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Sequential extraction and fractionation of four polysaccharides from cultivated brown algae *Saccharina latissima* and *Alaria esculenta*

Paulina S. Birgersson [a], Maren Oftebro [a], Wenche I. Strand [a], Olav A. Aarstad [a], Gerd Inger Sætrom [a], Håvard Sletta [b], Øystein Arlov [b], Finn Lillelund Aachmann [a,*]

[a] *Norwegian Biopolymer Laboratory (NOBIPOL), Department of Biotechnology and Food Science, NTNU Norwegian University of Science and Technology, Sem Sælands vei 6/8, N-7491 Trondheim, Norway*

[b] *Department of Biotechnology and Nanomedicine, SINTEF Industry, Richard Birkelands vei 3 B, NO 7491 Trondheim, Norway*

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ABSTRACT

Norway has a well-established seaweed industry based on extraction of alginates from wild harvested brown algae, *Laminaria hyperborea* (*LH*). However, further expansion of wild harvest is limited, and cultivated seaweed can contribute to meeting increasing demands for high value compounds in an environmentally sustainable manner. Herein, an integrated process for retrieving alginate, fucoidan, laminarin and cellulose was established for cultivated *Saccharina latissima* (*SL*) and *Alaria esculenta* (*AE*). Focus was to develop a process using mild chemical methods that do not compromise the molecular weights (MW) of the polysaccharides, particularly with respect to alginate. Response surface methodology (RSM) was applied to optimize the yield of fucoidan and laminarin and assess the effect of the applied extraction parameters: pH, temperature, and time, on the MW of subsequently extracted alginates. RSM confirmed that high yields of fucoidan and laminarin can be achieved while maintaining the alginate MW. Optimized conditions were applied in a pilot scale process, where all four polysaccharides were extracted. Purity (ranging from 40 up to 98 %) and composition of the components were evaluated combining several analytical techniques. Total yields of the polysaccharides were 23.4 % of the entering dry biomass from *SL* and 26.3 % from *AE*, representing around 55 % of the estimated maximal yield. However, all extractions were not optimized, and a mass balance revealed that <75 % more alginates could have been collected. The process can be adapted to simultaneously collect mannitol, which accounted for <15 % of the dry weight. The alginates were further examined by producing hydrogels, showing that the gel properties are comparable to alginates from *LH* fronds. The study demonstrated that mild chemical extraction techniques can be combined to extract all four polysaccharides from *SL* and *AE*, providing a foundation for a multicomponent biorefinery using cultivated brown algae.

1. Introduction

Macroalgae are found on rocky substrates or freely floating along coast lines all over the globe, from the Arctic to the Antarctic [1]. Currently, over 30 million tons of cultivated and wild seaweeds are harvested annually worldwide [2,3], and approximately 40 % of those are brown algae (*Phaeophyceae*) [3]. Compared to Asia, being the leading macroalgal producer, the algae industry and aquaculture in Europe is still in its infancy and only contributes with 0.57 % to the total global production. In Europe, 98 % of the harvested biomass originates from wild stocks, as opposed to the worldwide production where 97 % is based on aquaculture [2]. Cultivation of seaweed has many advantages compared to agriculture of terrestrials crops since it does not require arable land, irrigation, pesticides or fertilizers [4]. Additionally, the growth rates of seaweeds are generally higher than for terrestrial crops, such as rice, wheat [5], corn or switchgrass [6], which makes them good candidates for sophisticated multicomponent biorefineries. Cultivated seaweed can contribute to meeting increasing demands for alginate and other high value compounds, since large parts of the wild biomass populations are inaccessible for harvesting due to the topography of the seabed [7]. Furthermore, it is widely acknowledged that increased aquaculture will be necessary to meet the increasing demand for hydrocolloids and other products from seaweed, without over-exploiting wild seaweed resources [2].

* Corresponding author.
E-mail address: finn.l.aachmann@ntnu.no (F.L. Aachmann).

