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Acid preservation of cultivated brown algae *Saccharina latissima* and *Alaria esculenta* and characterization of extracted alginate and cellulose

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ABSTRACT

Cultivated brown algae represent an important potential source of carbohydrate polymers for packaging and other biobased materials. However, their exploitation is currently limited by a short harvest season and a lack of cost-effective and sustainable methods to preserve biopolymer quality. In the present study, cultivated *Saccharina latissima* (*SL*) and *Alaria esculenta* (*AE*) were preserved with formic acid at 4, 13 and 20 °C for up to 16 weeks prior to extraction and characterization of alginate and cellulose. The data show up to 40 % increased yield of alginate from preserved biomass compared with fresh and non-preserved biomass, primarily due to removal of minerals and other soluble compounds during the acid wash. Acid preservation and storage caused a reduction in alginate weight average molecular weight (M_w) that was mainly dependent on storage temperature and to a lesser extent on storage time; storage at 4 °C maintained the M_w of alginates at 350–500 kDa. Preservation had no effect on the guluronate block structure of the extracted alginates, but guluronic acid content and block length increased in the non-preserved samples, presumably due to enzymatic degradation of the alginate's M-rich regions. Preservation of the seaweed resulted in an increased cellulose yield compared with fresh and non-preserved biomass, again due to the biomass being reduced during acid wash. The molecular weight and crystallinity of cellulose were not altered by the process. Altogether our findings demonstrate that acid preservation at low temperatures can effectively stabilize seaweed biomass while preserving alginate and cellulose quality for biomaterials and other applications.

1. Introduction

>17 million tons of brown algae are produced globally, each year. The majority of this biomass (>95 %) comes from seaweed cultivation in Asia and is used directly as food or as food and feed additives [1]. Seaweed production in Europe and the Americas comes predominantly from wild harvest, where most of the harvested biomass is used for production of alginate [2]. Cultivation of brown algae in Europe has a great potential due to large available coastal areas and the cold, clean waters, and has seen substantial growth over the past few years. *Saccharina latissima* (*SL*) is the main cultivated brown algae species in Europe, whereas *Alaria esculenta* (*AE*) has been a favourable alternative for food applications due to its lower iodine content [3]. Seaweed cultivation can have environmental benefits by replacing less sustainable ingredients and materials and capturing CO_2 from the atmosphere [4,5]. To reach global impact, there is a need however for upscaling the seaweed cultivation industry outside of Asia, and exploring novel large-scale applications such as processed foods, household products, biomaterials, and packaging.

Alginates are the main structural component in brown algae. They are a class of linear 1 → 4 linked negatively charged polysaccharides consisting of the monomers D-mannuronic acid (M) and L-guluronic acid (G) (Fig. 1) [6,7]. The M and G residues are organized in various block structures and occur in varying relative proportions, depending on the

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algal species and anatomical part the alginates are isolated from [8]. Adjacent G residues can bind divalent cations (e.g., Ca^{2+}), cross-linking the alginate into hydrogels [9]. Alginates have several applications, particularly within the food industry as a thickening and gelling agent. Here, the most important quality parameters are the overall G-content and the molecular weight of the alginates, which affect gel strength and viscosity [10]. Commercial alginate production in Europe employs wild harvested (trawled) brown algae, where *Laminaria hyperborea (LH)* and *L. digitata (LD)* are the predominant species used due to their large and homogeneous wild stocks as well as the high G content of the alginates derived from their stipes. The demand for alginate is currently high and can increase further with new alginate-based innovations and the ongoing transition from fossil- to bio-based materials. As wild populations of brown algae may be threatened by environmental changes and other ecological factors, and reduced by excessive trawling, cultivated brown algae represent a promising alternative for alginate production at a large scale [11,12].

One of the present challenges of large-scale industrial applications of alginates from cultivated brown algae is the short harvest season, restricting year-round access to high-quality biomass. The quality of the algae also deteriorates fast from microbial and oxidative processes after harvest, necessitating cost-effective and scalable methods for stabilizing the biomass [13]. As *SL* and *AE* alginates have a lower G content than commercial alginates and thus slightly inferior gelling properties, maintaining a high molecular weight may be crucial to allow industrial applications [14]. However, the G content of alginates may also be enzymatically enhanced using C-5-epimerases, although these are presently not commercially available [15,16]. The higher cost of cultivating brown algae compared with wild harvest in Europe necessitates retrieval and commercial utilization of other fractions than alginates, which can be water soluble bioactive compounds such as fucoidan, phlorotannins or pigments [17].

Cellulose consists of a linear chain of hundreds to thousands of β-(1,4)-linked D-glucosyl units (Fig. 1). Owing to its unique properties, including excellent mechanical performance, hydrophilicity, biocompatibility and convenient processability, cellulose has gained great attention for its extensive industrial applications [18]. Cellulose I, or native cellulose, is the intrinsic polymorph in natural sources, containing two sub-allomorphs, which are classified as I_α (triclinic) and I_β (monoclinic) [19]. Typically, different from terrestrial plant cellulose, most algal cellulose is dominated by the I_α allomorph and its content can vary in different species of brown algae [20,21]. Further development and industrial implementation of cellulose-based materials from cultivated brown algae necessitates characterizing changes in features such as molecular weight and crystal structure of the cellulose associated with different storage conditions of the algal biomass. Residual raw materials from alginate production are rich in cellulose but are normally discarded as waste or low-value co-products. Reports related to the extraction and physical properties of cellulose from the residual materials are thus rather limited [22,23], particularly for cultivated brown algae which are yet to be implemented in large-scale biorefineries.

Cultivated seaweed is currently preserved by freezing, drying, fermentation or ensiling [24]. Outdoor drying of brown algae is unsuited in subarctic and cold-temperate regions. Thus, drying as a preservation method is energy-requiring, and will further increase the cost of processing brown algae. Several studies have preliminary assessed the effect of acid on conservation of seaweed biomass either by ensiling or fermentation, but the main focus has rather been on preserving compounds suitable for fermentation to biofuels, such as mannitol and laminarin [25,26], or assessing the potential feed quality of the conserved biomass [27,28].

In the present study we have preserved fresh cultivated brown algae (*SL* and *AE*) using weak formic acid and analysed the effects on the biomass, with particular emphasis on alginate quality over different storage times and temperatures. Furthermore, we present to our knowledge the first conducted structural characterization of cellulose from cultivated brown algae, including the influences of storage conditions on the structural properties.

2. Materials and methods

2.1. Biomass supply

The brown algae were cultivated and supplied by Seaweed Solutions AS. Sporophytes collected from mother plants were seeded onto 6 mm polyester ropes and maintained for 8 weeks under continuous water flow at 8–9 °C. The resulting seedlings were deployed at Frøya, Norway (N63° 44.713′ E8° 52.413′) in January 2021. *SL* was harvested on the 27th of April and the *AE* on the 11th of May. Correct species identification was guaranteed by the presence of a midrib in *AE*. Following harvest, the

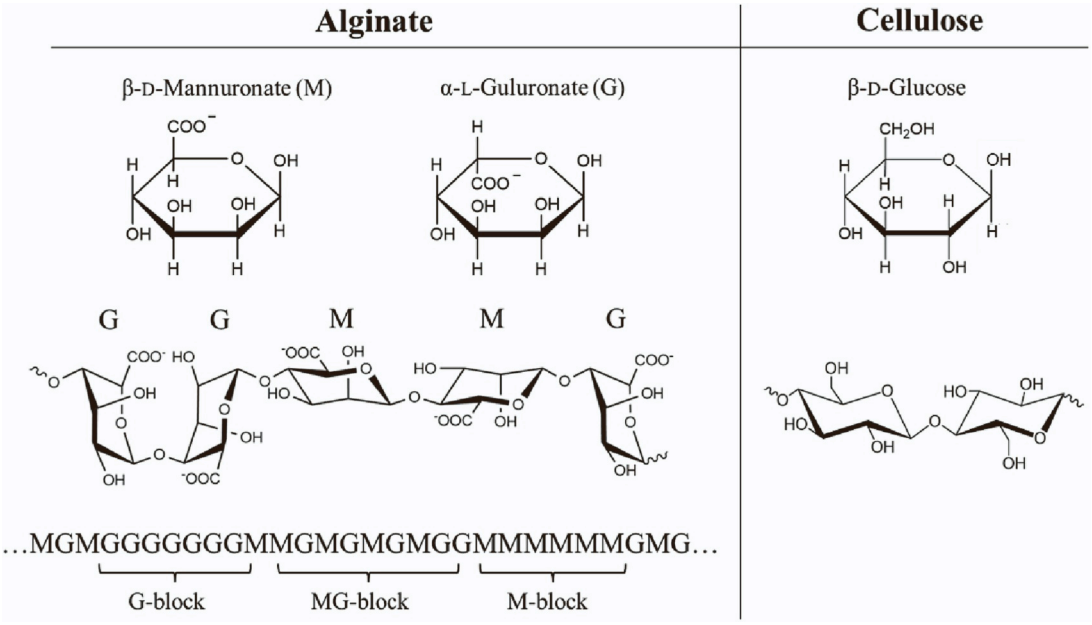

Fig. 1. Left: alginate structure and block patterns. Right: cellulose structure.

fresh algal biomass was transported in a cooled container (without seawater) for approximately 3 h, prior to milling using an industrial meat grinder with a grate hole diameter of 6 mm.

