for large batches in large scale production. Therefore, it is useful to investigate how a range of concentrations affect the final pH. To ensure food safety and avoid growth of pathogenic and spoilage bacteria it is necessary to have a sufficiently low pH, which is stable during storage. Løvdal et al. [39] concluded that a pH < 4.3 for refrigeration temperatures (4 °C) and <3.7 at higher storage temperatures is preferable to avoid growth of unwanted microorganisms and ensure food safety. In this study, the pH of acid-preserved samples remained relatively stable below 4.3 for LA3 and CA3, and below 3.7 for LA6 and LA9, during the storage period. Previous studies have investigated longer storage periods for acid-preserved *S. latissima* using different methods and acids. Larsen et al. [19] used lactic acid (7 and 9.2 g kg<sup>-1</sup>) and obtained relatively stable pH throughout a period of 12 months. Other studies have obtained successful preservation using formic and/or propionic acid as well as sulphuric acid during storage for 6.5 months [20,22,40]. The pH decrease observed for the seawater-preserved samples (from 7.3 to 4.3) was most likely due to the fermentative action of endogenous seaweed microbiota which converted available sugars to acids. This hypothesis was supported by the presence of lactic acid and small amounts of acetic acid analysed in the liquid fraction. Such spontaneous, or natural, fermentation have been reported earlier during storage of untreated *S. latissima* biomass. Sandbakken et al. [22] observed a decrease in pH to 4.0–4.2 after 50 days of storage of fresh *S. latissima* under anaerobic conditions. Similar behaviour was observed by Herrmann et al. [21] upon ensiling without additives where pH reached 4.0 after 14 days with a predominant production of lactic acid and some acetic acid shifting towards production of ethanol after 90 days of storage probably due to the presence of yeasts. The natural microbiota of seaweed is highly influenced by the microbial load and composition of the surrounding water [39]. The use of seawater as preservation media in this study would presumably lead to a higher microbial load compared to using tap water. In addition, cutting the seaweed into small pieces prior to preservation could increase the initial availability of water-soluble carbohydrates such as mannitol. The small amounts of acetic acid measured in all liquid fractions from acid-preserved samples is likely produced by endogenous microorganisms, which is interesting considering the low pH. However, the lack of off-smell indicates that there was no fermentative production of butyric or propionic acid [15,21]. Apart from lowering the pH, organic acids can have antimicrobial properties where the main mechanisms are coupled to the acids ability to penetrate the bacterial cell membrane and impair cell function [41]. The ability of the acid to penetrate the cell membrane is dependent on if it is in its undissociated or dissociated form. While lactic acid is a monocarboxylic (one functional carboxylic group) with a pKa of 3.7, citric acid is a tricarboxylic acid with three pKa values of 3.1, 4.7 and 6.4. The antimicrobial properties of monocarboxylic acids are known to be most effective at pH below the pKa where the acid is mainly in the undissociated form. Citric acid has been shown to function best at a pH between the first and second pKa, i.e., 3.1 and 4.7 [42]. Additionally, when acid dose is based on weight rather than molar concentration, the number of acid molecules differs due to difference in molecular mass (90.1 and 192.1 g/mol for lactic and citric acid, respectively). In general,

small molecules have a better capability to penetrate bacterial cell membranes [43]. It should also be noted that the presence of acetic acid, which was higher in CA3, show that there was also microbial production of acids. However, further studies are needed to fully understand the relationship between acid dose, pH and antimicrobial effect in seaweed preservation.