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Norway has the second longest coastline in the world [8] and is one of the largest seaweed biomass producers in Europe [2]. Norwegian seaweed industry today relies mainly on extraction of alginates from wild harvested *Laminaria hyperborea* (*LH*). Alginates are a group of linear copolymers composed of (1 → 4) linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) [9]. Alginates have a wide range of commercial applications due to possession of high intrinsic viscosity, water-binding capacity and the ability to form hydrogels [10]. However, alginates only account for around 15–40 % of the dry weight of brown algae, depending on species, seasonal fluctuations in the biomass composition as well as environmental conditions and maturity [11]. No substantial valorization of the remaining biomass is currently performed, leaving multiple side streams containing potential valuable polysaccharides and other components unexploited. Retrieval and application of multiple products while generating minimal waste will be necessary to improve the environmental and economic sustainability of seaweed biorefineries [12]. In addition to low-grade and high-volume products, at least one high-value chemical or material should be produced [13]. For seaweed biorefineries focusing on chemical production, polysaccharides are of main interest [3].

Apart from alginates, which are the most prevalent of the polysaccharides, all brown algae also contain cellulose and fucoidans [3]. Together, these three polysaccharides constitute the main building blocks of the algal cell wall [14]. Cellulose is a linear homopolymer composed of (1 → 4) linked β-D-glucose units [15], while fucoidan is a family of heterogenous fucose-rich sulfated polysaccharides with diverse structural compositions, often comprising several other sugar units and/or sugar acids [16]. Within the *Laminaria*, *Saccharina* and *Fucales* genera, a fourth polysaccharide, laminarin, can be found inside the cell vacuoles of the fronds. Laminarin is a low molecular weight biopolymer, with a linear β-(1 → 3)-glucan backbone having occasional β-(1 → 6)-branches and/or mannitol-substituents at the reducing end [17]. Cellulose has great commercial value, yet the cellulose industry relies entirely on terrestrial lignocellulosic feedstock from mainly wood and cotton. Macroalgal cellulose possesses many interesting features, due to absence of lignin [4] and high crystallinity compared to terrestrial cellulose [18,19]. Fucoidan and laminarin have in recent years been increasingly recognized for their potential diverse bioactivities [16,17], including anti-tumor, anti-apoptotic, anti-inflammatory, anti-coagulating, anti-viral etc. [20–22].

The first protocol for alginate extraction was developed and patented by Stanford in 1881 [23]. Although adaptions have been made, the general approach for alginate extraction remains the same. First, an acidic pre-treatment of the biomass is performed, followed by an alkaline incubation with sodium hydroxide or sodium carbonate to gain soluble Na-alginates [9,24]. Fucoidan and laminarin are water soluble biopolymers often extracted using mild to moderate acidic conditions, combined with elevated temperatures and water as solvent [17,25,26]. Most of the fucoidan and laminarin are therefore expected to be extracted during the acidic pre-treatment, while the residual biomass after alginate extraction mainly consists of a color-rich cellulose-protein complex [27,28]. Purified algal cellulose can be obtained using acidified sodium chlorite/hypochlorite bleaching [18,29] or alkaline hydrogen peroxide (H_2O_2) bleaching [30].

We propose a sequential extraction strategy that combines the above-mentioned methods, to extract and separate the four main polysaccharides: fucoidan, laminarin, alginate and cellulose in cultivated *Saccharina latissima* (SL) and *Alaria esculenta* (AE). Fresh biomass was used in the study to increase the relevance of the results for an industrial processing line. The aim was to develop a process using mild chemical methods that preserve the quality of the extracted components, particularly with respect to alginates. Alginates are prone to hydrolysis when influenced by low pH combined with elevated temperatures [9], and the molecular weight (MW) of alginates strongly correlates with their gel-forming and viscosifying properties [10]. Therefore, screening-trials using response surface methodology (RSM) was applied. The examined relationships by RSM were pH, time (t) and temperature (T) applied during an acidic pre-extraction of fucoidan and laminarin (Fuc/Lam), and the MW of consecutively extracted alginates as well as the yield of Fuc/Lam. The optimized conditions were used in pilot-scale extractions to sequentially extract all four polysaccharides. A mass balance (MB) covering the pilot-scale extraction was determined to identify possible valuable waste streams. To ensure high quality of the polysaccharides, a physiochemical characterization of the extracted fractions was made, and the gel-forming properties of the alginates were evaluated.