2.2. Acid preservation

10 L 0.1 M formic acid (HCOOH) was added to 7.8 kg milled seaweed. When the pH had stabilized at 3.2, the biomass was sieved to remove excess acid and distributed into anaerobic culture flasks at 200–300 g wet weight (ww) per flask in triplicates. The head space was flushed with N_2 to remove oxygen prior to capping the flasks. The flasks were stored in incubators at 4, 13 or 20 °C (to represent conditions for outdoor storage in Norway), over 4, 8 and 16 weeks. After incubation the pH was measured and the whole biomass (including leached liquid) was lyophilized and milled to a fine powder for subsequent analyses. One flask of fresh milled biomass was included for each temperature and time point (non-preserved control), while one batch of each species was immediately frozen after harvest and lyophilized (fresh control).

2.3. Determination of dry matter and ash content

Dry matter (DM) and ash content was measured in the fresh and acid washed material of both *AE* and *SL* prior to storage. For all 4 biomasses, approximately 5 g fresh, milled material was weighed out in crucibles in triplicates. The samples were dried at 105 °C over 22 h, and cooled down to room temperature in a desiccator. Each crucible was weighed, and the dry matter content calculated based on the seaweed ww and Eq. (1):

$$\mathrm{DM}(\%\text{of ww}) = \frac{\text{Total weight after drying} - \text{weight crucible}}{\text{ww}} \times 100\% \quad (1)$$

The crucibles with dried material were burned in an ashing furnace at 550 °C for 12 h. Ash content was calculated based on Eq. (2):

$$\mathrm{Ash}(\%\text{of DM}) = \frac{\text{Total weight after combustion} - \text{weight crucible}}{\text{DM}} \times 100\% \quad (2)$$

DM and ash were also measured in the formic acid solution after seaweed washing, to determine biomass loss during this process. A duplicate of the acid solution was lyophilized and weighed, and DM and ash were calculated based on the acid solution ww and Eqs. (1) and (2).

2.4. Polysaccharide extraction

The alginate and cellulose extraction processes are shown in Fig. 2.

2.4.1. Alginate

To extract alginate, 1 g of dried milled biomass was added to 50 mL centrifuge tubes, followed by the addition of 0.2 M HCl (40 mL). The samples were incubated at 20 °C while shaking (200 rpm, orbital movement 2.5 cm amplitude) over night. The biomass was centrifuged (3220 *g*, 15 min) and washed once with deionized water (40 mL) before adding 0.2 M $NaHCO_3$ (40 mL). The samples were again incubated overnight (200 rpm, orbital movement 2.5 cm amplitude, 20 °C), followed by centrifugation as described above. For this step, the pH of the biomass was approximately 7 after addition of $NaHCO_3$ and increased to around 7.2–7.5 after incubation. The supernatant was decanted and filtered (mesh size = 100 μm) while the solid residues were lyophilized. NaCl was added to the supernatant (2 mg/mL) followed by addition of 96 % ethanol in a 1:1 proportion to precipitate the alginate. The alginate was centrifuged (3220 *g*, 10 min) and the pellet washed once with 70 % ethanol and a second time with 96 % ethanol prior to lyophilization of the alginate.

2.4.2. Cellulose

A sequential process was utilized to extract cellulose from the *SL* and *AE* samples (Fig. 2). Dried and milled biomass (10 g) was first defatted with ethanol (95 %) at room temperature for 24 h to remove pigments and proteins. The insoluble fraction was washed with water and further treated with 250 mL of HCl (5 wt%) at room temperature for 12 h to convert soluble alginic acid sodium salt to insoluble alginic acid. Then the liquid phase was discarded, and the insoluble fraction was heated in 250 mL of NaOH (5 wt%) at 80 °C for 12 h. Subsequently, the sample was treated with 250 mL of H_2O_2 (4 wt%) at 60 °C for 8 h. After cooling to room temperature, the sample was washed thoroughly with H_2O until pH 7 was reached. The obtained extracted cellulose was dispersed in water and kept at 4 °C for further use, and the extraction yield was calculated based on the dry weight after heating at 105 °C.

2.5. Alginate characterization

2.5.1. Elemental analysis

The carbon (C), nitrogen (N) and sulfur (S) contents were determined

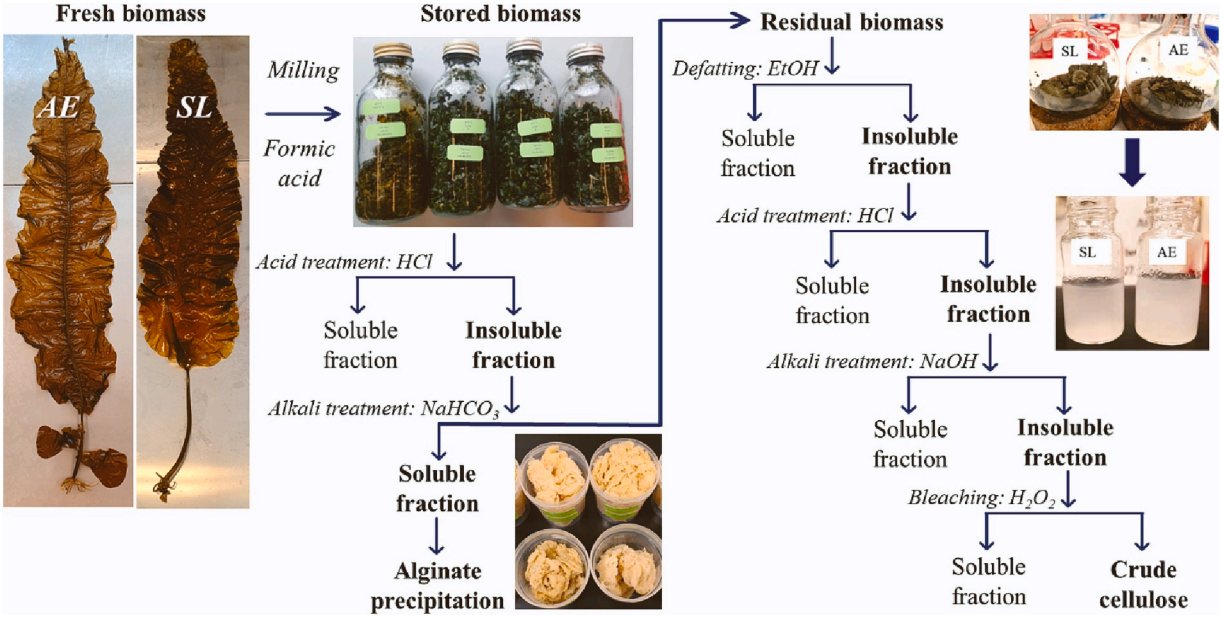

Fig. 2. Processing scheme for acid preservation of *Alaria esculenta* (AE) and *Saccharina latissima* (SL) and subsequent alginate and cellulose extraction.

by weighing approximately 5 mg lyophilized alginate in tin capsules. The samples were oxidised at 1150 °C and analysed on a Vario-El-Cube CNS element analyser (Elementar, Germany). The measured S content was used to estimate fucoidan levels in the precipitated alginate. The measured sulfur was used to estimate fucoidan levels in the precipitated alginate. The S and fucose contents (fc) have previously been measured in an extracted fucoidan from SL, and were 11.8 and 33.6 %, respectively (Birgersson, unpublished data). Assuming that only the fucose residues in the fucoidan are being sulphated, the sulphation degree (DS) is 1.68, translating to 84 % of the free hydroxyl groups being sulphated. These numbers are in good accordance with those reported by Bilan et al. [29]. The fucoidan content was also calculated in a commercial food-grade alginate from LH (LF10/60, DuPont Health & Nutrition, Norway), with DS 1.7 and fc 97.8, as reported by Kopplin et al. [30]. Nitrogen content was used to calculate protein content in the alginate samples, based on a nitrogen-protein factor (Kp) of 3.8, originating from work done by Forbord et al. [31].

The concentration of Na, Ca, K, Mg and P in the alginate was measured by inductively coupled plasma mass spectrometry (ICP-MS), on an Agilent 8800 Triple Quadrupole ICP-MS instrument (Agilent Technologies, USA). All solutions and dilutions were prepared using ultra-pure water (18.2 MΩ) from a OmniaTap 10 UV system (Stakpure, Germany) and concentrated nitric acid (65 % HNO_3) purified using a Savillex DST-100 Acid Purification System (Savillex, USA) or 25 % tetramethylammoniumhydroxide (TMAH, Acros). Lyophilized samples were hydrolyzed with HNO_3 at 250 °C in an UltraWAVE microwave oven (Milestone, Italy) and diluted to 5 % (*v*/v) prior to analysis.

