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Brining as an effective method to stabilise sea lettuce (*Ulva fenestrata*) -impact on colour, texture, chemical characteristics and microbial dynamics

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ABSTRACT

Brining as a cost-effective stabilising method to preserve the quality of fresh *Ulva fenestrata* was studied. The brines contained from 0 to 25 % (*w*/w) of sodium chloride or from 0 to 50 % sucrose and were combined with seaweed at a ratio of 1: 10 (*w/v*) prior to storage at 4 °C for up to 3 months. During this storage, the water activity of *U. fenestrata* was reduced from 0.94 to ≤0.89 with ≥15 % salt brines, which kept the microbial load <7 log (CFU/g) for 78 days. Among the sucrose brines, 50 % provided microbial shelf life <7 log (CFU/g) for 48 days. Further, 25 % salt or 50 % sucrose brines effectively retained the greenness (a*) of the *U. fenestrata* blades (< −20 a*-value for 80 days), while the tensile strength was only retained with 25 % salt brine (>3 Newton for 80 days). There was a time-dependent loss of crude proteins and fatty acids during storage, especially for 50 % sugar brined seaweed, where 58 % and 28 %, respectively, were lost after 20 days. Nutrients were best preserved in the 5 % salt-brine. Overall, the results indicate that brining with 25 % salt or 50 % sugar yields microbial stability and maintained colour of *U. fenestrata* for at least 48 days, with the former even exceeding 78 days at 4 °C, however, at a cost of nutritional value.

1. Introduction

Marine ecosystems are important for a resilient production of food raw materials and thus contribute significantly to the sustainable development goals (SDGs); not least goals 2, 3, 12 and 14 targeting hunger, good health and well-being, sustainable production and healthy seas and oceans [1]. Seaweed has been reported as the marine food resource having the lowest environmental footprint when farmed [2] and is from a consumer perspective considered nutritious because it contains protein, fibres, and bioactive compounds [3,4]. A recent review pin-pointed that seaweed is a particularly promising source of proteins providing all essential amino acids for human nutrition [5]. Economically, seaweed can be an opportunity for fisheries and fish aquaculture companies to diversify their activities and increase their revenues [6].

A growing interest, especially in green seaweeds from the genus *Ulva,* has been seen in the aquaculture industry. This is because *Ulva* spp. can tolerate significant variations in temperature, acidity, and nutrient accessibility in the ocean [7]. In addition to *Ulva* spp. resilience, they possess a rich nutrient profile. *Ulva fenestrata* cultivated in an off-shore seafarm was recently reported to reach a protein content between 16.6 and 20.75 % dry weight (dw), while the total fatty acid content varied between 3.2 and 3.5 % dw, and the polysaccharide level between 25 and 29 % dw [7]. One of the polysaccharides found in *U. fenestrata*, ulvan, has been linked to several different bioactivities e.g., anti-inflammatory effects in rats [8]. *U. fenestrata* thus has great potential to be used as a multifunctional food ingredient. However, as other seaweed produced biomasses, *U. fenestrata* after harvest rapidly perishes due to the sensitive nature of its nutrients and its high water activity, promoting microbial growth [4]. Therefore, tailor-made and scalable stabilising methods for the processing and preservation of *Ulva* spp. biomass are needed to ensure that, e.g., nutrients, colour, and texture are kept during storage [9].

Today, the most common stabilisation method for seaweed is drying. However, apart from when utilising sun and wind, this technique is relatively energy demanding [10]. Further, when applied to *Ulva* spp., colour changes and up to 37 % reduction of phenolic compounds have been recorded after oven drying, the latter to a larger extent with increased temperature and storage time [11]. In addition, significant losses of polyunsaturated fatty acids (PUFAs), were found during storage of oven-dried *U. fenestrata,* with higher losses (up to 84.1 %) seen during light exposure [12].

Another common stabilisation method for seaweed is freezing.

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Although it slows down enzymatic reactions and microbial growth, the formation of ice crystals that occur when the freezing is not fast enough can affect microstructure, texture and nutrient leakage of the seaweed after thawing [13].

Further, rapid freezing and subsequent frozen storage of large amounts of biomass harvested in a short period of time, imply high energy costs and require large investments for seaweed aquaculture companies, which commonly are of small to medium size from a European perspective [1]. In recent efforts to increase food preparedness in response to e.g. wars and the COVID-19 pandemic, factors such as robustness to extended power outages have also been brought into focus, calling for more diversified ways of storing our food [14]. Thus, cost-effective stabilising methods that can limit or minimise changes in texture, colour and nutrient loss while ensuring a longer shelf-life need to be explored for perishable raw materials such as seaweeds.

The fish industry still uses several ancient techniques for the preservation of small pelagic species, such as herring and anchovies, among others [15,16]. One of these techniques is brining, i.e., osmotic dehydration, which consists of adding either dry salt or salt brine to the raw material and storing it in a closed container. The closed environment and the salt itself decrease microbial growth and inhibit specific microorganisms that can be harmful to human consumption [17]. Brining as a stabilisation method could prolong the shelf-life of green seaweeds, such as *U. fenestrata*, while demanding low energy costs because it only requires access to a refrigerator or cold room. By avoiding either very low or high temperatures, as well as e.g., cutting or mincing, brining could potentially better maintain the seaweed's chemical composition and texture.

Only a handful of studies so far have focused on brining to stabilise seaweed. These comprise salt processing, via brining and dry salting of *Ulva rigida* to preserve the chemical composition and textural properties [9], dry salting of the brown seaweed *Alaria esculenta* to mantain texture and controlling shelf-life [18], submerging sea grape, *Caulerpa lentillifera* in salt brine to keep the nutritional composition as well as to reduce microbial load [19], and brining as a pretreatment prior to extraction of ulvan from *Ulva rotundata* [20]. No previous publications, however, have addressed the use of salt brining for the crop *U. fenestrata*.

Similarly, only one previous study focused on using sucrose as an osmotic agent for seaweed. This was done by immersion of blanched red seaweed, *Kappaphycus alvareii*; in a sugar bath at 30, 35 and 40 °C prior to drying [21]. To our knowledge, there is no other scientific literature on subjecting seaweed to sugar brines as a sole stabilising method under cold storage. However, sucrose, just like NaCl, provides L-shaped sorption isotherms when dissolved in water. This reflects their ability to bind large amounts of water, thereby effectively decreasing the water activity of foodstuff. In comparison to NaCl, sucrose is a non-ionic solute, which is primarily limited to the extracellular spaces, since the diffusion process through the membrane is low; thus it forms a surface layer [22]. Previously, a surface layer formed by coating *U. fenestrata* blades with whey protein solution prior to drying delayed lipid oxidation compared to non-treated samples [12].

Based on the identified knowledge gaps, and the needs for cost-effective and scalable seaweed preservation techniques, this study aimed at investigating salt and sugar brining as routes to stabilise *U. fenestrata* during refrigerated storage. Using NaCl and sucrose at different concentrations, the effects on water activity, microbial load, chemical composition, colour and tensile strength were determined. The outcome could aid seaweed producers to diversify the forms in which they sell seaweed to secondary food producers, and a possibility to compare pros and cons of differently preserved biomass in terms of nutritional, physical, chemical and safety characteristics.

2. Materials and methods

2.1. Seaweed raw material

U. fenestrata biomass from a long-term cultivation located in a thermo-stable room (90 L tanks, at 12 °C, under 16:8 h (L:D) light cycle, and at an irradiance of 90–110 μmol m^{-2} s^{-1}, under permanent aeration, with seawater flow through of 10–14 L h^{-}) at Tjärnö, Sweden (58°52′33.272″N, 11°8′47.202″E). The biomass was harvested at three different occasions, on the 17th January 2022, 6th April 2022 and on 9th June 2022. The biomass was kept in seawater while transported for 4 h to Chalmers University of Technology, Gothenburg, Sweden. Upon arrival, the seaweed was removed from the seawater and transferred into plastic containers for brining, see below.

2.1.1. Chemicals

The following chemicals were purchased from Sigma-Aldrich Sweden AB (Stockholm, Sweden): sodium chloride for the brines; plate count agar (PCA), gelatin, agar, potassium phosphate for microbiological analysis; chloroform, methanol, hexane, isooctane with HPLC grade, and toluene and acetyl chloride with ACS reagent grade, for fatty acids. Marine agar (MA) for microbial analysis and sucrose for brine preparation were acquired from Millipore (Merck, Darmstadt, Germany). Proteose-peptone no.2 for Long hammer (LH) media preparation was purchased from Thermo Fisher Scientific (Göteborg, Sweden),

2.1.2. Seaweed brining

The brines were made from deionised water (*w/*w) containing 5 % NaCl (5S), 15 % NaCl (15S), 25 % NaCl (25S), 15 % sucrose (15Sc), 25 % sucrose (25Sc) and 50 % sucrose (50Sc). Controls were prepared in the absence of ions with deionised water (C1) and with a natural content of ions in tap water (C2).

Ten grams of fresh weight (FW) seaweed were added to the brines or controls in a ratio of 1:10 seaweed to liquid (*w/v*). To ensure the seaweed would be covered by the brine, thereby reducing contact with air, a small lid was placed inside the container. The samples were then kept in a translucent container in a dark cold room at 4 °C. Sampling occurred every 2 days for the first 6 days and afterwards on days 13, 20, 40 and 80 days, or until spoilage. The latter was assessed based on odour at each sampling point using a small internal untrained sensory panel.