2. Materials and methods

2.1. Biomass supply

The cultivated seaweed used in this study was collected at the Seaweed Solutions (SES) farm located at Frøya in Norway (N 63°44.6720437′, E 8°53.1976789′). The seaweed was cultivated on ropes 2 m below the sea surface in euhaline (>30 PSU) and highly wave exposed water. All sporelings were deployed in the sea in January 2021. The biomass used in the screening trials was harvested on 26/04/21 and 12/05/21 for *SL* and *AE*, respectively. *AE* used in the pilot scale trials were harvested on 28/05/21 while *SL* were harvested on 07/06/2021. Following harvest, the seaweed was stored cold (4 °C) for 12–72 h prior to extraction experiments.

2.2. Sequential extraction of polysaccharides

2.2.1. Screening trials of fucoidan and laminarin extraction conditions

2.2.1.1. Experimental design. The selected independent variables of interest in the Fuc/Lam extraction were pH (3.5–5.5), T (50–70 °C) and t (1−3 h) and the analyzed responses were total yield (% dw) of crude Fuc/Lam and the MW (kDa) of the subsequently extracted alginates. Box-Behnken Design (BBD) was applied to reduce the number of experiments. For three factors (k = 3) on three levels (−1, 0, 1), the graphical representation of BBD can be described as a cube consisting of the central point and the middle points of the edges (see Fig. S1. Supplementary materials) [31]. The complete experimental design (Table 1) consisted of 15 experiments in total, including three replicates of the center point to estimate the experimental error (s^2).

The statistical software Minitab® (version 21.1) was used for all calculations. Initially, a full quadratic model was applied with a two-sided confidence interval. The over-determined system is solved using a least squares method where the coefficients of a model function (i.e. the response surface) is adjusted to optimally fit a data set (i.e. the re-

Table 1
Experimental design (BBD) used to determine the relationship between the independent variables (pH, T and t) and the responses Fuc/Lam yield (% dw) and MW of alginates in the screening trials. Real values: pH (3.5, 4.5, 5.5), T (50, 60, 70 °C) and t (1, 2, 3 h) together with coded values (−1, 0, 1) in brackets.

Experimental order	pH	T(°C)	t (h)
1	3.5 (−1)	50 (−1)	2 (0)
2	5.5 (+1)	50 (−1)	2 (0)
3	3.5 (−1)	70 (+1)	2 (0)
4	5.5 (+1)	70 (+1)	2 (0)
5	3.5 (−1)	60 (0)	1 (−1)
6	5.5 (+1)	60 (0)	1 (−1)
7	3.5 (−1)	60 (0)	3 (+1)
8	5.5 (+1)	60 (0)	3 (+1)
9	4.5 (0)	50 (−1)	1 (−1)
10	4.5 (0)	70 (+1)	1 (−1)
11	4.5 (0)	50 (−1)	3 (+1)
12	4.5 (0)	70 (+1)	3 (+1)
13–15	4.5 (0)	60 (0)	2 (0)

sults of the experiments) [32]. The obtained initial model follows the general equation:

$$y = const. + \sum_{1}^{k} \beta_i x_i + \sum_{1}^{k} \beta_{ii} x_i^2 + \sum_{i>j} \sum \beta_{ij} x_i x_j$$

To evaluate the relevance of the regression models ANOVA was conducted to determine the model significances (P_s), lack-of-fit (P_L) and significances (p) of all variables. Furthermore, the coefficient of determination (R^2) and adjusted R^2 (R^2-adj.) were used to determine the proportion of the variance in the dependent variable explained by the independent variable. Predicted R^2 (R^2-pred.) was calculated to evaluate if the models can be used to perform accurate predictions. The models are considered relevant if $P_s < 0.05$ and $P_L > 0.05$ [33]. According to guidelines developed by Lundstedt et al., for models containing data of chemical nature R^2 values ≥ 0.8 is considered acceptable, while R^2-pred. values > 0.8 is considered excellent and ≥ 0.5 acceptable [34].

2.2.1.2. Extraction procedure. Freshly harvested *SL* and *AE* were cut into approximately 1 × 1 cm pieces. 150 mL of deionized (DI) water was added to 100 g of biomass, and pH, T and t was set according to the experimental design (Table 1). pH was adjusted using 1 M HCl. The samples were placed in a water-bath with shaking at 60 rpm. At the end of the reaction the pH was neutralized using 1 M NaOH and the water phase and biomass were separated using a Büchner filtration set-up. The water phase was dialyzed with Spectra/Por®3 dialysis membranes with 3.5 kDa molecular weight cut-off (MWCO) and DI-water to remove low MW compounds such as mannitol and salts. Thereafter, the crude Fuc/Lam fraction was collected and lyophilized.