2.5.2. Molecular weight analysis

Alginate samples (0.5 mg/mL, 50 μL, 0.22 μm filtered) were analysed at room temperature on an HPLC system fitted with an OHpak LB 806 M size exclusion column using 0.15 $NaNO_3$ and 0.01 EDTA, pH 6.0, as elution buffer at a flow rate of 0.5 mL/min. The column outlet was connected to a Dawn Helios II multiangle laser light scattering photometer (Wyatt, USA) (λ_0 = 663.8 nm) and a Shodex RI-501 refractive index detector. Data were collected and processed using the ASTRA software v. 7.3 (dn/dc = 0.150 mL/g and $A_2 = 5.0 \times 10^{-3}$ mL mol g^{-2}).

2.5.3. Nuclear magnetic resonance (NMR)

The alginates were partially degraded by stepwise acid hydrolysis to an approximate degree of polymerization (DP) of 50 for ^{1}H NMR analysis as described in [32]. In short, 15 mg alginate was dissolved in ion-free water, the pH was adjusted to 5.6 with 0.1 M HCl, and the polymer was hydrolysed at 95 °C for 1 h. The solutions were immediately cooled to room temperature, the pH adjusted to 3.8, and the samples hydrolysed at 95 °C for 50 min. The solutions were cooled, neutralised with 0.1 NaOH (pH 6.8–7.5) and lyophilised.

Approximately 10 mg of the degraded samples were dissolved overnight in 600 μL D_2O (d-99.9 %) and dissolved overnight. Triethylenetetramine-hexaacetic acid (TTHA) in D_2O (0.3 M, 20 μL) was added as a chelator. The samples were centrifuged, and the supernatants were transferred to NMR tubes. 3-(Trimethylsilyl)-propionic-acid sodium salt (TSP) (Aldrich, Milwaukee, WI) in D_2O (1 %, 5 μL) was added for internal chemical shift reference. ^{1}H NMR spectra were recorded at 83 °C on a BRUKER NEO 600 MHz instrument equipped with a 5 mm iProbe (Bruker BioSpin). The spectra were recorded using the TopSpin 4.0.8 software (Bruker BioSpin) and processed and analysed with the TopSpin 4.0.7 software (Bruker BioSpin).

2.5.4. Rheological characterization

The viscosity of the alginates was measured using a Kinexus rheometer (Netzsch, Germany) equipped with an upper double gap bob geometry (25 mm OD) and a lower double gap system cup. The alginate was dissolved in water to a concentration of 1 % *w/v* and the viscosity was measured over a shear rate of 0.1–10 s^{-1} with 10 measurements per decade, at 25 °C.

2.6. Cellulose characterization

2.6.1. X-ray diffraction

The X-ray diffraction (XRD) patterns were recorded using a Philips X'Pert Pro diffractometer (model PW 3040/60) in the reflection mode (2θ angular range 5–40°). The CuKα radiation (λ = 1.5418 Å) was generated at 45 kV and 40 mA and monochromatised using a 20 μm Ni-filter. Diffractograms were recorded from rotating specimens using a position sensitive detector.

2.6.2. Fourier-transform infrared (FTIR) spectroscopy

The FT-IR spectra were obtained by using a PerkinElmer Spectrum 2000 instrument equipped with an MKII Golden Gate Single Reflection ATR system (Specac Ltd., UK) in a spectral range of 600–4000 cm^{-1} with a resolution of 4 cm^{-1}. The cellulose I_α allomorph content was determined from the FTIR spectra [23]. The characteristic peaks of cellulose I_α and I_β in cellulose samples from fresh *AE* and *SL* were identified at 3233 $cm^{-1}$and 3276 cm^{-1}, respectively, based on the second derivative spectra of the OH stretching vibration at 3150–3500 cm^{-1}. The OH bands were deconvoluted and cellulose I_α content was calculated from the integral area of the I_α and I_β bands.

2.6.3. Degree of polymerization (DP) measurement

To measure the degree of polymerization (DP) of the extracted cellulose, lyophilized cellulose samples (40 mg each) were dissolved in 0.5 M copper ethylenediamine (CED, 50 mL) for 30 min. Intrinsic viscosity data [η] were obtained using a Cannon-Ubbelohde semi-micro viscometer and the DP of each cellulose sample was calculated from [η] value using $[\eta] = 0.42\ DP$ for $DP < 950$ or $[\eta] = 2.28\ DP^{0.76}$ for $DP > 950$.

3. Results and discussion

3.1. Biomass characterization

The chemical composition of cultivated brown algae varies with deployment and harvest time, location, and other environmental conditions [8]. The biomasses studied had an average dry matter content of 8.3 % for *SL* and 11.7 % for *AE*. Fresh *SL* had a higher ash content than *AE,* i.e. 50.8 % and 36.3 %, respectively (Fig. 3), which is consistent with previously published literature [3]. Analysis of acid washed biomasses prior to storage showed a reduced relative ash content compared with the fresh biomass, primarily due to partial removal of salts (Na, K, Cl). Based on the ratio between organic matter and ash in the fresh biomass, the formic acid solution after washing, and the sieved biomass after

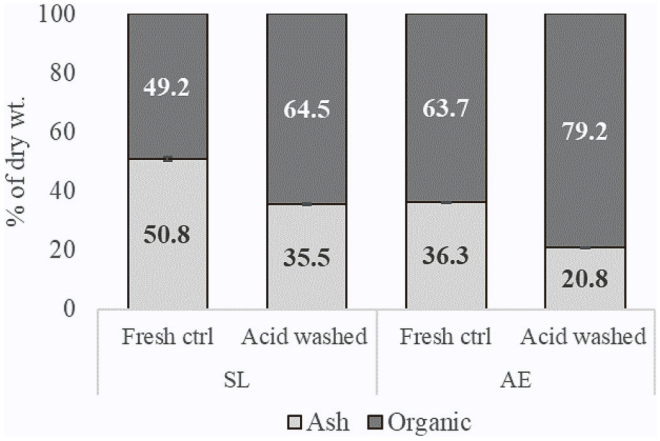

Fig. 3. Ash content of fresh and acid washed *Saccharina latissima* (SL) and *Alaria esculenta* (AE) prior to storage.

washing, the biomass loss during processing was 34.0 ± 1.5 % and 30.5 ± 0.5 % in *SL* and *AE,* respectively. Ash accounted for between 70 and 80 % of this loss and was highest in *SL*. Biomass loss estimations within the same range were also obtained when the calculations were based on the dry matter content in the acid solution. Thus, it is important to note that at the start of the experiment the acid-preserved samples had a higher dry matter content and organic fraction compared with the non-preserved controls that were transferred directly to flasks for storage after milling.

After storage, the pH of the acid-preserved sample had increased from 3.2 to around 3.3 for all *SL* samples with no difference from 4 to 16 weeks (Table 1). The acid-preserved algae had a dark green appearance and no noticeable smell of spoilage, except for *AE* (20 °C, 16 weeks) which had a slight brown tint and a weak odour compared to the other samples. For the *SL* control samples an additional decrease in pH over time was observed, dependent on the storage temperature. The preserved *AE* samples showed an increase in pH at 4 weeks from the initial adjustment but then decreased again to 3.2–3.3 by 16 weeks storage. As observed for *SL*, there was observed no effect of storage temperature on the pH of the acid preserved *AE*. For the non-preserved controls, the pH was decreased with time and increasing storage temperature, and there was a greater reduction in pH in *AE* compared with *SL*. This can be due to a higher content of fermentable compounds, particularly mannitol, and/or a lower buffering capacity in the *AE* biomass used for the present study. There was further observed a small degree of gas production in the bottles containing the non-preserved samples, and the biomass had a brown colour and an unpleasant odour which was similar for all time points and temperatures in both species.

3.2. Alginate characterization

3.2.1. Alginate yield and purity

Following alkali treatment of the biomass, alginate was precipitated from the supernatant and dried and weighed to assess differences in yield based on storage conditions and time (Fig. 4). While ethanol is less selective than acid for precipitating alginate, the precipitated matter typically contains traces of protein and other carbohydrates. The precipitate is referred to as alginate in this context, and the levels of impurities are discussed further below.

Except for one outlier (AE, 4w, 4 °C) a higher alginate yield was observed in all acid preserved samples compared with the fresh *SL/AE* controls and the non-preserved stored samples. This is mainly attributed to the higher relative alginate content per weight biomass in the preserved samples, as the acid pre-treatment had washed out minerals (see Section 3.1 above) and other water-soluble compounds (fucoidan, laminarin, mannitol). As alginate is converted to its acid form (alginic acid) during standard alginate extraction, the acidic preservation may contribute further to this conversion and dissolving the gelling ions [8]. Together with the higher effective alginate concentration in the substrate, it could reduce the time and costs of subsequent steps in the alginate production line. For acid-preserved *SL* (20 °C) and *AE* (13 and 20 °C) a small decrease in the alginate yield was seen between 4 and 16 weeks, which can be due to partial depolymerization leading to material loss during precipitation and subsequent washing. This was also evident in the non-preserved *AE* samples stored for 16 weeks where there was a visible alginate loss during precipitation and subsequent washing.