2.2. Water activity (a_w)

The water activity (a_w) of the blades from brined *U. fenestrata* was measured using a hydrometer LabTouch aw, (Novasina, Lachen, Switzerland), based on AOAC method 978.18 [23]. At each time point, approximately 0.3 g were taken from the *U. fenestrata* blades (same location every time), which was enough to cover the bottom of the measuring plate. This plate was then placed into the measuring chamber kept at 25 °C. The instrument was calibrated with NaCl standards provided by the manufacturer having a_w of 0.985, 0.760 and 0.362. The measurements were done in duplicates per sample ($n = 2$), and the results of a_w are expressed without unit since it is a relative measure [11].

2.3. Brine analysis; pH, ionic strength and °brix

At each time point, 1 milliliter (mL) of brine was withdrawn from the beakers and analysed for pH using a pH meter (PHM210, MeterLab, Radiometer analytica, Villeurbanne, France). Measurements were done at room temperature; ~21 °C. One mL of brine was transferred to a falcon tub, and a conductivity meter probe (CDM210, MeterLab, Radiometer analytica, Villeurbanne, France) was introduced in order to measure the conductivity of the brine over time in (mS/cm). The ionic strength was then calculated against a calibration curve based on NaCl %. Thirty microliters (μL) of the brine were also subjected to analysis using a hand-held refractometer (Master-53α, Atago, Japan) to

determine the °brix (°Bx), i.e., solids in suspension, based on a sucrose scale. All measurements were done at room temperature and in duplicates ($n = 2$). Samples were taken randomly from the brine.

2.4. Microbiological analysis

At days 0, 13, 20, 34 and 48, around 10 g of brined seaweed were removed from the containers and placed into a stomacher bag with 90 mL sterile 2 % NaCl. Afterwards, the bag was placed into a stomacher for 3 min, and 50 mL of stomached liquid was then taken into a falcon tube. Serial dilutions (10^{-1},10^{-2}.10^{-4},10^{-6}) were thereafter prepared using sterile peptone water as a dilutant. Each dilution was plated into 3 different media: MA, PCA and LH; the latest was prepared as indicated by NMKL [24]. MA and PCA plates were incubated at 25 °C for 48 h to determine marine microbial and total viable counts, respectively. While LH plates were used to study psychrotrophic bacteria, and were incubated at 12 °C for 7 days. Each sample was plated in 6 different spaces as replicates.

2.5. Colour analysis

Colour of the *U. fenestrata* blade was analysed using a colourimeter (CR-400 Chroma Meter Konica Minolta sensing, NJ, USA), which was calibrated with a white tile. At each time point, a blade was selected randomly from the brine container and placed into a petri dish having a diameter of 30 mm. Four replicate measurements were then performed at a 2° view angle and D65 illuminant, and the results were expressed as lightness from 0 to 100 (L*), green (−a*) to red (+a*) and from blue (−b*) to yellow (+b*). Total colour (ΔE*) was also calculated based on the following equation:

$$\Delta E = \sqrt{\left(L_2^* - L_1^*\right)^2 + \left(a_2^* - a_1^*\right)^2 + \left(b_2^* - b_1^*\right)^2} \quad (1)$$

2.6. Tensile strength

The tensile strength was measured using a texture analyser (Perthment Instruments, Shelton, CT, USA) with two grips to hold the blade. The method was based on previous studies on seaweeds [25,26], with some modifications. In this case, a 7 cm × 3 cm rectangle was cut per blade, which was placed between the grips of the instrument. Two centimeters were placed between the grips, leaving 3 cm available for the measurement. The tensile strength analysis was set with 10 % extension or until breakage of the blade, with a speed of 1.6 mm·s^{-1}. The lower grip was fixed, while the upper grip moved upwards until the rupture of the blade. The analyses were performed on four different blades per sample type ($n = 4$), and the results are expressed in newtons (N).

2.7. Proximate composition

The Dumas method [27] was used to determine the total nitrogen content of the differently brined *U. fenestrata* using the LECO Turmac nitrogen analyser (St Joseph, MI, USA). Fifty mg of freeze-dried and milled samples were measured. The protein content of the samples was calculated using a conversion factor of 5 [7,28]. The moisture content was measured based on the AOAC Method 925.10, by the weight difference of the samples before and after being placed at 105 °C overnight. Afterwards, the selected samples (fresh seaweed and seaweed brined 40 days) were transferred to a furnace for 6 h at 550 °C to determine the ash content, based on the AOAC method 938.08 [29].

2.8. Fatty acid composition

Based on previous research [12,30], the fatty acid composition was determined using direct methylation of freeze-dried and milled seaweed samples. Fifty mg sample was placed into glass tubes spiked with 20 μL of C23:0 (1000 ppm) dissolved in chloroform. Subsequently, 2 mL of toluene and 2 mL of 10 % methyl acetyl chloride (*v*/v) in methanol were added to the sample, which was heated at 70 °C for 120 min in a table-top bench incubator. Afterwards, the samples were left to cool down to 20 °C, and 2 mL of water was added, followed by 2 mL of hexane. The mixture was then vortexed for 60 s and placed into a centrifuge at 100 ×*g* for 6 min. After centrifugation, 3 mL of the upper phase was transferred into a glass tube to be evaporated under nitrogen gas. The dried sample was then re-dissolved in 4 mL isooctane, where after 300 μL was transferred to glass vials (GC–MS Agilent, Santa Clara, CA, USA).

The different samples were analysed using an Agilent 7890A GC system with an Agilent 5975C triple axis MS detector (Santa Clara, CA, USA). One μL of sample was injected with split 15:1 into the system, and the fatty acids were separated in a VF-WAX column (30 m × 250 μm × 0.25 μm) (Phenomenex, Torrance, CA, USA). The inlet temperature was set at 275 °C, and the carrier gas used was helium, with a constant flow of 1 mL/min. The temperature was increased in 4 °C steps from 100 °C to 205 °C. Then, a 1 °C increment was applied up to 230 °C, where the temperature was kept for 5 min. The fatty acids were identified using an external fatty acid standard mixture GLC-463 (Nu-Check, Prep, Inc., Elysian, MN, USA). The standard mixture, however, did not contain all fatty acids found in seaweed; specifically, C16:3n3 and C20:4n3, which were identified using the NIST08 library search.

2.9. Statistical analysis

Statistical analysis was performed using the R Studio software version 4.2.2 with the packages "Agricolae" [31] and "stats" [32]. The data was first tested for homogeneity variance and distribution with the Shapiro and Bartlett tests. If complied, a one-way ANOVA and *t*-test were performed, with a set maximum *p*-value of 0.05. In case of the tensile strength measurements, the results did not follow a normal distribution nor homogeneity, therefore a root square was done in the data prior to performing an ANOVA.

3. Results and discussion

3.1. Changes of the brine during storage

In the water brines, i.e., C1 and C2, and in the 15Sc, there were no significant rises in the °Bx measure (Supplementary - Fig. A). For the rest of the sugar brines, 25Sc and 50Sc, significant decreases were seen in °Bx on day 2 and onwards until day 6. Thereafter, the brines reached an equilibrium with the seaweed until the end of storage on day 50.

Among the two controls, only the tap water-based brine (C2) showed a significant change in ionic strength (Supplementary - Fig. B). On day 2, it had decreased from 9 to 0.3 % NaCl equivalents, after which it remained stable. Similarly, the sugar brines also decreased in ionic strength during the first 2 days, as seen in the °Bx results discussed above.

Differently from the controls and sugar brines, the salt brines presented significant differences in their ionic strength over the storage time. From day 0 to day 4, the 5S and 15S-brines showed a decrease in ionic strength to 40 % and 42 %, respectively, after which values stabilised at around 2.9 and 14 % NaCl equivalents, respectively, until the end of the storage. On day 6, the strongest salt brine, 25S, had significantly decreased to 50 % of its ionic strength, reaching a value of 14 % NaCl equivalents.

A possible explanation for the variation in time to reach equilibrium between brine and seaweed (from 0 to 6 days) could be the different concentrations of salt or sugar used, which lead to differences in the osmotic pressure. Further, sucrose being a larger molecule than NaCl and being a non-ionic solute, can reduce movement of water from the intracellular spaces towards the brine, which could explain the small variations in ionic strength of sugar brines over the first days compared

to the large variations in the salt brines [22,33].

After 6 to 8 days storage, all brines except 50Sc had become significantly more acidic than at the start of the storage, when the seaweed had a pH of 6.96 (Fig. 1). The fast decrease in pH suggests the formation of e.g., lactic acid or acetic acid, likely due to enzymatic degradation of cellulose by possible associated microorganism found in seaweed such as lactic acid bacteria [34,35]. There can also be displacement of H^+ in the seaweed by Na^+, leading to acidification [36]. Beyond day 8, the pH of the controls (i.e., C1 and C2), rapidly increased towards slightly acidic or neutral pH, around 5.9 and 6.5, respectively, where they remained stable for the rest of the storage time. In contrast, all sugar brines continued to acidify over time, especially in the case of 15Sc, where the pH dropped to 4.5 (Fig. 1).

Similar to the controls, the pH of salt brines decreased up to day 13; afterwards, the pH of the salt brines remained stable throughout storage. Only a slight increase was seen for the 25S brine from 5 to 6 after 20 days. The increase could be explained by mould or yeast growth which can use substrate from i.e. acidic compounds [37].

A decrease in pH, from 5.3 to 5 after 30 days, was also seen by Perry et al. [18] in dry salted brown seaweed (*Alaria esculenta*). Others have reported lack of pH changes in brines [9,19], or an increase of 0.2 units after 180 days dry salting of *Laminaria ochroleuca* [37]. In brining of other seafood such as fish, changes in pH over the storage time has been attributed to the effect of salt on the diffusion of its ions and other soluble components from the tissue towards the brine [38], for example the displacement of H+ by Na+ [36].