Remaining seaweed residues were used to extract alginates. 230 mL 0.2 M HCl were added per 100 g of residues and incubated at room temperature (RT) with 120 rpm shaking for 4 h. The acid (aq) was discarded, and the residues washed with 230 mL DI-water. Next, 230 mL of 0.2 M $NaHCO_3$ per 100 g residues was added, incubated at RT, 120 rpm shaking, 12 h. Crude alginate fractions were obtained by centrifugation (10,000*g*, 10 min) and collection of the supernatant. Three additional crude extracts were collected and combined, by sequentially adding 120-, 100- and 40-mL DI-water and repeating centrifugation and collection of the supernatants between each addition. Alginates were precipitated by adding NaCl (0.2 % w/v) and 96 % EtOH in a 1:1 ratio to the supernatant. Solvents were removed, and the precipitate washed three times with 100 mL 70 % EtOH and one time with 96 % EtOH before air drying.

2.2.2. Pilot-scale extraction of polysaccharides from SL and AE

The chemical extraction of polysaccharides from *SL* and *AE* was performed in a 100 L steel tank (Fig. 1) with a two bladed stirrer and a heating mantle. All extraction steps were performed with tap water if not stated otherwise.

2.2.2.1. Extraction of crude Fuc/Lam. In two separate experiments, 17.16 kg of fresh harvested *SL* and 18.99 kg of *AE* were cut into 5 × 5 cm pieces and transferred to the processing tank. 30 L of water and 1 M HCl was added to each biomass and pH, t, and T were adjusted according to the optimized conditions: pH = 4.5, t = 3 h and T = 50 °C. After 3 h the supernatant containing crude Fuc/Lam was removed. Approximately 15 L of tap water were added and mixed thoroughly with the biomass before being removed to gain more of the diffused Fuc/Lam. The crude fractions were neutralized using 1 M NaOH and stored at −20 °C prior to further separation and purification.

2.2.2.2. Alginate extraction. To convert the insoluble Ca-alginates into water-soluble Na-alginates, 0.2 M HCl was first added to the residual *SL* biomass (30 L, 60 rpm stirring, 12 h, RT). The acid was removed, and the biomass washed twice with tap water (30 L) before addition of 0.2 M $NaHCO_3$ (30 L, 12 h, RT). This resulted in a partial dissolution of the seaweed and a viscous mass that could not be separated by sieving. To collect the alginate-rich supernatant, the mass was centrifuged (10,000*g*, 10 min). The pellet was washed with tap water (15 L) and centrifuged, and the supernatant was combined with the first fraction. To remove impurities (including residual fucoidans), the alginates were precipitated first in acid, then redissolved and precipitated in EtOH. This was performed by firstly reducing the pH to 2, using HCl to precipitate the alginates. Thereafter, the acidic supernatant was removed, and the alginates washed once using 17 L of HCl (aq), pH < 2.5. Then, the alginates were redissolved by adding 15 L of tap water and 1 M NaOH (aq) until reaching a neutral pH. At last, EtOH precipitation and washing were performed as described in the screening trials.

Fig. 1. Processing tank used for the sequential extractions: 100 L AISI 904L stainless steel tank, with two bladed stirrer and heating mantle, custom built by Skala Fabrikk, Norway.

2.2.2.3. Cellulose extraction. Five kg of the remaining residual biomass were washed with 96 % EtOH (10 L, 12 h, RT). EtOH was discarded, and residues washed twice with in total 25 L of water. Bleaching to obtain cellulose was carried out using a similar method as suggested by Wahlström and coworkers [4] developed for the green macroalgae *Ulva fenestrata*, with some modifications. The residues were suspended in 15 L, 4 % hydrogen peroxide (H_2O_2), pH was adjusted to 10 using 1 M NaOH and heated at 50 °C for 24 h. The liquid fraction was removed, and residues soaked in 10 L of 96 % EtOH for 30 min to remove remaining colorants. Separation was performed using Büchner filtration (grade 113 Whatman filter) and EtOH-washing (5 L) was repeated until all visible colorants were removed. The cellulose fraction was soaked in 5 L of 0.5 M NaOH at 50 °C for 3 h. Alkali was removed using Büchner filtration and the procedure were repeated using 5 L 1 M HCl at 50 °C, 12 h. The purified cellulose was washed five times with 5 L DI-water.