Table 1
Measured pH in formic acid-preserved and non-preserved control samples of *Saccharina latissima* and *Alaria esculenta* stored for 4, 8 and 16 weeks at 4, 13 or 20 °C. The pH of the acid preserved samples was set at 3.2 prior to storage.

	Temp.	*SL*			*AE*		
		4 w	8 w	16 w	4 w	8 w	16 w
Control	4 °C	5.28	5.25	5.28	4.87	4.88	4.61
	13 °C	5.43	5.19	4.95	4.20	4.23	4.19
	20 °C	5.09	4.82	4.75	4.31	4.30	4.16
Acid preserved	4 °C	3.33	3.29	3.33	3.53	3.45	3.26
	13 °C	3.32	3.26	3.28	3.42	3.42	3.33
	20 °C	3.31	3.27	3.26	3.48	3.44	3.25

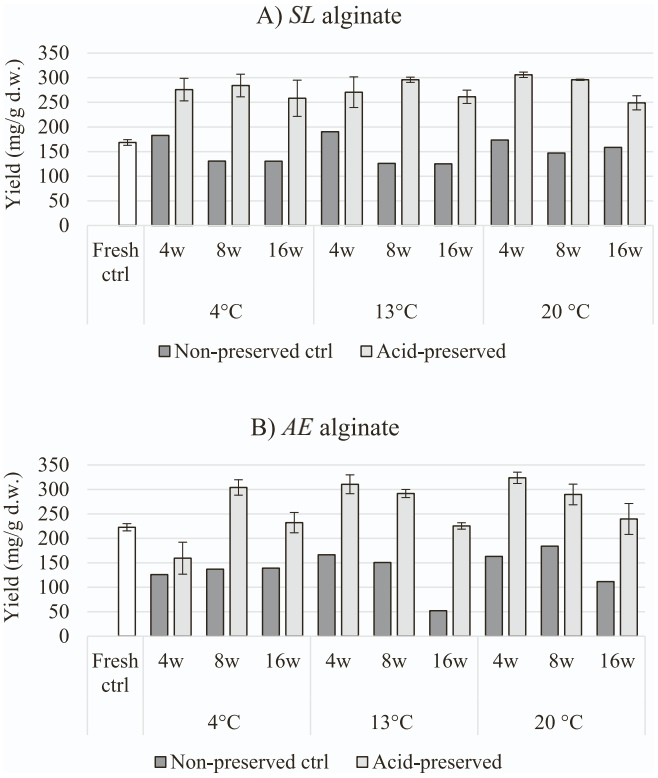

Fig. 4. Estimated yield of alginate from A) *Saccharina latissima* (SL) and B) *Alaria esculenta* (AE), determined as the weight of ethanol-precipitated matter from an alkali extract. Samples were stored at 4, 13 and 20 °C for 4, 8 and 16 weeks with or without acid preservation. The Fresh SL/AE control was immediately frozen and lyophilized after harvest. Fresh and acid preserved samples were analysed at n = 3 and the non-preserved samples at n = 1.

It should be noted that the obtained yields were based on a one-step alkali extraction and do not represent the total alginate content of the samples, which can be increased by tuning the extraction conditions (time, temperature, pH) as well as washing the residues with alkali or water [14,33].

To assess the purity of the alginates, elemental analysis was performed on the alginates extracted after 16 weeks storage and compared with a commercial food-grade alginate (LF10/60, DuPont Health & Nutrition, Norway). The contents of coextracted fucoidan and protein were estimated based on C/N/S analysis and are shown in Table 2. The C/N/S data and basis for protein and fucoidan estimation are shown in Supplementary material S1.

The extracted alginates showed an overall high level of purity with relatively small differences between the stored samples and the fresh controls. Of note, the protein content of the alginates decreased with increasing storage temperature, for both preserved and non-preserved biomass. This indicates that less protein is co-extracted/precipitated with the alginate which can be due to partial hydrolysis of proteins. The N content was higher in all *SL* and *AE* alginate samples compared with the commercial reference sample, which is presumably due to the alginate in the present study being precipitated with ethanol alone. A more selective acid precipitation of alginate followed by additional washing steps would have resulted in less protein contamination.

Alginates extracted from *SL* showed overall higher estimated levels of fucoidan compared with alginates from *AE*, whereas no significant differences were observed between acid-preserved and non-preserved

Table 2
Content of protein and fucoidan impurities (estimated from C/N/S analysis) in alginates extracted from acid-preserved or non-preserved controls of Saccharina latissima (SL) and *Alaria esculenta* (AE) biomass, stored for 16 weeks at 4, 13 or 20 °C. The Fresh SL/AE control was immediately frozen and lyophilized after harvest. Fresh and acid preserved samples were analysed at $n = 3$ and the non-preserved samples at $n = 1$.

Sample		Temp.	Content (mg/g d.w.) in alginates	
			Protein	Fucoidan
SL	Fresh	–	15.6 ± 4.1	30.5 ± 10.3
	Control	4 °C	13.3	49.2
	16 w	13 °C	9.1	52.3
		20 °C	8.0	34.8
	Acid pres.	4 °C	17.6 ± 2.9	38.1 ± 10.3
	16 w	13 °C	14.6 ± 1.6	39.1 ± 10.2
		20 °C	10.3 ± 1.4	39.5 ± 7.4
AE	Fresh	–	11.6 ± 2.4	25.2 ± 4.6
	Control	4 °C	14.1	32.8
	16 w	13 °C	14.1	26.5
		20 °C	8.7	18.8
	Acid pres.	4 °C	12.8 ± 1.9	22.6 ± 5.3
	16 w	13 °C	11.0 ± 0.4	22.8 ± 1.4
LH		20 °C	8.4 ± 0.4	22.3 ± 5.4
	LF 10/60	–	5.1 ± 0.6	9.2 ± 0.3

samples. Storage at high temperature (20 °C) resulted in a decreased S content in non-preserved biomass compared with lower temperatures, which can be due to higher microbial/enzymatic activity and was expectedly not observed for the acid-preserved samples.

The high purity of the extracted alginates was also confirmed by 1D NMR spectra (Supplementary material, Figs. S1–S4). Only minor traces of fucoidan (detected as the methyl signal from fucose) were observed in all spectres regardless of whether they were control, acid-preserved, or not.

Additional elements were selected based on their prevalence in the biomass and the data measured by ICP-MS are shown in Supplementary material, Table S2. All alginate samples showed a mineral content similar to that of the commercial reference sample, demonstrating that minerals are effectively washed out during the alginate extraction process in the present. Furthermore, no significant differences were observed between the stored samples and the fresh control, nor between the different storage conditions.

3.2.2. Alginate molecular weight and viscosity analysis

The weight average molecular weight (M_w) of the extracted alginates was estimated using SEC-MALS (Fig. 5). Alginates from the fresh controls showed a high M_w of approximately 700 kDa for both species, which was consistent with previous observations [14] and expectations due to the relatively mild extraction conditions (see Section 2.4.1). All stored samples showed a relative reduction in the M_w depending on the storage conditions. Alginate has a pK_a-value of approximately 3.6, and thus at pH < 3.6 the alginate is partially converted to its acid form and is more susceptible to depolymerization due to intramolecular hydrolysis [34–36], which explains the alginate depolymerization in the acid preserved samples. Non-preserved seaweed samples, on the other hand, are susceptible to depolymerization due to microbial growth and thus potential lyase activity. The pH in the acid preserved samples is presumably too low for any enzymatic depolymerization of the alginate.

Acid preserved *SL* alginates had an overall higher M_w than *AE* alginates in all conditions. For both species, the M_w of the alginates was reduced with storage temperature, which was expected due to increased rate of acid hydrolysis and enzymatic activity in the preserved and non-preserved samples, respectively. For *SL*, increasing the storage temperature from 4 to 20 °C resulted in a drop in M_w from around 500 to 200 kDa. For each temperature, an additional reduction in M_w was observed with increasing storage time for most samples. Interestingly, there was no reduction in M_w from 4 to 16 weeks at 4 °C for either species in the