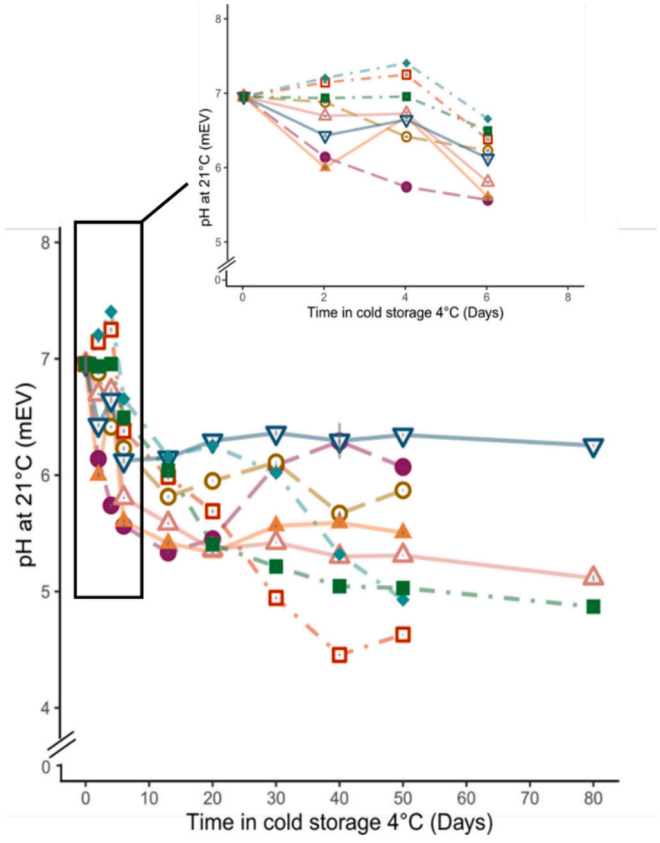

Fig. 1. pH of the different brines during storage. Water-derived brines (dotted line) ●C1, ○ C2; salt brines (full line), ▲ 5S, △15S, ▽25S; and sugar brines (long dash line), □15Sc, ◆ 25Sc, ■ 50Sc over the storage time (Days) at 4 °C. The insert refers to the first days of storage from day 0 to day 8. Data show average values ± standard deviation ($n = 2$).

3.2. Water activity (a_w) of *Ulva fenestrata* over time

The a_w of brined *U. fenestrata* reflected the concentration of salt and sugar (Fig. 2). When incubated in 5S, 15Sc and 25Sc for 6 days, the a_w was 0.95, which is similar to the a_w measured in the fresh *U. fenestrata*, 0.98. Likewise, the two controls, C1 and C2, had an a_w of 0.96 after 6 days. The small variations seen in a_w between fresh and brined *U. fenestrata* in ≤25 % sucrose, and 5 % NaCl could be due to the ionic strength being similar or lower compared to the fresh seaweed. Thus, solutes likely leaked from the seaweed to the brine rather than the opposite.

Differently, salt brines ≥15 % NaCl and the strongest sugar brine (50Sc), provided a considerable reduction in a_w (Fig. 2). For instance, *U. fenestrata* in 15S and 25S brines reached 0.91 and 0.83, already on day 2 while the 50Sc reached a significantly lower a_w compared to fresh *U. fenestrata*, 0.89 after 30 days. The lowest a_w was detected for the *U. fenestrata* in 25S brine, which reached 0.82 on day 2. Thereafter, a peak of 0.85 appeared on day 6 after which it remained at ~0.80 during the whole storage. Thus, the large difference in ionic strength between the brine and the *U. fenestrata* blades resulted in higher exchange of water and salt between these two, with a net salt uptake into the blades.

Other researchers have reported a similar effect of dry salting and brining on the a_w of seaweed over storage time. For instance, Perry et al. [18] investigated the effect of dry salting *Alaria esculenta* with 200 g and 180 g NaCl/ kg followed by cold storage. After 13 weeks, they observed a decrease in a_w from 0.99 in the fresh seaweed to 0.84 and 0.85 a_w with 200 g and 180 g NaCl, respectively [18]. Likewise, del Olmo et al. [37] investigated the effect of dry salting *Laminaria ochroleuca* with 400 g of NaCl/ kg and found that after 1 day, a_w was significantly lowered from 0.98 to 0.74 [37]. Interestingly, little research is reported on sugar brining compared to salt brining or dry salting. However, sugar has been used as an osmotic dehydration agent in fruits to prevent browning [39,40]. The a_w results of this study indicate that different ionic strengths are provided by sugar and salt brines, and that salt has the capability to penetrate inside the cell walls. Sugar brines thus require higher sucrose concentration to reduce a_w to similar values as those displayed by the salt brines.

Bacillus cereus, Bacillus subtilis, Bacillus pumilus, Listeria

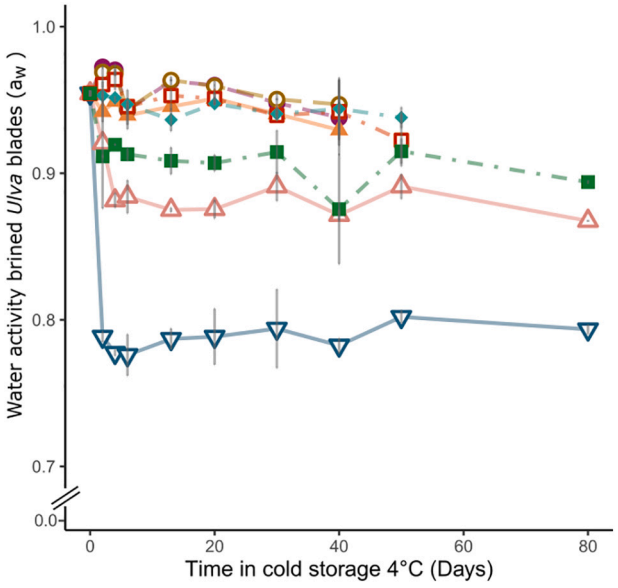

Fig. 2. The water activity of *U. fenestrata* brined in water (dotted line) ●C1, ○ C2; salt brines (full line), ▲ 5S, △15S, ▽25S; and sugar brines (long dash line), □15Sc, ◆ 25Sc, ■ 50Sc over the storage time (Days) at 4 °C. Data show average ± standard deviation ($n = 2$).

monocytogenes, Staphylococcus aureus, Salmonella and *Vibrio parahaemolyticus* have all been reported as relevant pathogenic bacteria found in seaweed [41]. The minimum a_w for these bacteria to grow ranges between 0.83 and 0.97; thus, only the 25S brine could fully inhibit the growth of pathogenic bacteria in Nordic cultivated seaweed [41]. The 15S and 50Sc brines, which reduced the a_w of *U. fenestrata* to ≤0.91, could not prevent all pathogens, but likely *Salmonella*, *Bacillus cereus* and *Vibrio parahaemolyticus*, all three has been suggested as most concerning for human consumption [38].

3.3. Microbial quality

In the two controls, i.e., C1 and C2, the microbial growth increased significantly over the storage trial in all three medias; PCA, LH and MA, the two latter comprising psychrotrophic bacteria and marine bacteria, respectively (Table 1). This was likely related to the low salt content of these samples (~2 %), which were ideal conditions for previously reported microorganism found in seaweed i.e., *Salmonella* or *Bacillus* spp. to thrive [41].

For sugar-brined *U. fenestrata*, the microbial count over time was 10-fold higher than that for salt-brined *U. fenestrata* of the same strength. Particularly the 15Sc and 25Sc brines gave rise to high counts of psychrotrophic bacteria (Table 1). *U. fenestrata* in 15Sc brine enumerated psychrotrophic bacteria of 6 log (CFU/g) after 13 days and > 300 log (CFU/g) at day 20. *U. fenestrata* in 25Sc presented a similar value on day 13, 6.1 log (CFU/g), after which there was no increase until day 34. After 48 days, values of 6.6 log (CFU/g) were reached. Meanwhile, the 50Sc brine gave rise to the lowest growth log (CFU/g) among the sugar brines for both marine and psychrotrophic microbial counts, and there was even a reduction in growth over time (Table 1). This decline in microbial count could be explained by the reduced a_w of the *U. fenestrata* in 50Sc brine (from 0.96 at start to 0.84 during the storage). Interestingly, based on isolation and gram staining of different colonies, the sugar-brined *U. fenestrata* had a predominant presence of yeast compared to the salt-brined *U. fenestrata*. The gram staining also revealed a mixed culture of gram positive and gram negative strains (Data not shown).

Table 1
Microbial load log colony forming units per gram of brined *U. fenestrata* (CFU/g) ± standard deviation (n = 6), at different days over storage at 4 °C in three different media: Long hammer (LH), marina agar (MA) and plate count agar (PCA).