For residual *AE* biomass, the cellulose extraction protocol was modified slightly in that no additional water wash (gaining fractions C2 and C3 in Fig. 2) was performed prior cellulose extraction. Furthermore,

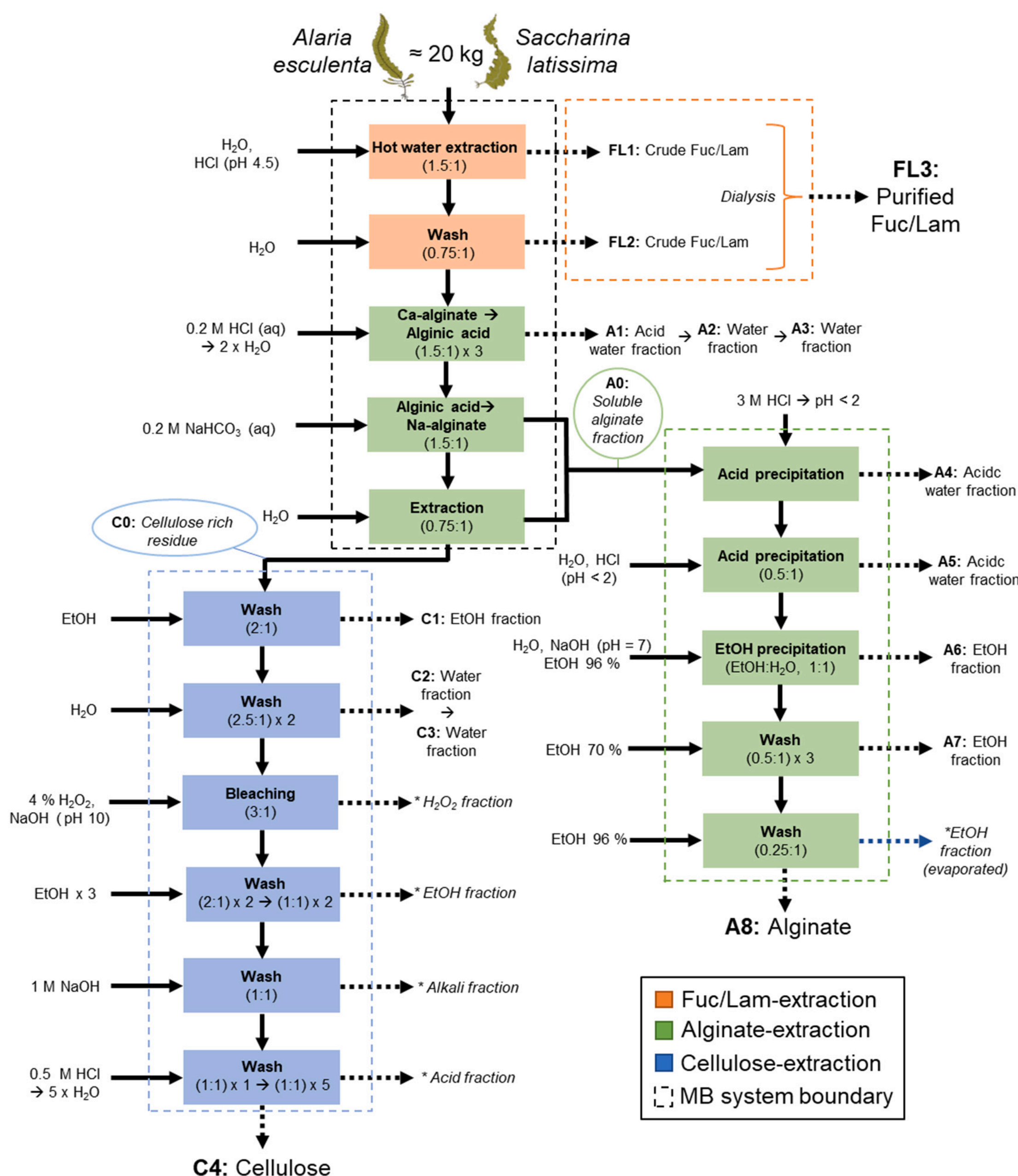

Fig. 2. Flow diagram illustrating the sequential pilot-scale extraction of the four polysaccharides: fucoidan, laminarin, alginate and cellulose. The ratio of entering biomass:solvent is expressed in brackets. The indexes FL1-3, A0-8, and C0-4 describe the sampling points for the mass balances (MBs). Outflows marked with asterisks were not collected for the MBs.

pre-trials had shown that extraction of cellulose from *AE* required harsher chemical treatment to remove pigments (results not presented here). Thus, the duration of the H_2O_2-bleaching was increased from 24 to 48 h and the additional alkali wash was removed.