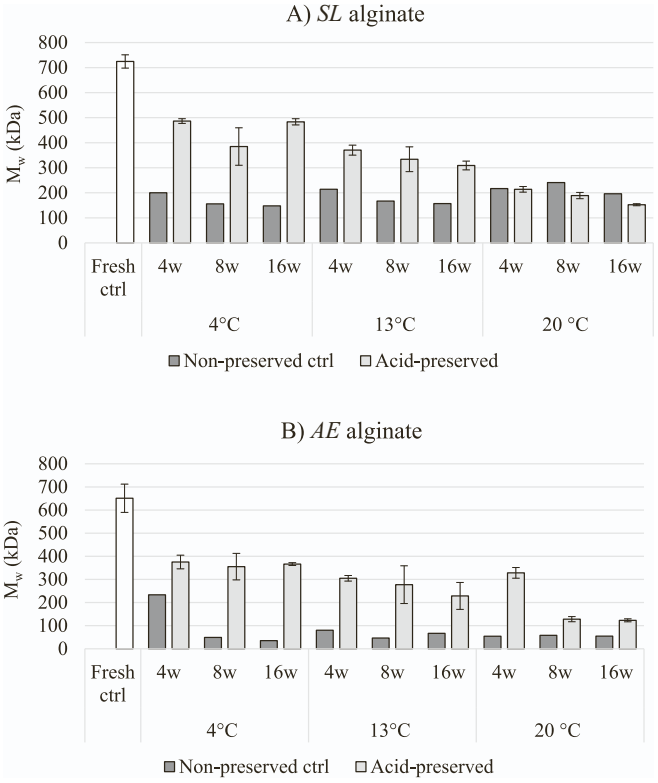

Fig. 5. Average molecular weight (M_w) of alginates as determined by SEC-MALS. Alginates were extracted from A) *Saccharina latissima* (SL) and B) *Alaria esculenta* (AE), stored at 4, 13 and 20 °C for 4, 8 and 16 weeks with or without acid preservation. The Fresh SL/AE control was immediately frozen and lyophilized after harvest. Fresh and acid preserved samples were analysed at n = 3 and the non-preserved samples at n = 1.

preserved samples, indicating long-term stability at low storage temperatures. The alginates from the non-preserved controls all showed a lower M_w compared with their corresponding preserved samples, and no clear dependence on storage temperature or time. Here, it must be noted that the analyses of non-preserved samples were based on only one replicate but showed that the depolymerization occurred earlier than 4 weeks and predominantly by enzymatic activity as the pH (measured between 4.7 and 5.3, Table 1) was too high for acid hydrolysis.

Most commercial alginates have a M_w lower than 240 kDa, as the strength of alginate gels does not increase significantly with M_w beyond this point [37], while the high viscosity of higher-M_w alginates can be disadvantageous during biomass processing. The intended reduction in M_w occurs in the acid wash, which is usually performed at a higher temperature (40–100 °C) than used in the present study (20 °C) [33]. Hence, the results show that acid preservation of the biomass can maintain the molecular weight of the alginates at an acceptable level for most commercial applications, and that the natural reduction in M_w occurring during storage of the biomass can remove the need for energy-demanding alginate depolymerization in an industrial process line.

It should be noted that most commercial alginates are based on other brown algal species and have a higher G-content than *SL* and *AE* alginates (see Section 3.2.3 above), and that the M_w dependence of alginates with a lower G-content is a less researched subject.

The replicates of the acid-preserved biomass stored for 16 weeks were combined, followed by alginate extraction as described in Section 2.4.1 to obtain larger quantities for subsequent analyses. The viscosity of the alginates was measured on 1 % *w/v* solutions in water at room temperature (Fig. 6). The results were consistent with the molecular weight analyses, showing a decrease in viscosity as a function of storage temperature and overall lower viscosities for *AE* alginates compared

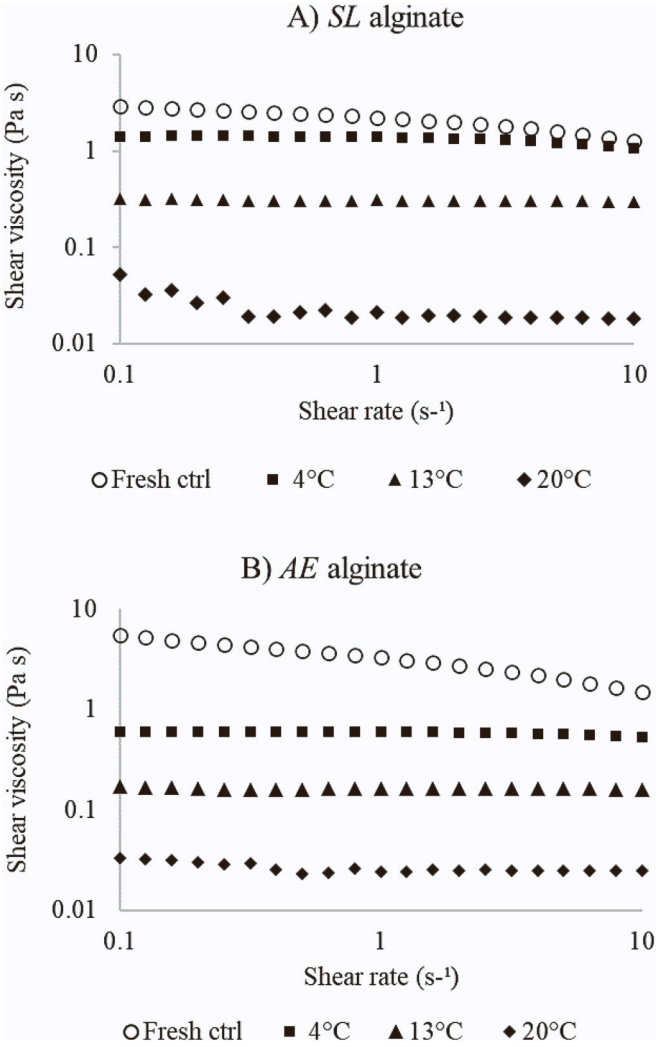

Fig. 6. Shear viscosity of alginates extracted from (A) *Saccharina latissima* (SL) and (B) *Alaria esculenta* (AE) biomass acid preserved for 16 weeks at 4, 13 and 20 °C. Viscosity was measured at room temperature on 1 % w/v solutions in water.

with *SL* alginate. The high-molecular weight alginates from the fresh control samples exhibited shear-thinning behaviour where the viscosity decreased with increasing shear rate, which is commonly due to breaking of molecular entanglements. This phenomenon was not evident in the lower-M_w acid preserved samples at the applied shear range.

3.2.3. Alginate structure

The monosaccharide composition of the alginates was analysed using ^{1}H NMR (Fig. 7 and S1–S4). Table 3 shows the relative fractions of G and M, GG/MG/MM diads and the GGG triad, as well as the estimated average length of G blocks ($N_{G>1}$). The G content of the alginate in the starting material was found to be slightly higher in *AE* ($F_G = 0.55$) than in *SL* ($F_G = 0.49$), and have longer G-blocks as indicated by $N_{G>1}$ and F_{GGG}. The G content of brown algae has been shown to increase with the age of the thallus [8] and can depend on deployment and harvest times for cultivated seaweed (unpublished data). For reference, most commercial alginates from *Laminaria* species have a G content of 60–85 % [38].

Acid preservation of the biomass was not found to have a large impact on the structure of the alginates compared with the fresh control. The rate of hydrolysis increases with temperature and type of glycosidic linkage (rate decreasing in the order G-M > M-M > M-G > G-G) [40,41]. The minor reduction in F_{GG} and F_{GGG} in the acid-preserved samples compared with the fresh controls, can be explained by the highest rate of hydrolysis of the G-M linkages. When a linkage between G and M is hydrolysed, this creates a new reducing end which is not accounted for in the NMR block composition analysis.

Interestingly, an increase in F_G and $N_{G>1}$ and a corresponding decrease in F_M and F_{MM} was observed in both species for the non-preserved control samples. Here, the alginates are mainly in their calcium-crosslinked form in the biomass but are subjected to degradation by alginate lyases resulting from microbial activity in the samples. The results indicate that less cross-linked M-rich sections of the alginates are more susceptible to enzymatic degradation, and that the degraded alginates are consumed by microorganisms and/or too fragmented to precipitate with the extracted alginate. This effectively increases the G content and average G-block length of the precipitated and analysed alginate. Fig. 7 (and S5-S8) shows a signal at 5.8 ppm explained by the unsaturated Δ-4 end (4-deoxy-L-erythro-hex-4-enopeyranosyluronate) created by an alginate lyase [39] in the non-preserved *AE* and for some *SL* samples. The signal is strongest in samples stored at 4 °C, implying that activity here is prolonged or that turnover and transformation of the unsaturated end residue is slower than at higher temperatures. This is also supported by the continuous depolymerization in the non-preserved AE-alginate stored at 4 °C after 8 and 16 weeks (Fig. 4 and S5-S8), whereas at higher temperatures, the depolymerization seems to have ended after 4 weeks. It should be noted that the same alginate lyase activity is not observed to the same extent in non-preserved *SL*, implying that this alginate lyase is endogenous from *AE* rather than microbial activity. However, here further investigations are encouraged.

3.3. Cellulose characterization

3.3.1. Cellulose yield and DP

Cellulose was extracted from dried residual material after alginate extraction, following 16 weeks storage of the biomass. The FT-IR spectra (Supplementary material, Fig. S9) of extracted cellulose showed that alginate has been efficiently removed, as neither protonated (at 1730 cm^{-1}) or deprotonated (at 1600 cm^{-1}) carboxylate groups were detected. For both *SL* and *AE* species, nearly all stored samples had a higher cellulose yield compared with the fresh control and the yield was further found to increase with storage temperature (Fig. 8). The highest cellulose yields from the residues were 180 mg/g and 138 mg/g for *SL* and *AE* samples, respectively, which were both found in non-preserved samples stored at 20 °C. This variation of cellulose content may be due to the release of soluble components such as proteins, fucoidan, and alginate during preservation. While the yields were slightly lower, the overall contents and interspecific differences were consistent with recently published literature using *SL* and *AE* from the same cultivation site and approximate time of harvest [42].