Brine	Day	log (CFU/g) in incubation media		
		PCA	MA	LH
Fresh	0	7.57 ± 0.00	5.36 ± 0.00	6.20 ± 0.00
C1	13	6.58 ± 0.27	<25*	6.75 ± 0.04
	20	>300*	6.59 ± 0.03	7.75 ± 0.02*
	34	>300*	>300*	>300*
C2	13	6.59 ± 0.25	6.58 ± 0.27	6.40 ± 0.08
	20	>300*	>300 *	8.61 ± 0.00*
	34	>300*	>300 *	>300 *
5S	13	<25*	5.97 ± 0.00	<25*
	20	5.09 ± 0.05*	5.22 ± 0.00	<25*
	34	5.53 ± 0.11*	6.41 ± 0.05*	<25*
	48	6.29 ± 0.06*	6.59 ± 0.08*	<25*
15S	13	5.09 ± 0.73*	<25*± 0.00*	0.00 ± 0.00*
	20	<25*	<25*	<25*
	34	<25*	5.21 ± 0.11*	0.00 ± 0.00*
	48	<25*	<25*	0.00 ± 0.00*
25S	13	<25*	5.30 ± 0.18	0.00 ± 0.00*
	20	<25*	<25*	<25**
	34	<25*	5.22 ± 0.00	0.00 ± 0.00*
	48	0.00 ± 0.00*	<25 *	0.00 ± 0.00*
15Sc	13	>300*	6.03 ± 0.07	>300*
	20	>300*	>300*	>300*
	34	>300*	>300*	>300*
	48	>300*	>300*	>300*
25Sc	13	<25*	6.13 ± 0.08*	6.40 ± 0.17
	20	6.16 ± 0.07	6.13 ± 0.03*	6.53 ± 0.05
	34	<25*	6.21 ± 0.06*	6.47 ± 0.06
	48	6.41 ± 0.05	6.49 ± 0.09*	6.58 ± 0.04
50Sc	13	<25*	<25*	<25*
	20	<25*	<25*	<25*
	34	<25*	0.00 ± 0.00*	0.00 ± 0.00*
	48	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*

* Indicate a significant difference compared to fresh *U. fenestrata* values, with a p-value of 0.05. Results indicated as "<25" indicates growth but below 25 colonies, below the limit of quantification. While ">300" indicates that growth was above 300 colonies. When the value is "0.00 ", no growth was detected in the media.

U. fenestrata stored in salt brines exhibited a difference in the microbial count depending on the salt content of the brine. Only 25S showed a decrease in the colonies counted over the storage time in all three media (Table 1). In the case of 15S, slow, although significant, growth of microorganisms was seen for marine bacteria using MA and total count PCA agar (Table 1). *U. fenestrata* in this brine had an a_w of 0.86 after 4 days, and the pH of the brine decreased from 6 to 5 over time. The latent phase of bacterial growth could thus be due to the a_w, pH and salt content, separately or combined [42].

Using 5 % NaCl, a significant increase in microorganisms was detected over time using PCA media (Table 1). Similarly, using MA media, a significant increase in log (CFU/g) was detected over time, with values of 6.6 log (CFU/g) after 48 days. However, the growth of psychrotrophic bacteria on LH media was significantly reduced during storage and reached below the detection limit of <25 (CFU/g). These results align with earlier findings that low concentration of salts does not limit growth of e.g. *Salmonella*, *S. aureus* and *Vibrio* at 25 °C [17,41,43]. In the above mentioned study of dry salted kombu by del Olmo et al. [43], where 400 g NaCl was used per kg seaweed, results also revealed a reduction of microbial growth on MA during storage at 4 °C. Differently, Perry et al. [18] studied the effect of dry salting kelp and did not see a significant reduction in the microbial load over the storage time at 4 °C. However, the initial load of aerobic bacteria reported by Perry et al. [18] was only half the amount found for fresh *U. fenestrata* in the present study, which could be a reason for different responses to salt.

In the European Union, no specific regulation determines the maximum allowed microbial load in seaweeds, except for France, which has established a maximum of 10^5 (CFU/g) for aerobic mesophilic bacteria in dried seaweed. Thus, this threshold is not applicable to the wet/brined biomass used in this study. There are different thresholds reported for when fresh fish is considered unacceptable for consumption; numbers of aerobic place count (APC) from $>10^5$ to $>10^7$ can be found [44]. However, a firm threshold has not yet been established for fresh seaweed biomass.

A recent study on stability of washed and blanched *Saccharina latissima* suggested using 7 to 7.7 log (CFU/g) as a microbial limit based on MA to determine the sensory shelf life under cold storage (4 °C) [45]. This threshold has also been used in previous studies on cold storage of minimally treated vegetables [46].

Taking into consideration 7 log (CFU/g) as a limit, the resulting shelf life of both controls, i.e., C1 and C2, as well as *U. fenestrata* brined in 15 % sugar was limited to 13 days, based on the values obtained for psychrotrophic and marine microbial counts, incubated at 4 °C and 25 °C, respectively. As stated above, gram staining indicated that the short shelf life of sugar-brined *U. fenestrata* was possibly due to marine yeast, using sugar as a substrate. Previous research suggests the survival of marine yeast at an a_w of 0.93 [17], which agrees with the water activity of the 15Sc brine at day 13 (0.95). In addition, the pH this brine was 6.5, a value at which marine yeast has been reported to proliferate [47].

The more concentrated sugar-brines 25Sc and 50Sc gave a shelf life of 48 days based on MA. On day 48, *U. fenestrata* in 25Sc brine reached values of 6.5 and 6.6 log (CFU/g) for marine microbial count and psychrotrophic bacteria, respectively, and thus were expected to exceed the limit of 7 log (CFU/g) shortly after. In a repeated experiment, *U. fenestrata* in 25Sc showed for both marine counts and psychotropic bacteria >7 log (CFU/g) already after 20 days. In the same experiment

the 50Sc reached this limit after 50 days (Supplementary data, Table A).

The seaweed brined in 5S, 15S, 25S had a shelf life of ≥48 days based on the growth of marine and psychotropic microorganisms (Table 1).

Overall, the results thus indicate that highly concentrated sugar-brines (25–50 %), and salt brines between 5 and 25 % extended shelf-life of *U. fenestrata* from 13 days to ≥48 days. A repeated trial done by the authors on *U. fenestrata* brined with 15S and 25S, did not show any microbial counts until day 78 (Supplementary data, Table A). Thus, the shelf-life for both 15S and 25S was in this trial at least 78 days.

3.4. Colour changes of brined Ulva fenestrata

The brining process was expected to also affect leakage of pigments to the brine or degradation of pigments e.g., via oxidation [48]. The three dimensions of colour, L*, b* and a*, of the brined *U. fenestrata* over the storage are presented in Fig. 3A, B and C, respectively, while the total colour difference delta E in Fig. 3D. Images of the brined *U. fenestrata* were also taken during the storage and can be found in Supplementary - Fig. D.

For *U. fenestrata* in all brines, the L* values significantly increased throughout the storage compared to fresh seaweed (Fig. 3A). For sugar-brined *U. fenestrata*, the increase in lightness could be due to the formation of a surface layer of sugar on the seaweed blades when the seaweed was taken out of the brine for measurements. This has also been seen previously during osmotic dehydration of fruits using sugar brine [48]. In the case of the salt brines, a likely explanation could be that residual crystals from salt, that have not been diffused into the seaweed, can reflect the light, a phenomenon described by Del Olmo et al. [37] for dry salted Kombu.

As shown in Fig. 3B, the *U. fenestrata* brined in 15Sc, 25Sc, and 5S attained significantly higher b*-values than the fresh seaweed already after 2 days, i.e., it was more yellow. The same was true for both the C1 and the C2-controls, although after 2 days, they re-gained similar b*-value as the fresh *U. fenestrata*. In 5S brine, yellowness increased