### 4.2. Effect of preservation on composition and compound retention

Preservation clearly had an effect on the biomass composition, mainly through the decrease in ash content and water soluble carbohydrates (i.e., mannitol) which is in accordance with previous studies investigating blanching and fermentation of *S. latissima* [12,44,45] While expressing the chemical composition as a fraction of DW provides important information regarding the nutritional quality it does not fully inform about the loss of compounds to the liquid phase during processing and subsequent storage. In an industrial context, minimal processing losses are important from an economical perspective. Processes that produce high-quality and safe seaweed with low biomass losses are thus desirable. Therefore, the effect of preservation on total biomass loss, and the retention of compounds related to the initial composition was investigated. In an industrial drying process, the seaweed would typically be drained, and the liquid fraction considered a side stream. Generally, the effect on the biomass and loss of compounds correlated positively with acid dose. Low dose of lactic acid and citric acid seemed to better preserve the biomass in terms of biomass loss and thus higher retention of compounds. Probably, partial hydrolysis of structural cell wall elements promoted the release of compounds in all samples, but most likely higher acid dose led to a more extensive degradation, thus a greater release. Soaking seaweed biomass in acid solution has earlier been investigated as a pretreatment to enhance the effect of subsequent mechanical dewatering techniques [46]. In our study, there was a clear effect of increasing lactic acid concentration on increased moisture loss as seen from the decreasing retentions. An increase in Na content and decrease in K, I and As content is in line with previous studies using seawater/salt water in processing [26,47]. Katayama et al. [48] reported an additional effect of acidic treatment water (4 % acetic acid) as well as increased temperatures, both factors reducing the total As content in the brown seaweed Hijiki. Despite relatively low retention of I in all samples (between 21.3 ± 1.0 and 37.5 ± 0.9) the content related to the dry weight was still above the available recommendations of 2 g kg<sup>-1</sup> DW [49]. The amount of I extracted during processing depends on several factors, such as temperature and biomass-to-liquid ratio [50]. However, the results obtained in this study indicate no additional effect of acid addition on iodine reduction. Future studies should investigate whether increasing the amount of liquid during acid preservation can result in lower I contents. Divalent cations Ca, Mg and Cd have been seen to be less prone to extraction during processing such as blanching or boiling [45,51] which is most likely due to their close interaction with alginate and other cell wall components. Stévant et al. [47], however, reported significant loss of Cd when soaking *S. latissima* in hypersaline water (2.0 mM NaCl). In addition, LAB fermentation have been shown to reduce Cd contents with 35 % [44]. Moreover, soaking the green seaweed *Ulva* sp. in 0.5 % citric acid solution for 15 min reduced the Cd content with up to 96 %, while blanching in deionized water rather increased the concentration due to loss of other compounds [52]. At low pH, alginate is partly converted to its acid form and is more susceptible to depolymerization [20]. Consequently, divalent cations bound to the alginate are released. Nøklung-Eide et al. [20] found a reduction in mannuronic acid content in untreated compared to acid-preserved samples, suggesting that alginate sections rich in mannuronic acid are more susceptible to enzymatic degradation resulting from microbial activity. This is in accordance with the lower retentions and content of mannuronic acid in the naturally fermented seawater-preserved sample which presumably had higher microbial activity compared to the acid-preserved samples. The sugar alcohol mannitol is a small, water-soluble molecule which functions as

an osmolyte and is readily released from the seaweed when exposed to salinity changes [53]. Thus, it is also highly susceptible to loss upon processing, which was evident following the low retention of mannitol in all samples. The almost complete removal in the seawater-preserved sample is likely due to the ability of fermentative microorganisms to use mannitol as carbon source. Sandbakken et al. [22] found that mannitol was highly stable in *S. latissima* stored anaerobically at or below pH 3.7 (by adding sulphuric and/or formic acid). Our study also showed preservation of mannitol in samples treated with acid but also partial loss due to leakage to the liquid fraction. The loss of fucose (Table 4), largely representing the sulphated polysaccharide fucoidan, was expected since it is water-soluble and usually extracted under mild to moderate acidic conditions [54]. Larsen et al. [19] stored *S. latissima* anaerobically with lactic acid (7 g kg<sup>-1</sup> FW) and reported loss of approximately half of the mass to the liquid fraction. Ensiling using lactic acid bacteria of the seaweed species *Laminaria digitata* resulted in WW loss of 45–48 % after 12 months [55]. In this study, the total loss of WW was lower, which could be due to shorter storage time but potentially also the use of seawater as preservation medium.