Acid preservation was found to maintain the DP of cellulose extracted from both *SL* and *AE* residual biomass, compared with the fresh control (Fig. 9). Compared to the starting fresh material, the DP of *SL* cellulose was around 1700 glucosyl units, significantly higher than that of *AE* cellulose (1130 glucosyl units). Interestingly, a significant decrease in DP was observed for the cellulose from non-preserved *SL* compared with fresh and acid preserved samples, while this was not seen for the *AE* samples.

3.3.2. Cellulose structure

According to the deconvolution of OH stretching bands (Fig. S10, Supplementary material), most of the extracted cellulose was of the I_α allomorph, for both species. *AE* cellulose extracted from fresh material showed a cellulose I_α content of 73.1 %, higher than that (64.1 %) for *SL* cellulose. The I_α cellulose content of both *SL* and *AE* samples was unchanged after storage for 16 weeks with and without acid preservation (Table S3, Supplementary material).

The XRD diffractograms for the corresponding to cellulose extracted

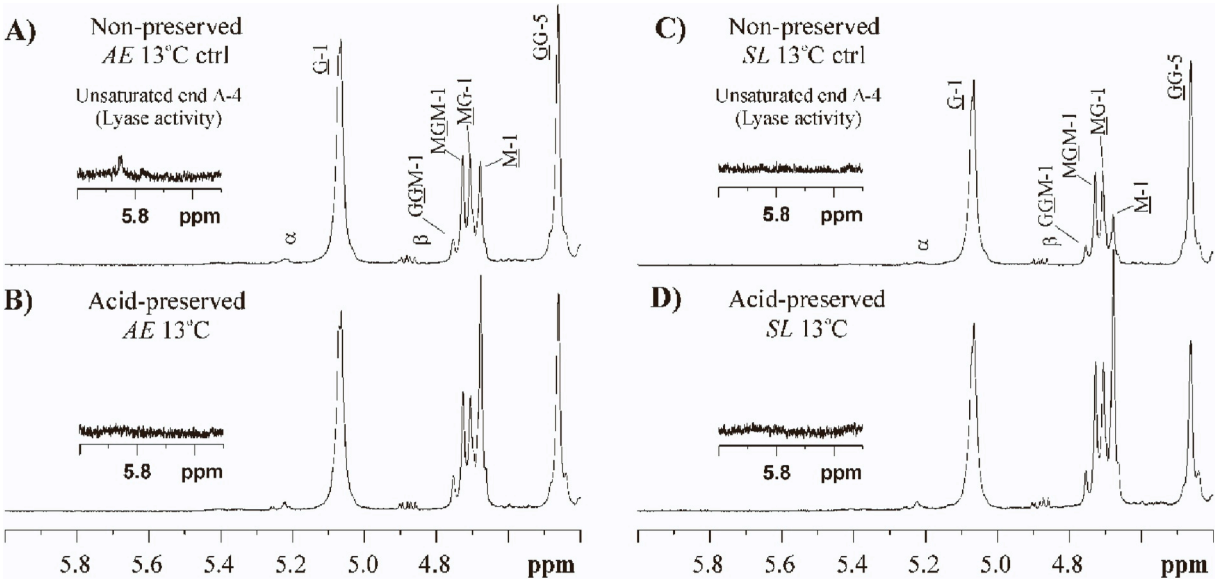

Fig. 7. 1D proton of the anomeric region in NMR spectra of alginates extracted from *Alaria esculenta* (AE) and *Saccharina latissima* (SL) biomass acid preserved for 16 weeks at 13 °C recorded at 600 MHz and 83 °C. Alginate from parallel 2 A) Non-preserved AE ctrl at 13 °C, B) acid-preserved AE at 13 °C, C) Non-preserved SL ctrl at 13 °C, D) acid-preserved SL at 13 °C. Full data set can be found in supplementary materials Figs. S5-S8. The resonance assignment of spectra is based previously published assignments for alginate [32,39]. The inlay panel shows the region where the proton signal of Δ-4 (4-deoxy-l-erythro-hex-4-enopeyranosyluronate) residue, which is indicative of alginate lyase activity. α and β indicate the signals for the reducing end of alginate, G is guluronate, M is mannuronate, # indicate proton number in alginate sugar ring, underlined indicate the residue giving rise to the signal.

Table 3
Average fraction of guluronic acid (F_G), mannuronic acid (F_M), all possible diads, the triad of guluronic acid (F_{GGG}) and the average length of G-blocks ($N_{G>1}$) in alginates extracted from fresh-, acid preserved- or non-preserved biomass after 16 weeks storage at 4, 13 or 20 °C.

Sample		Temp.	F_G	F_M	F_{GG}	$F_{GM,MG}$	F_{MM}	F_{GGG}	$N_{G>1}$
SL	Fresh	–	0.49	0.51	0.30	0.18	0.33	0.27	9
	Control 16 w	4 °C	0.67	0.33	0.47	0.20	0.14	0.43	13
		13 °C	0.70	0.30	0.52	0.19	0.11	0.48	16
		20 °C	0.69	0.31	0.53	0.16	0.14	0.49	16
	Acid pres 16 w	4 °C	0.47	0.53	0.28	0.19	0.34	0.24	8
		13 °C	0.47	0.53	0.27	0.19	0.34	0.24	9
		20 °C	0.46	0.54	0.27	0.19	0.34	0.24	9
AE	Fresh	–	0.55	0.42	0.40	0.18	0.25	0.36	11
	Control 16 w	4 °C	0.69	0.31	0.52	0.17	0.15	0.49	21
		13 °C	0.66	0.34	0.50	0.17	0.17	0.46	15
		20 °C	0.71	0.29	0.53	0.18	0.11	0.49	13
	Acid pres. 16 w	4 °C	0.53	0.47	0.36	0.17	0.31	0.32	10
		13 °C	0.52	0.48	0.35	0.17	0.30	0.32	10
		20 °C	0.53	0.47	0.36	0.17	0.31	0.33	11

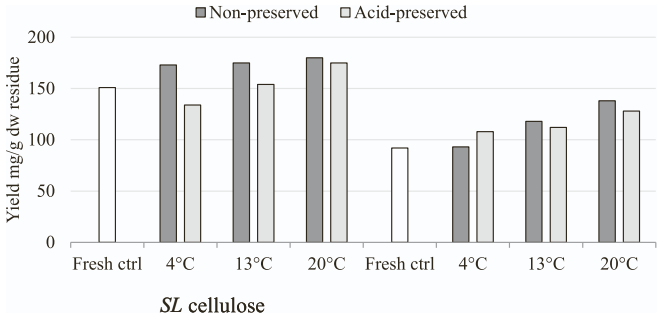

Fig. 8. Estimated yield of cellulose extracted from *Saccharina latissima* (SL) and *Alaria esculenta* (AE), following storage biomass at 4, 13 and 20 °C for 16 weeks with or without acid preservation. The Fresh SL/AE control was immediately frozen and lyophilized after harvest, without storage. The yield was calculated based on the dry weight of the residues after alginate extraction.

from the fresh, non-preserved, and acid preserved *SL* and *AE* samples showed a typical pattern of cellulose I (Fig. 10). By using the curve-fitting approach with the OriginPro program, four peaks arising from the crystalline phase (101, 10$\bar{1}$, 021, and 002) were deconvoluted using the Gaussian function with an amorphous background profile using a fifth-degree polynomial function (Fig. S11, Supplementary materials). The crystallinity index (CI) was calculated as the ratio between the area of the crystalline contribution and the total area (Table S3, Supplementary materials). The CI of *AE* cellulose from fresh control was 53.2 %, similar to the CI of *SL* cellulose from the fresh control (51.2 %). All preserved and non-preserved *AE* samples maintained their crystallinity after 16 weeks storage, whereas the non-preserved *SL* samples stored at 13 and 20 °C decomposed significantly, as judged by the decreased intensity of the characteristic peaks of cellulose I (Fig. 10). While these results may indicate increased microbial activity in the *SL* control samples, it is interesting to note that for alginate the most extensive depolymerization and stable lyase activity was observed in *AE* (Fig. 5 and 7, respectively).