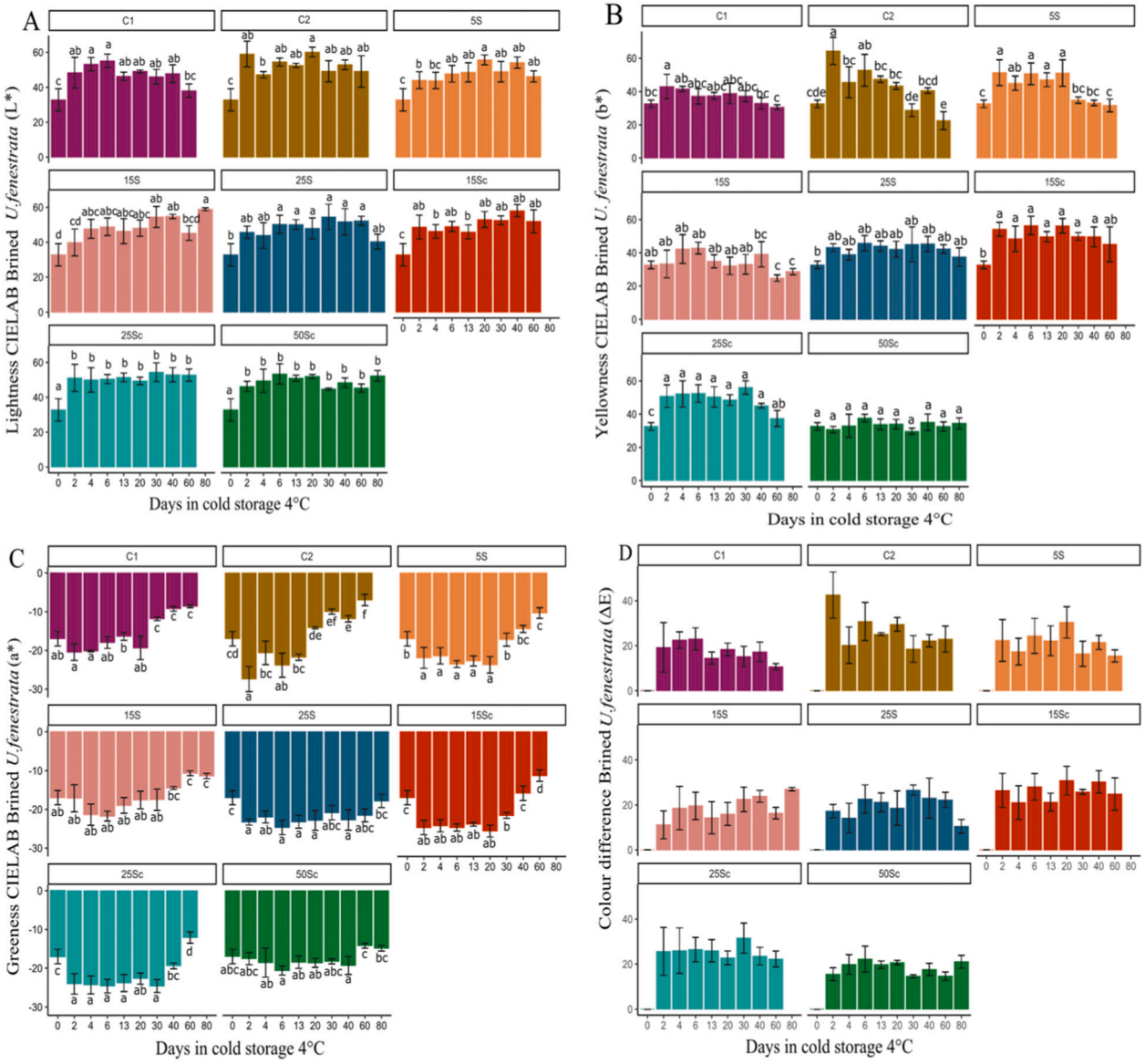

Fig. 3. Colour differences of the brined *U. fenestrata* in different brine solutions over the storage time. A. (L*) Lightness values for the different brines (L* 0-black to L* 100-white). B. b* values for the different brines (+b*) associated with yellow while (−b*) indicates blue tones. C. a* values for the brines, (+a*) indicates red tones while (−a*) indicates green tones. D. Delta E*, colour difference of the different brined *U. fenestrata*. Data show average values ± standard deviation ($n = 4$). Letters indicate a significant difference of each brine compared to the fresh (Day 0). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significantly from day 2 until 20 days while for the 15Sc- and 25Sc-brines, significant increases continued until day 40. Differently, the *U. fenestrata* kept in 50Sc, 25S brines had a stable b*-value of around 45 over the entire storage time. The changes in yellowness of all samples except 50Sc and 25S could be due to the loss of chlorophyll and the persistence of yellow carotenoids within the cell walls of *U. fenestrata* [9].

In the case of green seaweeds such as *Ulva* spp. the green colour is considered a quality parameter and indicates freshness [49]. However, as recently shown by Stedt et al. [50] for *U. fenestrata*, green colour, i.e., presence of chlorophyll, also correlates positively with the protein content. The fresh *U. fenestrata* used in this study had a mean a*-value of −17 (Fig. 3C), which is within the range found by Stedt et al. [50]. Within 30 days, the *U. fenestrata* in the control brines C1 and C2 showed a significant decrease in their green tones raising a*-values to −11.4 and − 10, respectively. A delay in the greenness loss was seen for the brines with low sugar content, 15Sc and 25Sc, which did not give rise to significant increases in a*-value until after 60 days, reaching −11.3 and − 12.1, respectively. Similarly, the salt brines 5S and 15S obtained significantly higher a* values, −14.4 and − 14.5, respectively, after 40 days.

Although most brines lead to a loss of greenness over time, *U. fenestrata* brined in 25S and 50Sc exhibited stable a*-values over the entire storage period. This could be due to inhibition of the chlorophyll degrading enzyme chlorophyllase. In case of 25S, even higher greenness was seen during parts of the storage, compared to the fresh samples, specifically between day 2 and 60. A reason could be osmotic effects up-concentrating the thylakoids where chlorophyll is located [51].

The significant loss of greenness for *U. fenestrata* brined in 15S could be due to the degradation or leakage of chlorophyll to the brine. For example, magnesium can be lost from the porphyrin ring at pH < 6 [52], yielding brownish colours. The 15S presented pH < 6 until day 30 of storage. Chlorophyll can also be subjected to oxidation, which disrupts the conjugated double bonds and thus, causes bleaching. Another route to the loss of the bright green colour is enzymatic release of the phytol group by chlorophyllase [53]. Previous studies also reported on a loss of greenness when brining *Ulva rigida* in 25 % salt-brine [9]. These authors further saw a higher degradation of chlorophyll with higher water content, which in our study could be translated to a higher greenness loss in samples with $a_w > 0.85$, i.e., controls as well as *U. fenestrata* in the 15Sc, 25Sc, 5S, and 15S brines. *U. fenestrata* in sugar brine with the highest concentration, 50Sc, was an exception from this logics as it presented a stable green colour. Further, retention of phycoerythrin and phycocyanin pigments was seen in the red seaweed *Kappaphycus alvarezii* after being submerged in a sucrose solution of 50 °Bx at 40 °C. Authors stated that water loss induced by osmotic pressure led to pigment up-concentration [21].

The total colour changes (ΔE*) over time for the differently brined *U. fenestrata* compared to fresh *U. fenestrata* (Fig. 3D) varied the most for the controls and the low concentration brines (5-15S and 15-25Sc), while 50Sc and 25S, showed significant colour difference over time. Reasons for this could be the mentioned decrease in a*-values found for *U. fenestrata* in 25S brine over time, and the increase in L* that both brines presented over the storage.

Others who have studied the effect of brining *Ulva rigida* with 25 % salt revealed an increase in a* values from −15.4 a* to −10.4 a* after 180 days at 4 °C, i.e., a loss of greeness [9]. A major discrepancy to our study was however that the authors kept the seaweed for only 10 min in the brine, whereafter it was stored at 4 °C. This indicates that dipping in saturated salt solutions is not enough to protect chlorophyll from degradation. The same study also reported an increase in L*-values from 48.78 to 54.5 after 180 days.

Further, when the effect of dry salting on the colour of brown seaweed, kelp (*Alaria esculenta*) was studied over time at 4 °C [18], the authors disclosed that a* values were inversely correlated to the salt concentration. This agreed with the present study, where higher salt concentration in brines for *U. fenestrata* stabilised its green colour during cold storage.

Likewise, kombu (*Laminaria ochroleuca),* dry salted with 400 g NaCl per kg biomass presented no changes in a*- values compared to fresh kombu after 180 days at 4 °C [37]. b*-values, on the other hand, decreased over storage time when salted, the decrease on yellowness was reported for both kelp and kombu, which according to the authors reflect a loss of the yellow pigment fucoxanthin from the kombu. Similar to our study, lightness (L*) for the dry salted kombu also increased significantly over time, which was ascribed to salt crystals that were unable to diffuse into the seaweed.

3.5. Tensile strength

During the storage, the *U. fenestrata* in water (C1 and C2), a significant loss of the blade strength was seen after 6 days (Fig. 4). At this time point, *U. fenestrata* in the salt and sugar brines did not present any significant losses of the tensile strength. However, beyond 6 days, storage-induced changes in the tensile strength of the brined *U. fenestrata* blades were detected depending on the type and concentration of brine.

During the first 6 days, *U. fenestrata* in 15Sc sugar-brine showed the highest strength among the three sugar brines tested, which was similar to fresh *U. fenestrata* (Fig. 4). However, after 20 days, the *U. fenestrata* brined in 15Sc, had lost 50 % of the tensile strength it displayed on day 6. The 25Sc brined *U. fenestrata* had higher tensile strength during early storage compared to 15Sc, but it lost its strength at a slower rate, and not until day 30 it showed a 50 % loss. During extended storage, i.e., >30 days, *U. fenestrata* both in 15Sc and 25Sc were subjected to further significant losses in tensile strength. The retention of tensile strength during storage was highest in 50Sc-brined *U. fenestrata.* Although significantly lower than fresh seaweed, it had only lost 50 % on day 80, compared to 100 % and 75 % in 15Sc and 25Sc. A similar relation between sucrose concentration and maintained blade strength was reported by Lee et al. [21] in *Kappaphycus alvareii* after submerging in 50 °Bx and 70 °Bx sugar solutions. With decreased sugar saturation, they reported a loss of texture of the seaweed.

The *U. fenestrata* in salt brines decreased its tensile strength significantly over time when the salt concentration in the brine was ≤15 % (Fig. 4). After 80 days of storage, the highest tensile strength was seen for the blades stored in 25S brine, followed by the 15S brine. At that time point, the 5S brined *U. fenestrata* was too fragile to allow measurements

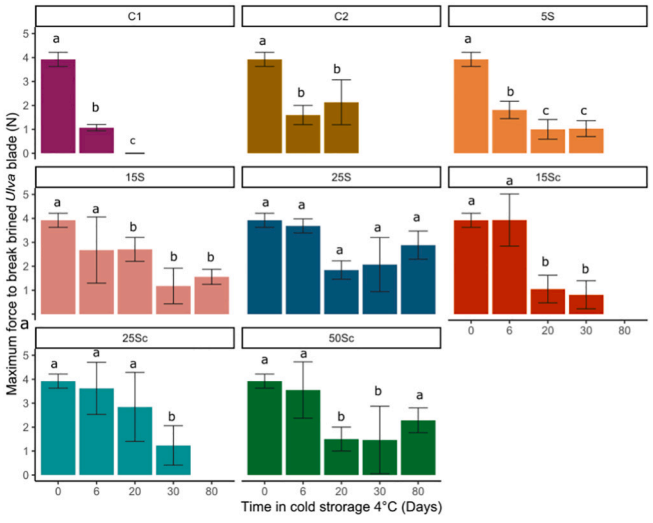

Fig. 4. Total force needed, measured in Newtons (N), to break the blade of brined *U. fenestrata* over the cold storage time at 4 °C over days. The small letters refer to each graph individually. Different letters indicate significands (*p*-value <0.05) compared to fresh (Day 0).

of the tensile strength.