#### 4.3. Effect of preservation and storage on sensory properties

The sensory profile of *S. latissima* have earlier been described as having a high saltiness, moderate-to-high umami flavour, low bitterness and sourness [56,57] as well as an intense flavour and aroma of fresh sea [57]. Bruhn et al. [44] reported that heat-treatment and LAB fermentation reduced the saltiness and marine flavour attributes of *S. latissima*. Apart from the sourness, the preserved samples seemed to have a similar profile as those previously described. However, it was clear for all samples that either the addition of lactic or citric acid or the production of lactic acid resulted in a sour taste otherwise not present. The intensity of the sour taste increased with increasing concentration of lactic acid and citric acid was perceived as more sour than lactic acid. The sour taste of organic acids is a complex mechanism and several attempts have been made to connect acid properties to the sour taste they elicit as reviewed by Da Conceicao Neta et al. [58]. The difference in properties between citric and lactic acid could explain the difference in perceived sour taste between CA3 and LA3. The physicochemical characteristics also play a role in the physiological response to sour taste stimuli. The sour taste of citric acid has been described as fresh and associated to citrus fruits while lactic acid has been described as tart and acrid [58]. Moreover, the presence of sodium ions has been seen to modulate the perception of sour taste [59]. Here, sour and salty taste were well separated by PC, indicating a correlation between higher saltiness and lower sourness. Less salty taste also correlated with lower content of Na and K. Moreover, the use of seawater in processing of *S. latissima* has earlier been seen to result in a more intense salty taste compared to processing in freshwater [26]. The natural fermentation in the seawater-preserved sample seemed to modulate the marine flavour and result in a more fermented flavour, which was in agreement with the previously mentioned results reported by Bruhn et al. [44]. In this study, the hypothesized effect on sensory properties had a large impact on the choice to test lactic acid as preservative, as it was thought to resemble the profile obtained during fermentation. However, despite the similar levels of lactic acid found in the liquid fractions after 56 days of storage, LA3 had a higher saltiness, more umami taste, and seemingly less sour taste than the seawater-preserved sample. Proposedly, these differences reflect the different modes of preservation, e.g., direct acidification or fermentation, which could lead to production of other taste-active compounds as an effect of microbial activity. Variations in the FAA composition could to some extent be correlated with differences in sensory profile. For instance, the FAA profile of LA3 (both after 7 and 56 days of storage) had a higher content of Asp, Gln and Asn, which can be associated to umami taste [60]. Interestingly, a higher contribution of Glu to the FAA profiles of the SW samples and CA3\_56 did not seem to be reflected in the perceived umami taste. A significant loss of Ala as a

result of freshwater blanching of *S. latissima* has been seen in previous studies [12], which was also the main difference between the control and preserved samples here. Overall, longer storage did not affect the sensory profiles of the acid-preserved samples, which is positive from an industrial perspective. Both due to the possibility to store biomass for longer periods but also for the potential use as ingredient in food products where predictability and stability of sensory attributes are of great importance.

## 5. Conclusion

Direct acidification of fresh *S. latissima* biomass using lactic acid and citric acid resulted in a rapid decrease in pH which remained stable throughout the storage period of 56 days. Storage in seawater without acid led to a gradual decrease in pH due to the production of lactic acid through spontaneous fermentation. All treatments, including the seawater treatment, resulted in a loss of biomass and nutrient retention below 100 %. Overall, low concentrations of lactic or citric acid gave the best preservation of both biomass and DM whereas higher concentrations of lactic acid led to higher mass loss during storage and thus lower nutrient retention. Citric acid has not traditionally been used in seaweed processing but proved very promising in our study. Thus, this should be followed up by further studies. The use of seawater and acids resulted in sensory profiles characterized by high saltiness and sour taste that was significantly different between treatments. However, the effect of the different acids on the sour taste need be considered in relation to the final food application. Using acidic seawater as preservation medium is beneficial as the processing can be started directly on the harvesting vessel. However, storage of seaweed biomass submerged in liquid would require additional storage space and potentially increased costs compared to storage of drained biomass. Overall, this study shows that acid preservation in seawater is a good method to store *S. latissima* for at least 56 days at room temperature.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.algal.2024.103524>.

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## CRedit authorship contribution statement

**Johanna Liberg Krook:** Writing – original draft, Investigation, Formal analysis, Conceptualization. **Luca Riboldi:** Writing – review & editing, Resources, Investigation, Conceptualization. **Ingrid Mjelde Birkeland:** Writing – review & editing, Investigation. **Pierrick Stévant:** Writing – review & editing, Investigation, Formal analysis. **Wenche Emblem Larsen:** Writing – review & editing, Investigation, Formal analysis. **Nanna Rhein-Knudsen:** Writing – review & editing, Investigation, Formal analysis. **Siv Skeie:** Writing – review & editing, Supervision. **Svein Jarle Horn:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

Johanna Liberg Krook and Ingrid Mjelde Birkeland are employed by Orkla Foods Norway, a company using seaweed as a food ingredient. Luca Riboldi is employed by Arctic Seaweed, a seaweed producer.

## Data availability

Data will be made available on request.

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