In summary, the results gave important evidence on the effect of acid

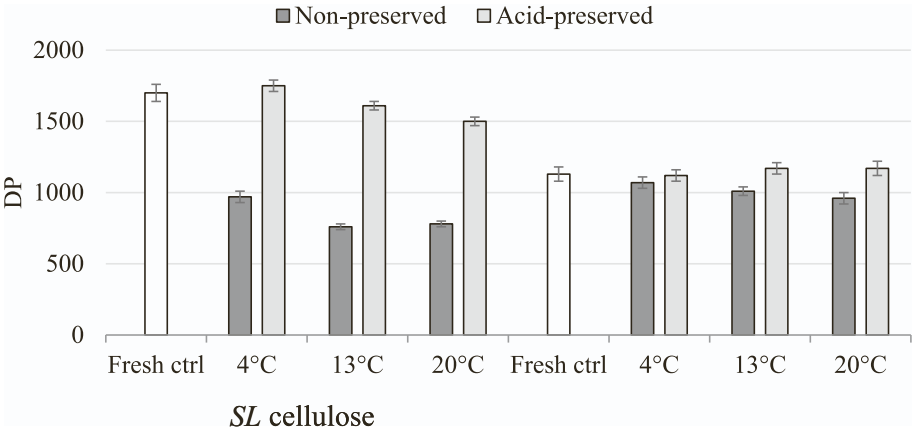

Fig. 9. Degree of polymerization (DP) of cellulose samples extracted from A) *Saccharina latissima* (SL) and B) *Alaria esculenta* (AE) residual biomass, following storage at 4, 13 and 20 °C for 16 weeks with or without acid preservation, and extraction of alginate. The Fresh SL/AE control was immediately frozen and lyophilized after harvest, without storage.

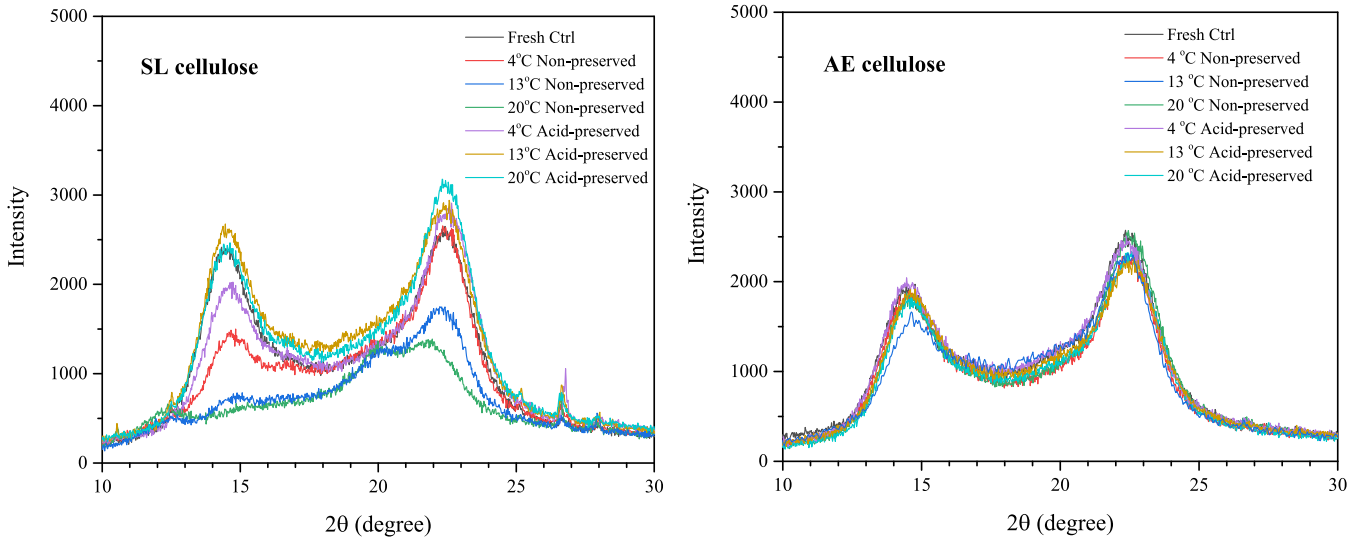

Fig. 10. XRD pattern of the extracted *Saccharina latissima* (SL) and *Alaria esculenta* (AE) cellulose.

preservation on the structure and properties of cellulose extracted from *SL* and *AE* residual biomass. The acid treatment is a useful method to store the seaweed samples without compromising cellulose yield. Moreover, the acid addition and low storage temperature were beneficial for producing cellulose with high DP.

4. Conclusions and future perspectives

Utilization of brown algal biomass for large-scale material applications necessitates cost-effective means for preservation which that do not compromise the properties of structural polysaccharides. This is particularly the case for cultivated seaweed which has a short harvest window characterized by rapid deterioration after harvest. The present study has assessed the effects of preservation of two cultivated brown algal species with formic acid, which prevented spoilage of the biomass and resulted in a low level of depolymerization of alginates where the extent depended on storage time and temperature. It is indicated that the acid pre-treatment can improve downstream processing and potentially the quality of alginates. With more extensive depolymerization in the non-preserved biomass, an increase in the G content of the alginates was observed, presumably due to selective degradation of less-crosslinked regions. The preserved biomass was kept at a pH below the pK_a of alginate (3.6), but increasing the pH to 4 could potentially still prevent microbial growth and reduce non-specific depolymerization. The effects of storage temperature on alginate depolymerization are of particular importance for outside storage of biomass under different climates. Finally, acid preservation was found to have no negative impact on the cellulose yield and molecular weight, and can contribute to developing applications from components of residual raw material.

CRediT authorship contribution statement

Katharina Nøkling-Eide: Conceptualization, Investigation, Analysis, Visualization, Writing – Original Draft Preparation. **Fangchang Tan:** Investigation, Analysis. **Shennan Wang**: Investigation. **Qi Zhou:** Analysis, Supervision, writing – review & editing. **Mina Gravdahl:** Analysis. **Anne-Mari Langeng:** Investigation, Analysis. **Vincent Bulone:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Finn Lillelund Aachmann:** Analysis, Supervision, Writing – review & editing. **Håvard Sletta:** Funding acquisition, Project administration, Supervision, Writing – review & editing, **Øystein Arlov:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2023.103057.