The high variation in tensile strength seen at all time points could be due to variations among individual blades sampled from each brine. Seaweed is not a homogenous product, and in each batch, there were variations in thickness and length of the whole *U. fenestrata* blades, which created differences in the tensile strength [25].

Previous research on texture changes of dry salted kombu used compression tests rather than tensile strength tests as used here [37]. Similar results were, however, retrieved in the sense that the dry salted kombu was subjected to softening of the texture after 60 days at 4 °C, as was seen here for *U. fenestrata* in the 15S and 5S brines [37]. The explanation given was caused by the action of enzymatic hydrolysis of cell wall components.

Other studies on brined seaweed have found an increase in firmness over storage as measured by the force needed to penetrate the seaweed from a downward probe [9,18]. Pinheiro et al. [9] focused on *Ulva rigida* brined in 25 % NaCl for 5 min or dry salted at 28 and 48 % (*w*/w) where after it was stored at 5 °C [9]. Both the brined and the dry salted *Ulva rigida* became firmer during the 180 days of storage [9]. Similar results were seen by Perry et al. [18] who studied the effect of dry salting on the storage stability of *Alaria esculenta* at 4 °C [18]. Explanations given in these studies were based on previous research on osmotic dehydrated fruits, in which they refer to softening of the tissue during osmotic treatment of strawberry [54–56]. Intact pectin might also be solubilised into the brine due to disruption of other components in the cell walls. According to this principle, e.g. ulvan, which makes up 9–36 % of *Ulva* spp. dry weight [8] could be degraded by ulvan lyase, softening the tissue during brining.

The effect of e.g., osmotic pressure on cell walls can differ depending on the time period the tissue is submerged into a hypotonic or hypertonic solution. The present study kept the seaweed in the brine over the entire storage period, while the study of Pinheiro et al. [9] removed the seaweed from the brine after a short time period (5 min), or applied dry salting. The latter, however, also forms a brine with time when water is released from the seaweed tissue. Extended exposure time to elevated osmotic pressure caused by sucrose and salt can increase swelling or shrinkage of the cell walls, which has been reported for berries and vegetables such as strawberry tissue [54], cabbage [57] and eggplants [58].

The difference in textural properties of seaweed in sugar brine compared to salt brine could be related to the capacity of salt to promote interaction between ions and carboxyl groups from components of the cell wall, increasing the blades' texture and strength [33]. Sucrose, on the other hand, does not interact with e.g., pectin or cellulose carboxyl groups as strongly as salt [33].

Table 2
Moisture and ash percentage of brined *U. fenestrata* on Day 0 and after 40 days in 4 °C storage in brine. Data are shown as mean value ± SD ($n = 3$).

Sample	Moisture%		Ash%	
	Day 0	Day 40	Day 0	Day 40
Fresh Ulva	88.14 ± 0.89[a]	–	25.78 ± 0.31[a]	–
C1		98.53 ± 0.90[b]		12.71 ± 0.81[b]
C2		94.00 ± 0.99[c]		11.72 ± 0.63[b]
5S		92.07 ± 0.45[c]		38.29 ± 1.45[c]
15S		81.93 ± 0.74[d]		54.89 ± 0.42[d]
25S		78.73 ± 1.15[e]		72.12 ± 0.95[e]
15Sc		89.14 ± 0.20[a]		4.16 ± 0.07[f]
25Sc		78.04 ± 0.26[e]		2.38 ± 0.15[fg]
50Sc		60.82 ± 0.08[f]		1.25 ± 0.12[f]

[a]Mean value with a different alphabet indicates significant differences (p-value <0.05).

3.6. Proximate composition

3.6.1. Ash and moisture

The moisture content of the brined *U. fenestrata* was monitored throughout the storage (Table 2). The results indicated that incubation in water alone significantly increased the moisture content of the controls, C1 and C2, after 40 days at 4 °C. Likewise, incubation in low concentration salt brines, 5S increased significantly the moisture from 88.14 % of fresh seaweed to 92.07. At higher concentrations, salt and sugar affected the moisture content equally after 40 days, 25S and 25Sc had a 79.7 and 78 % moister, respectively. The lowest moister content was found for *U. fenestrata* in 50Sc brine, reaching 60.8 % after 40 days. Decreases in moisture content over time with sugar and salt brines of increasing strength has previously been reported for *Caulera lentillifera* [19] and *Kappaphycus alvarezii* [21], respectively.

The brining process significantly affected the ash content of all samples (Table 2). The two controls, C1 and C2, presented a 50 % and 55 % loss of inorganic material after 40 days compared to fresh biomass, respectively. Higher ash loss was found for *U. fenestrata* stored in sugar than salt brines, and the loss was enhanced at higher sugar concentrations. *U. fenestrata* n 50Sc brine only had 1.3 % ash after 40 days compared to 25.8 % in the fresh *U. fenestrata*. The higher loss of ash in *U. fenestrata* brined with sugar is likely due to the lower ash content of sucrose compared to NaCl.

The salt-brined *U. fenestrata*, contrary to controls and *U. fenestrata* in sugar brines, showed increased ash content with increased NaCl concentration and increased storage time. *U. fenestrata* in 5S, 15S and 25S obtained 0.5, 1.1 and 1.5 times higher ash content on day 40 compared to fresh seaweed, respectively. These results, together with the loss of moisture indicates that there was a migration of salt from brines to the *U. fenestrata* biomass and the opposite was true for water.

Similar increases in ash content with increasing salt concentration have been described for *Caulera lentillifera* stored in salt brine for 10 days [19], and for dry salted *Alaria esculenta* [18].

3.7. Protein content

Fresh *U. fenestrata* had a protein content of 17 % dw, which after storage in water (i.e., C1 and C2), was significantly up-concentrated (Fig. 5). After 20 days, C1 and C2 had a protein content of 19.6 % and 19.2 % dw *U. fenestrata*, respectively. This phenomenon was also earlier found by Harrysson et al., [12] when rinsing *U. fenestrata* in fresh water prior to drying, and it is explained by the leaching of minerals into the water, reducing the ash of the biomass. As discussed above, the ash content was reduced to half for both of the controls C1 and C2 after 40 days.

Contrary to storage in water, the sugar brines 15Sc and 50Sc, gave rise to significant decreases in protein content (41.8 % and 58 %, respectively) after 20 days (Fig. 5). After 40 days, the loss was as high as 62 % and 79 %, respectively. This was likely due to the osmotic pressure stimulating water uptake and water loss, respectively (Table 2), the latter which could imply losses of soluble proteins. Further, it is possible that there was an increase in proteolytic enzyme activity, which can be enhanced by the presence of sugar [59]. The more soluble peptides or free amino acids could then leak out to the brine.

U. fenestrata in the 25Sc brine behaved differently in the sense that at day 20, it had similar protein content as the fresh seaweed (16.4 % dw, vs 16.5 % dw) (Fig. 5). This could be due to this brine having a similar ionic strength to the *U. fenestrata* biomass (5 % NaCl equivalents vs 7 % NaCl equivalents in the fresh *U. fenestrata*), resulting in minimal exchange of water/sucrose within that period, and thus, minimal leakage of soluble proteins to the brine.

Compared to the sugar-brined *U. fenestrata*, salt brines with low concentrations of NaCl (5S and 15S) reduced and delayed *U. fenestrata 's* protein loss during storage. After 40 days, the protein content significantly decreased to 14.4 % and 9.9 % dw, respectively. For the most

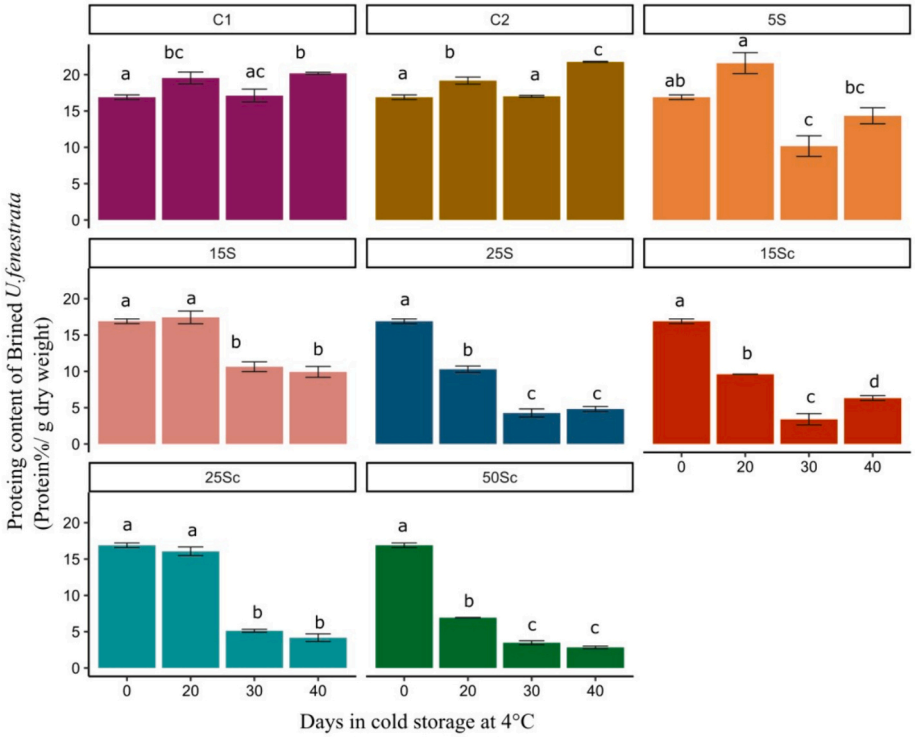

Fig. 5. Protein content as a percentage on dw-basis of brined *U. fenestrata* over the storage time (0–40 days). Lower case letters indicate significance between fresh Ulva and timepoints for each brine separately, and significance was set at p-value <0.05.

concentrated brine, 25S, a protein loss of 37 % was detected already at day 20, which increased to 71 % loss after 40 days, based on the initial protein content of fresh *U. fenestrata*.