2.2.3. Mass balance of the pilot-scale processing of *Saccharina latissima*

The sample points for the mass balance are presented in Fig. 2. All in- and outflows were recorded during extraction of crude Fuc/Lam (FL1-FL3) and purified alginates (A0-A8), while only the first three fractions during cellulose extraction (C0-C3 and C4) were collected. For each sample point the total mass flow (g) and dry weight (dw) was determined as well as the fraction of organic material (OM (% dw)) in the dry matter. To determine dw, the collected samples were freeze dried and then oven dried at 105 °C (Eq. (1)) and the fraction of OM was obtained by determining the ash content (Eq. (2)) in duplicate samples.

$$DW\ (\%) = \frac{Dry\ mass\ after\ freeze\ drying\ (\mathrm{g})}{Wet\ mass\ (\mathrm{g})} \times \frac{Dry\ mass\ after\ oven\ drying\ (\mathrm{g})}{Dry\ mass\ after\ freeze\ drying\ (\mathrm{g})} \times 100\% \tag{1}$$

$$OM\ (\%) = \frac{DW\ (g) - Ash\ (g)}{DW\ (g)} \times 100\% \quad (2)$$

Mass balances were analyzed over four different system boundaries: 1) separation of the polysaccharides into crude Fuc/Lam, crude alginate and crude cellulose, 2) alginate precipitation and purification, 3) purification of crude Fuc/Lam, FL1 and FL2, by dialysis (pore size, 3.5 kDa), and 4) cellulose extraction from cellulose rich seaweed residues.

2.2.4. Fractionation of fucoidan and laminarin from crude Fuc/Lam

Precipitation of laminarin followed by tangential-flow filtration (TFF), sometimes referred as crossflow filtration, was used to separate fucoidan and laminarin from 1 L of crude FL1. To enhance precipitation of laminarin, samples were placed on an ice-bath and stored at 4 °C for 48 h. The samples were centrifuged at 10,000*g*, 10 min, 0 °C to collect the precipitate. Only FL1 from *AE* resulted in sufficient precipitate for further purification. The precipitate from *AE* was washed 3 times with 400 mL of 96 % EtOH, then re-dissolved in ion-free water and freeze dried.

TFF was performed using Millipores Pellicon 2 Mini Biomax® membranes made of modified polyethersulfone (PES). The nominal molecular weight cut-offs (MWCO) provided by the manufacturer were 10 kDa and 50 kDa. A Millipore Masterflex I/P Peristalitic pump was used to pump the feed. The feed-flux was kept constant at 1.2 L/min and the initial transmembrane pressure was set to 0.5 bar. Changes in pressure were not recorded. Firstly, the volume was reduced by 50 %, then DI-water was used as buffer and added to the sample feed reservoir at the same rate as the filtrate was generated. In total 8 filtration volumes (FV) were added, but only the first 2.5 permeate FV were collected, dialyzed (Spectra/Por®3 dialysis membrane, 3.5 kDa MWCO, DI-water) and freeze-dried. The retentate was directly freeze-dried. To prevent remaining laminarin from precipitating, the samples were kept in a water bath at 50 °C.

The supernatant (after precipitation) from *AE* was fractionated with a 50 kDa cassettes. The laminarin from SL was separated using 10 kDa cassettes for 2.5 FV. The permeate (containing laminarin) were collected, dialyzed, and freeze dried, while the remaining FVs of the retentate was filtered with a 50 kDa cassette to separate fucoidan.

2.2.5. Preparation and characterization of alginate hydrogels

Alginate solutions were mixed with $CaCO_3$ (4 µm particle size) and degassed for 10 min before addition of glucono-δ-lactone (GDL) to final concentrations of 1 % alginate, 15 mM $CaCO_3$, and 30 mM GDL [35]. The solution was transferred to 24 well plates and set to hydrogel for 24 h and thereafter saturated in 50 mM $CaCl_2$ with 200 mM NaCl for 24 h at 4 °C.