References

[1] J. Cai, in: Global Status of Seaweed Production, Trade And Utilization, 2021.
[2] J. Cai, A. Lovatelli, J. Aguilar-Manjarrez, L. Cornish, L. Dabbadie, A. Desrochers, S. Diffey, G.E. Garrido, J. Geehan, A. Hurtado, D. Lucente, G. Mair, W. Miao, P. Potin, C. Przybyla, M. Reantaso, R. Roubach, M. Tauati, X. Yuan, Seaweeds and microalgae: an overview for unlocking their potential in global aquaculture development, in: FAO Fisheries and Aquaculture Circular 1229, 2021, https://doi.org/10.4060/cb5670en, 48 pp.
[3] P. Schiener, K.D. Black, M.S. Stanly, D.H. Green, The seasonal variation in the chemical composition of the kelp species Laminaria digitata, Laminaria hyperborea, Saccharina latissima and Alaria esculenta, J. Appl. Phycol. 27 (2015) 363–373, https://doi.org/10.1007/s10811-014-0327-1.
[4] C.M. Duarte, J. Wu, X. Xiao, A. Bruhn, D. Krause-Jensen, Can seaweed farming play a role in climate change mitigation and adaptation? <sb:contribution><sb:title>Front. Mar. </sb:title></sb:contribution><sb:host><sb:issue><sb:series><sb:title>Sci.</sb:title></sb:series></sb:issue></sb:host> 4 (100) (2017) https://doi.org/10.3389/fmars.2017.00100.
[5] D. Krause-Jensen, P. Lavery, O. Serrano, N. Marbà, P. Masque, C.M. Duarte, Sequestration of macroalgal carbon: the elephant in the Blue Carbon room, Biol. Lett. 14 (6) (2018), https://doi.org/10.1098/rsbl.2018.0236.
[6] F.G. Fischer, H. Dörfel, Die polyuronsäuren der Braunalgen (Kohlenhydrate der Algen I), Biol.Chem. 302 (Jahresband) (1955) 186–203, https://doi.org/10.1515/bchm2.1955.302.1-2.186.
[7] P.A.J. Gorin, J.F.T. Spencer, Exocellular alginic acid from Azotobacter vinelandii, Can. J. Chem. 44 (9) (1966) 993–998, https://doi.org/10.1139/v66-147.
[8] A. Haug, Composition And Properties of Alginates, Norwegian Institute of Seaweed Research, 1964. Doctoral dissertation.
[9] O. Smidsrød, A. Haug, A light scattering study of alginate, Acta Chem. Scand. 22 (1968) 797–810.
[10] A. Haug, S. Myklestad, B. Larsen, O. Smidsrød, Correlation between chemical structure and physical properties of alginates, Acta Chem. Scand. 21 (3) (1967) 768–778.
[11] M. Mac Monagail, L. Cornish, L. Morrison, R. Araújo, A.T. Critchley, in: Sustainable harvesting of wild seaweed resources 52(4), 2017, pp. 371–390, https://doi.org/10.1080/09670262.2017.1365273.
[12] P. Stévant, C. Rebours, A. Chapman, Seaweed aquaculture in Norway: recent industrial developments and future perspectives, Aquac. Int. 25 (2017) 1373–1390, https://doi.org/10.1007/s10499-017-0120-7.
[13] S. Enríquez, C.M. Duarte, K. Sand-Jensen, Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content, Oecologia 94 (1993) 457–471, https://doi.org/10.1007/BF00566960.
[14] K. Nøkling-Eide, A.M. Langeng, A. Åslund, F.L. Aachmann, H. Sletta, Ø. Arlov, An assessment of physical and chemical conditions in alginate extraction from two cultivated brown algal species in Norway: Alaria esculenta and saccharina latissima, Algal Res. 69 (2023), 102951, https://doi.org/10.1016/j.algal.2022.102951.
[15] H. Ertesvåg, H.K. Høidal, H. Schjerven, B.I.G. Svanem, S. Valla, Mannuronan C-5-epimerases and their application for in vitro and in vivo design of new alginates useful in biotechnology, Metab. Eng. 1 (3) (1999) 262–269, https://doi.org/10.1006/mben.1999.0130.
[16] A. Tøndervik, O.A. Aarstad, R. Aune, S. Maleki, Philip D. Rye, A. Dessen, G. Skjåk-Bræk, H. Sletta, Exploiting mannuronan C-5 epimerases in commercial alginate production, Mar.Drugs 18 (11) (2020) 565, https://doi.org/10.3390/md18110565.
[17] S.L. Holdt, S. Kraan, Bioactive compounds in seaweed: functional food applications and legislation, J. Appl. Phycol. 23 (2011) 543–597, https://doi.org/10.1007/s10811-010-9632-5.
[18] B. Thomas, M.C. Raj, K.B. Athira, M.H. Rubiyah, J. Joy, A. Moores, G.L. Drisko, C. Sanchez, Nanocellulose, a versatile green platform: from biosources to materials and their applications, Chem. Rev. 118 (2018) 11575–11625, https://doi.org/10.1021/acs.chemrev.7b00627.
[19] A.M. Donald, Polysaccharide crystallization, in: Encyclopedia of Materials: Science And Technology, 2011, pp. 7714–7718, https://doi.org/10.1016/B0-08-043152-6/01385-1.
[20] E. Zanchetta, E. Damergi, B. Patel, T. Borgmeyer, H. Pick, A. Pulgarin, C. Ludwig, Algal cellulose, production and potential use in plastics: challenges and opportunities, Algal Res. (2021) 56, https://doi.org/10.1016/j.algal.2021.102288.
[21] R.S. Baghel, C.R.K. Reddy, R.P. Singh, Seaweed-based cellulose: applications, and future perspectives, Carbohydr. Polym. (2021) 267, https://doi.org/10.1016/j.carbpol.2021.118241.
[22] H. Gao, B. Duan, H. Deng, Y. Du, X. Shi, L. Zhang, Fabrication of cellulose nanofibers from waste brown algae and their potential application as milk thickeners, Food Hydrocoll. 79 (2018) 473–481, https://doi.org/10.1016/j.foodhyd.2018.01.023.
[23] A.J. Onyianta, D. O'Rourke, D. Sun, C.M. Popescu, M. Dorris, High aspect ratio cellulose nanofibrils from macroalgae Laminaria hyperborea cellulose extract via a zero-waste low energy process, Cellulose 27 (2020) 7997–8010, https://doi.org/10.1007/s10570-020-03223-5.
[24] P. Stévant, C. Rebours, Landing facilities for processing of cultivated seaweed biomass: a Norwegian perspective with strategic considerations for the European seaweed industry, J. Appl. Phycol. 33 (2021) 3199–3214, https://doi.org/10.1007/s10811-021-02525-w.
[25] I.S. Sandbakken, M. Sæther, J. Funderud, I.M. Aasen, Acid preservation of Saccharina latissima for application as a carbon source for fermentation to biofuels and chemicals, J. Appl. Phycol. 30 (2018) 3581–3588, https://doi.org/10.1007/s10811-018-1489-z.
[26] C. Herrmann, J. FitzGerald, R. O'Shea, A. Xia, P. O'Kiely, J.D. Murphy, in: Ensiling of seaweed for a seaweed biofuel industry 196, 2015, pp. 301–313, https://doi.org/10.1016/j.biortech.2015.07.098.
[27] A.R.J. Cabrita, M.R.G. Maia, I. Sousa-Pinto, A.J.M. Fonseca, Ensilage of seaweeds from an integrated multi-trophic aquaculture system, Algal Res. 24 (2017) 290–298, https://doi.org/10.1016/j.algal.2017.04.024.
[28] M. Campbell, J. Ortuño, L. Ford, D.R. Davies, A. Koidis, P.J. Walsh, K. Theodoridou, The effect of ensiling on the nutritional composition and fermentation characteristics of brown seaweeds as a ruminant feed ingredient, Animals 10 (6) (2020), https://doi.org/10.3390/ani10061019.
[29] M.I. Bilan, A.A. Grachev, A.S. Shashkov, M. Kelly, C.J. Sanderson, N.E. Nifantiev, A.I. Usov, Further studies on the composition and structure of a fucoidan preparation from the brown alga Saccharina latissima, Carbohydr. Res. 345 (14) (2010) 2038–2047, https://doi.org/10.1016/j.carres.2010.07.009.
[30] G. Kopplin, A.M. Rokstad, H. Mélida, V. Bulone, G. Skjåk-Bræk, F.L. Aachmann, Structural characterization of fucoidan from Laminaria hyperborea: assessment of coagulation and inflammatory properties and their structure–function relationship, ACS Appl.Bio Mater. 1 (6) (2018) 1880–1892, https://doi.org/10.1021/acsabm.8b00436.
[31] S. Forbord, S. Matsson, G.E. Brodahl, B.A. Bluhm, O.J. Broch, A. Handå, A. Metaxas, J. Skjermo, K.B. Steinhovden, Y. Olsen, Latitudinal, seasonal and depth-dependent variation in growth, chemical composition and biofouling of cultivated Saccharina latissima (Phaeophyceae) along the Norwegian coast, J. Appl. Phycol. 32 (2020) 2215–2232, https://doi.org/10.1007/s10811-020-02038-y.
[32] H. Ertesvåg, G. Skjåk-Bræk, Modification of alginate using mannuronan C-5-epimerases, in: C. Bucke (Ed.), Carbohydrate Biotechnology Protocols. Methods in Biotechnology™, vol. 10, Humana Press, 1999, https://doi.org/10.1007/978-1-59259-261-6_6.
[33] S. Saji, A. Hebden, P. Goswami, C. Du, A brief review on the development of alginate extraction process and its sustainability, Sustainability 14 (9) (2022), https://doi.org/10.3390/su14095181.
[34] B. Capon, Intramolecular catalysis in glucoside hydrolysis, Tetrahedron Lett. 4 (14) (1963) 911–913, https://doi.org/10.1016/S0040-4039(01)90742-7.
[35] A. Haug, B. Larsen, O. Smidsrød, The degradation of alginates at different pH values, Acta Chem. Scand. 17 (5) (1963) 1466–1468.
[36] O. Smidsrød, A. Haug, B. Larsen, The influence of pH on the rate of hydrolysis of acidic polysaccharides, Acta Chem. Scand. 20 (1966) 1026–1034.
[37] A. Martinsen, G. Skjåk-Bræk, O. Smidsrød, Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate gel beads, Biotechnol. Bioeng. 33 (1989) 79–89, https://doi.org/10.1002/bit.260330111.
[38] G. Skjåk-Bræk, I. Donati, S. Paoletti, Alginate hydrogels: properties and applications, in: P. Matricardi, F. Alhaique, T. Coviello (Eds.), Polysaccharide Hydrogels: Characterization And Biomedical Applications, Pan Stanford Publishing, 2015.
[39] C. Campa, S. Holtan, N. Nilsen, T.M. Bjerkan, B.T. Stokke, G. Skjåk-Bræk, Biochemical analysis of the processive mechanism for epimerization of alginate by mannuronan C-5 epimerase AlgE4, Biochem. J. 381 (1) (2004) 155–164, https://doi.org/10.1042/BJ20031265.

[40] S. Ballance, S. Holtan, O.A. Aarstad, P. Sikorski, G. Skjåk-Bræk, B.E. Christensen, Application of high-performance anion-exchange chromatography with pulsed amperometric detection and statistical analysis to study oligosaccharide distributions – a complementary method to investigate the structure and some properties of alginates, J. Chromatogr. A 1093 (1–2) (2005) 59–68, https://doi.org/10.1016/j.chroma.2005.07.051.

[41] S. Holtan, Q. Zhang, W.I. Strand, Characterization of the hydrolysis mechanism of polyalternating alginate in weak acid and assignment of the resulting MG-oligosaccharides by NMR spectroscopy and ESI−mass spectrometry, Biomacromolecules 7 (7) (2006) 2108–2121, https://doi.org/10.1021/bm050984q.

[42] V. Cebrián-Lloret, M. Metz, A. Martínez-Abad, S.H. Knutsen, S. Ballance, A. López-Rubio, M. Martínez-Sanz, Valorization of alginate-extracted seaweed biomass for the development of cellulose-based packaging films, Algal Res. (2022) 61, https://doi.org/10.1016/j.algal.2021.102576.