Our results differed from the previously reported study of *Ulva rigida* brined in 25 % salt for 5 min and then stored at 4 °C for 180 days [9]. The authors did not find a significant difference in protein content after completed storage compared to fresh seaweed, which likely is ascribed to the very short submerging in the saturated salt brine.

Brining of fish products, e.g., herring, also leads to a loss of proteinaceous material during maturation at 4 °C [44]. Previous studies on the brine composition after maturation of herring in e.g., 25 % NaCl, have reported that the loss of proteinaceous material increases with time and is dependent both on the brine composition, i.e. salt level, acids and species [60]. In another study, a higher nitrogen loss from herring was reported with more concentrated salt brines in the range from 5 to 15 % salt [61]. The loss of nitrogen from herring can be due to soluble proteins leaking into the brine because of a salting-in effect. Further, it is well known that proteolysis proceeds during herring maturation, rendering the tissue softer as peptides and free amino acids are formed [62]; both which can leak to the brine [63].

Reduced levels of crude proteins during brining of *U. fenestrata* in the present study, could thus be explained by three parallel phenomena; (i) loss of soluble protein into the different brines, (ii) loss of peptides into the brine as a result of proteolytic reactions within the *U. fenestrata* during brining, and (iii), uptake of NaCl and sucrose into the *U. fenestrata*, changing the mass balance and thereby diluting the protein. In (i) reasons could be salt-induced solubilization of membrane protein or leakage from the cytoplasm caused by osmotic pressure. These actions could be facilitated by the movement of water from the seaweed to the brine [64]. To confirm (i) and (ii) the brines formed after 13 days were analysed with the Lowry method [65,66] to determine soluble proteins and peptides (see Supplementary data, Fig. C). Protein/peptide concentrations after 13 days in 4 °C were significantly different ($p < 0.05$), Highest were C1 and 15S, followed by 15Sc, C2, 25S, 25Sc, 50Sc and last 5S. There was an increase in soluble proteins in the brine over time for all brines except for 5S. After 20 days, all brines presented a decrease in soluble protein/peptides (Supplementary data, Fig. C) which could be due to proteolysis in the brine, forming free amino acids which are not detectable with the Lowry method.

3.8. Fatty acid content and composition

In this study, the starting *U. fenestrata* biomass only contained 13.8 mg total fatty acids on a dw basis (Fig. 6). When kept in water, i.e., C1 and C2, the total fatty acid content increased over time, similar to what was seen for crude protein. The predominant increase was seen for the

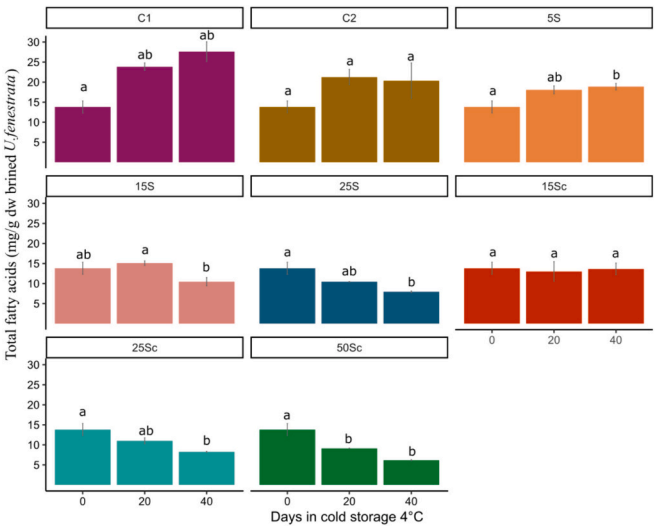

Fig. 6. Total fatty acid content (mg/g dw) of the differently brined *U. fenestrata* over the storage time (0–40 days). Different lowercase letters indicate significance (p-value <0.05) between time points and Fresh *Ulva* (Day 0) within the same brine individually.

U. fenestrata in deionised water (C1) in which the fatty acid content rose from 13.8 mg/g dw, fresh *U. fenestrata* to 27.6 mg/g dw after 40 days. At the same time point, *U. fenestrata* in tap water (C2) presented 20.3 mg/g dw. As discussed in the protein section (3.7), the dilution of minerals into the water reduces the ash content of *U. fenestrata* (Table 2), thereby up-concentrating macronutrients as proteins and lipids [12].

The *U. fenestrata* brined in either sugar or salt, decreased its fatty acid content over time, a phenomenon that increased with increases in the brine concentration (Fig. 6). An exception was the 15Sc brine where the fatty acid content was preserved. The highest loss of fatty acids (55 %) was seen in the 50Sc brine after 40 days. At this time point, the 50Sc brined *U. fenestrata* had 6.2 mg fatty acids/g dw compared to fresh *U. fenestrata,* which had 13.8 mg/g dw. The difference between 15Sc and 50Sc could be due to lower diffusion of aqueous molecules from *U. fenestrata* to the brine in 15Sc compared to 50Sc, translating into a lower exchange of components i.e. trace metals with a pro-oxidant activity, similarly to the reported effect of salt in brine fish [67].

U. fenestrata stored in salt brines responded similarly to those in 50Sc and 25Sc brines. The *U. fenestrata* preserved in 25S had lost 42 % of the fatty acids after 40 days, from 13.8 mg/g dw in the fresh biomass to 7.9 mg/ g dw (Fig. 6). *U. fenestrata* kept in 5S, however, just as the controls, significantly increased its fatty acid content over time, reaching 18.9 mg/g dw after 40 days in the brine. As this brine was relatively isotonic to the seaweed (5 % NaCl vs 7 % NaCl equivalents), this could reflect a slight leach out of carbohydrates as has seen for brined *Ulva rigida* [9].

A previous study from Tolentino and Sorio [19], reported a similar trends to ours based on measurements of crude lipid content on a dw basis when storing *Caulerpa lentillifera* in different salt brines over 10 days at 4 °C. Specifically, they saw a decrease in crude lipids after storage in 10 % and 15 % NaCl brine while there was an increase in crude lipids, when 5 % salt-brine was applied [19]. The authors explained this by 5 % salt being isotonic to sea water, therefore, being in equilibrium with *Caulerpa lentillifera.* The lipid loss in 10 % and 15 % salt-brine was explained by the increased osmotic pressure having high effect on the ramuli, in turn inducing biomass shrinkage.

Apart from changes in mass balances, i.e., uptake/decreases of NaCl or sucrose, oxidation of fatty acids likely also contributed to their losses in some of the salt and sugar brines over time. The concentration of 3–5 % NaCl has been found to be a pro-oxidant for fish lipids according to different mechanisms, e.g. salt can increase disruption of cells, facilitating the access for pro-oxidants to the lipids found in the muscle. Further, the salt used could contain pro-oxidative trace metals such as iron or copper [67]. For sucrose, to our knowledge there are no earlier studies on its pro-oxidative ability.

The fatty acid composition of the fresh and differently brined *U. fenestrata* is shown in Table 3. In total, the authors were able to identify sixteen different fatty acids, five saturated fatty acids (SFA), four monounsaturated fatty acids (MUFA) and seven PUFAs. The fresh *U. fenestrata* contained 5.14, 2.2, and 6.5 mg/g dw SFA, MUFA and PUFA, respectively (Table 3). Among total PUFAs, 6.7, 13.4 and 7 % were the n-3 fatty acids C16:3n-3, C18:3n-3 and C20:5n-3, respectively, and among the n-6 fatty acids, 36.3 and 24.1 % were the fatty acids C18:2n-6 and C18:3n-6. A relative dominance of PUFAs, followed by SFAs and then MUFAs, was also earlier reported from dried *Ulva rigida* [11].

The fatty acid content and profiles found in this study also indicates that C16:0, palmitic acid, and C18:2n6, linoleic acid were the major fatty acids for all samples, which was maintained throughout the storage. Palmitic acid was also reported earlier to dominate the fatty acid profile of brined [9] and dried [11,12] *Ulva* spp. For instance, previous studies of dried *U. fenestrata* [11,12] reported 17 % and 13 % of total fatty acids to be C18:2n-6 while in our study it was 17 %.

Overall, losses of fatty acids in salt and sugar brines were primarily explained by losses of PUFAs. As an example, for 50Sc, which showed the largest fatty acids losses; the relative loss of SFAs, MUFAs and PUFAs over 40 days was 54.0, 32.8 and 63.7, respectively. Among the individual PUFAs, particularly large losses were seen for C16:3n-3, C18:3n-3. Regarding C20:5n-3, i.e. eicosapentaenoic acid (EPA), no significant losses were seen in the two controls, 5S and 15Sc brines while there were significant losses after already 20 days in the 15S, 25S and 25Sc and 50Sc brines. The initial levels of EPA were lower than others have found in *U. fenestrata*, here 0.5 mg/g dw compared to 1.1 mg/ g dw found by Harrysson et al. [12]. This can be due to different harvesting times; Harrysson et al. [12] collected the *U. fenestrata* in October, while the seaweed used for this study was collected in June.