Measurements of gradient and rupture strength were performed using a texture analyzer (Stable Micro Systems, UK) with a P/35 flat probe. A 5 kg load cell with trigger force of 1 g was used for the gradient measurements, and a 30 kg load cell with trigger force of 5 g for the rupture strength. The Youngs modulus, E, was calculated from the initial slope of the gradient curves [36]. Syneresis (%) was determined by weighing the hydrogel at the end of saturation after removing excess water (w) and relating this value to the original gelling weight (w_0) (Eq. (3)).

$$Syneresis\ (\%) = \frac{w_0 - w}{w_0} \times 100\% \quad (3)$$

The properties of the hydrogels produced by the extracted alginates from *SL* and *AE* were compared to hydrogels produced from a commercial alginate extracted from *LH* fronds provided by FMC Biopolymer A/S.

2.3. Characterization

2.3.1. NMR spectroscopy

All ^{1}H NMR experiments were acquired on a BRUKER NEO 600 MHz equipped with 5 mm iProbe TBO (Bruker BioSpin AG, Fälladen, Switzerland) and recorded at 83 °C. The spectra were recorded using TopSpin 4.0.8 software (Bruker BioSpin) and processed and analyzed with TopSpin 4.0.7 software (Bruker BioSpin).

Alginate samples were prepared by stepwise acid hydrolysis and freeze-dried prior to analysis as previously described [37]. Approximately 12–15 mg of the degraded samples was dissolved in 600 µL D_2O (d-99.9 % Sigma-Aldrich). 3-(Trimethylsilyl)-propionic-acid sodium salt (TSP) (Aldrich, Milwaukee, WI) in D_2O (1 %, 5 µL) was added for internal chemical shift reference, and triethylenetetramine-hexaacetic acid (TTHA) (Sigma-Aldrich) in D_2O (0.3 M, 20 µL) was added as chelator. Fucoidan and laminarin were prepared by dissolving 5–10 mg sample in 600 µL D_2O (d-99.9 % Sigma-Aldrich). For chemical shift reference 1 µL 1 % TSP is added to the sample. Volumes of 600 µL or 160 µL of the sample were transferred into 5 mm or 3 mm NMR tube, respectively.

2.3.2. SEC-MALLS

Molar masses of the alginates were analyzed using size-exclusion chromatography (SEC) with online multi-angle static laser light scattering (MALLS). The analyses were performed at ambient temperature on an HPLC system consisting of a solvent reservoir, on-line degasser, HPLC isocratic pump, automatic sample injector, serially connected OHpak LB-G 6Bguard column and OHpak LB 806 M main columns (Shodex). The column outlet was connected to a Dawn HELEOS-II multi-angle laser light scattering photometer (Wyatt, U.S.A.) ($\lambda_0 = 663.8$ nm) followed by a Shodex RI-501 differential refractometer. The eluent was 0.15 M NaNO3/0.01 M EDTA (pH = 6.0) and the flow rate was 0.5 mL/min. Samples (0.5 mg/mL) were filtered (pore size 0.45 µm) before injection. As a control, the polysaccharides dextran (MW 10 kDa) and pullulan (MW 137 kDa) were run in the same sequence as the samples. The injection volume was 50–100 µL. Data were collected and processed (with dn/dc = 0.150 mL/g and $A^2 = 5 \cdot 10^{-3}$ mL·mol/g^2) using the Astra (v. 7.3.2.21) software (Wyatt, U.S.A.).

2.3.3. Monosaccharide analysis

Dried samples (10–50 mg) were grinded with a mortar before wetting in 0.5 mL 12 M sulfuric acid, for 60 min at 30 °C. DI-water was added to a concentration of 2 M sulfuric acid and the sample was hydrolysed at 100 °C for 4 h. 6 mL of DI-water was added and 180 µL were transferred to an Eppendorf tube together with 850 µL of 0.15 M $Ba(OH)_2$ to neutralize the samples and remove free sulfates. A factor to note is that the amount of $Ba(OH)_2$ is not necessarily stoichiometric but dependent on the age of the $Ba(OH)_2$ solution due to accumulation of carbonate. For a freshly made solution it was necessary to reduce the amount to 750 µL since epimerization of glucose to mannose was otherwise observed at pH 10.5 (some of the initially analyzed samples might have been subjected to a high pH, leading to an overestimation of the amount of mannose and underestimation of glucose). The samples were centrifuged for 10 min at 2000*g*. Prior to analysis, the supernatant was diluted with DI-water 1–20 times to obtain monosaccharide concentrations within levels of the standard curve.