The selective loss of PUFAs during storage could be due to their high susceptibility to oxidation, their higher polarity compared to SFAs [68], and/or to their specific location within the *U. fenestrata* tissue, for instance in plasmids or lipid bodies [69]. Significant losses of fatty acids, specifically PUFAs, were also seen in dried *U. fenestrata* (+/− presoaking in fresh water before drying) over storage at room temperature with and without light [12]. In that study, lipid oxidation was confirmed in terms of increases in malondialdehyde (MDA) and 4-hydroxyhexenal (HHE).

For the two controls C1 and C2, as well as for 5S, PUFAs remained the major contributor to the total fatty acids throughout the entire storage, while at high salt and sugar concentrations there were selective losses of PUFAs. In case of large intake of *U. fenestrata*, a selective loss of PUFA indeed has a nutritional consequence.

4. Conclusions

The results from this study showed that the 25S and 50Sc brines were most promising for preservation of *U. fenestrata* at 4 °C since they i) maintained microbial counts below the shelf-life threshold of 7 log (CFU/g) for an extended time, especially 25S which provided a shelf-life of 78 days ii) kept the green colour, and for 25S, iii) prevented significant losses in tensile strength over a period of 30 days. Drawbacks for all these concentrated brines were the documented losses of nutrients, and for the 50Sc, also losses in texture properties throughout the storage. The nutrient losses were higher for the 50Sc brine than for 25S. Altogether, the results thus indicate that the 25 % NaCl brine could best stabilise *U. fenestrata* from a safety aspect, while also preserving colour and texture over storage for up to 80 days. Under these conditions, however, 70 % of the proteins were lost, and 42 % and 57 % of the total fatty acids and PUFA, respectively.

An interesting simultaneous finding was that the biomasses stored in water (i.e. controls) or in salt and sugar brines of lower concentrations (5S and 15Sc), obtained increased protein and fatty acids content. However, to take advantage of such up-concentration, short incubation periods in the mentioned solutions prior to other forms of preservation could be recommended as the samples per se showed high microbial growth and large losses of colour and texture over time.

Further research on the microbial communities in *U. fenestrata* using 16S RNA or full genome sequencing could help understanding which microorganisms that best dictates safety of differently brined *U. fenestrata*. Also, the dynamics of polysaccharide content in both biomass and brines during storage of *U. fenestrata* is highly relevant, not least based on its content of ulvan. The sensory aspects were not explored, but further research on the impact of brining on the sensory attributes would be relevant to understand future applicabilities of this new product in the food industry. Finally, potential post-brining treatments of *U. fenestrata* prior to its inclusion in different food products could be an interesting continuation of this study. For e.g., salted cod and herring, it is common to apply a leaching step in fresh water prior to use in order to reduce the salt content and thereby improve sensory properties. If salt- or sugar-brined *U. fenestrata* is to be included at a high level in foods, such treatments could be expected. If only added as a taste enhancer in small quantities, the presence of salt or sugar could, however, be advantageous. The sugar-brined *U. fenestrata* would be a new product on the market, which could have possibilities e.g., in bakeries and desserts, but also in regular cooking. This study has thus opened up

Table 3
Fatty acid content in mg/g dry weight (dw) of brined *U. fenestrata* during cold storage (4 °C) at different time points (0, 20 and 40 days). Polyunsaturated fatty acids (PUFA), Monosaturated fatty acids (MUFA), Saturated fatty acids (SFA), n-3 (omega-3) PUFA and n-6 PUFA.

Fatty acids mg/g of Ulva in dw	Fresh	C1		C2		5S		15S		25S		15Sc		25Sc		50Sc	
	Start	20	40	20	40	20	40	20	40	20	40	20	40	20	40	20	40
C14:0	0.486	0.567	0.554	0.545	0.544	0.507	0.541	0.494	0.468	0.477	0.459	0.492	0.494	0.482	0.421	0.452	0.427
C15:0	0.126	0.160	0.159	0.150	0.167	0.156	0.157	0.147	0.127	0.128	0.121	0.130	0.133	0.142	0.109	0.115	0.111
C16:0	3.619	8.138	9.229	6.610	6.017	5.015	5.677	4.463	2.809	2.931	1.999	3.860	3.874	2.950	1.854	2.129	1.031
C16:3n-3	0.434	0.600	0.806	0.563	0.434	0.517	0.517	0.308	0.184	0.129	0.068	0.272	0.233	0.249	0.243	0.157	0.050
C18:0	0.501	0.581	0.558	0.539	0.605	0.512	0.556	0.523	0.514	0.526	0.512	0.527	0.522	0.516	0.463	0.491	0.480
C18:1n-9	0.237	0.288	0.313	0.293	0.343	0.271	0.272	0.247	0.240	0.236	0.226	0.248	0.256	0.245	0.209	0.216	0.207
C19:1n-7	0.885	1.824	2.347	1.514	1.533	1.127	1.262	0.947	0.611	0.638	0.439	0.862	0.926	0.595	0.455	0.528	0.246
C18:2n-6	2.351	4.114	4.501	3.638	3.432	3.412	3.168	2.691	1.582	1.587	0.949	1.995	2.039	1.886	1.112	1.452	0.862
C18:3n-6	1.564	2.542	2.973	2.490	2.463	2.372	2.091	1.799	1.077	1.009	0.634	1.194	1.515	1.234	0.796	0.823	0.623
C18:3n-3	0.871	1.565	1.994	1.392	1.223	0.971	1.331	0.730	0.434	0.392	0.281	0.693	0.855	0.399	0.425	0.429	0.201
c20:4n-6	0.317	0.492	0.626	0.524	0.520	0.464	0.404	0.340	0.235	0.230	0.164	0.280	0.316	0.281	0.195	0.223	0.160
C20:5n-3	0.452	0.640	0.783	0.741	0.663	0.583	0.547	0.431	0.364	0.337	0.332	0.457	0.479	0.402	0.351	0.348	0.310
C22:0	0.413	0.577	0.666	0.538	0.590	0.501	0.553	0.492	0.388	0.411	0.377	0.433	0.424	0.415	0.330	0.341	0.317
C22:5	0.491	0.594	0.956	0.610	0.666	0.600	0.628	0.408	0.356	0.340	0.330	0.471	0.480	0.398	0.331	0.375	0.143
C22:1n-6	0.553	0.604	0.583	0.569	0.581	0.565	0.626	0.561	0.562	0.567	0.560	0.574	0.574	0.564	0.511	0.547	0.531
C20:1n-5/9	0.515	0.566	0.565	0.546	0.566	0.514	0.549	0.524	0.505	0.523	0.510	0.530	0.524	0.271	0.467	0.510	0.487
PUFA (mg/ g DW)	6.481[a]	10.547[bA]	12.639 [bA]	9.958[aA]	9.401[aA]	8.919[aA]	8.658[aA]	6.708[aA]	4.232[aB]	4.023[bA]	2.759[bB]	5.361[aA]	5.917[aA]	4.849[aA]	3.453[bA]	3.806[bA]	2.350[bA]
MUFA (mg/ g DW)	2.189[a]	3.282 [bA]	3.808 [bA]	2.922 [aA]	3.023 [aA]	2.477[aA]	2.709[bA]	2.278 [aA]	1.917 [aA]	1.965[aA]	1.735[bA]	2.214[aA]	2.280 [aA]	1.675 [aA]	1.641[aA]	1.801[aA]	1.470 [bA]
SFA (mg/ g DW)	5.144[a]	10.023[bA]	11.165[bA]	8.38 [aA]	7.922[aA]	6.692[aA]	7.484[bA]	6.119 [aA]	4.307 [aA]	4.473[aA]	3.469 [bA]	5.442[aA]	5.447 [aA]	4.505 [aA]	3.178[bA]	3.529 [aA]	2.365 [bA]
n3 PUFA (mg/g DW)	2.869	4.707	5.772	4.446	4.121	3.859	3.938	2.837	1.695	1.529	0.983	2.159	2.603	1.882	1.464	1.408	0.875
n6 PUFA /mg/g DW)	4.233	7.148	8.100	6.652	6.414	6.248	5.663	4.831	2.894	2.825	1.747	3.468	3.870	3.400	2.103	2.499	1.646
%Loss PUFA		−62.729	−95.000	−56.633	−45.037	−37.606	−34.002	−3.497	34.705	37.932	57.439	17.292	8.702	25.192	46.725	41.275	63.741
%Loss MUFA		−49.920	−73.940	−33.462	−38.086	−13.167	−23.760	−4.056	12.413	10.252	20.751	−1.154	−4.146	23.486	25.025	17.721	32.834
%l Loss saturated		−94.834	−117.031	−62.946	−53.994	−30.092	−45.489	−18.952	16.279	13.052	32.563	−5.782	−5.886	12.423	38.219	31.400	54.033
%Total Loss		−72.654	−99.867	−53.904	−47.271	−30.935	−36.656	−9.341	24.311	24.281	42.362	5.777	1.234	20.167	40.119	33.865	55.228

[a]The statistical analysis has been done for PUFA, MUFA and SFA results. The significance compared to fresh is indicated with lowercase letters, while the difference between time points of the same brine is indicated with uppercase letters. The p-value was set at 0.05.

for further diversification of the routes by which seaweed can reach consumers. The presented strategies are not least interesting from an energy consumption perspective, given the fact that drying and freezing are highly energy-demanding operations.

CRediT authorship contribution statement

Mar Vall-llosera Juanola: Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation. **Sophie Steinhagen:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Henrik Pavia:** Writing – review & editing, Resources. **Ingrid Undeland:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

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