The samples were analyzed using high-performance anion-exchange chromatography (HPAEC) with pulsed electrochemical detector (PAD) on a Dionex ICS 5000+ system (Thermo Scientific) with a 4 × 250 mm CarboPac SA10 main column and 4 × 50 mm SA10 guard. 25 µL sample was injected and eluted with 1 mM NaOH and a flow rate of 1.2 mL/min at 28 °C (complete elution conditions are given in Supplementary materials, Table S4). Post column addition of 0.4 M NaOH, 0.3 mL/min from a LC-20Ai pump was used to give a concentration of 80 mM NaOH to ensure high and stable detector response. A monosaccharide std. mix (mannitol, fucose, arabinose, galactose, rhamnose, glucose, mannose,

Table 2
Factors correcting for degradation of monosaccharides during acid hydrolysis.

Monosaccharide	Mannitol	Fuc	Ara	Gal	Rha	Glc	Xyl	Man
Corr. fact.	1.00	1.13	1.11	1.12	1.00	1.19	1.57	1.16

xylose) from 0.1 to 10 mg/L were analyzed before and after the samples for quantification. Data was collected and processed with Chromeleon 7.2 software. Factors correcting for the degradation of released monosaccharides during hydrolysis (Table 2) has previously been determined as the ratio of the peak areas for 5 mg/L standard before and after hydrolysis and was included when calculating the eluted weights (details in Supplementary materials, Section S2.2).

2.3.4. Element analysis and estimation of contaminants using CNS and ICP-MS

The contents of nitrogen (N) and sulfur (S) in the raw biomass and the extracted polysaccharides were determined using a Vario-El-Cube CNS element analyzer (Elementar). Approximately 5 mg of dried samples were weighed out in tin capsules and oxidized at 1150 °C. A conversion factor of 2.5 was used to convert the S into estimated sulfite (-SO_3^-). The amount of protein in the samples was estimated using a nitrogen-to-protein conversion factor determined for cultivated *SL* of 3.8 [8]. To determine the concentration of the elements Na, Mg, P, S, K, Ca, Fe and Cu co-extracted during the fucoidan-laminarin extraction and in the waste streams during alginate extraction, an Agilent 8800 Triple Quadropole ICP-MS with SPS 4 Autosampler was used. In the raw biomass the additional elements Cl, Br and I were also analyzed. 200 mg freeze dried samples were digested with 5 mL 50 % (v/v) nitric acid (HNO_3) at 250 °C in an UltraWAVE microwave oven (Milestone, Italy) and diluted to 5 % (v/v) upon analysis. Samples for Cl, Br and I analysis were extracted in parallel with 5 mL 20 % (v/v) TMAH at 80 °C in a bead bath overnight and diluted to 1 % (v/v) upon analysis.

The amount of S analyzed by ICP-MS was used to estimate the amount of fucoidan in the alginate waste streams. According to structural elucidation of fucoidan from *SL*, performed by Bilan et al. [38], the main fraction comprises fucose and galactose in a molar ratio of 5:1, with approximately 1.2 -SO_3Na per sugar unit. Estimating the amount of fucoidan using the structure suggested by Bilan et al. one arrives at a sulfur-to-fucoidan conversion factor of 8.5.

3. Results & discussion

3.1. Consolidated polysaccharide extraction

Cultivated seaweed biomass is currently a lot more expensive than wild harvested. Hence, to increase the valorization of cultivated *SL* and *AE*, a sequential extraction process that besides alginate recovers the additional polysaccharides fucoidan, laminarin and cellulose, was developed.

The conventional acidic pre-treatment (pH < 2) executed prior to alginate extraction facilitates the conversion of Ca-alginates to soluble Na-alginates [9,24], while also washing out large fractions of water-soluble minerals and organic compounds, including fucoidan and laminarin. Recovery of fucoidan and laminarin was, however, not considered feasible within this step for two reasons. Firstly, preliminary experiments have shown that a temperature above 40 °C is needed to extract linear laminarins (results not presented), whereas branched laminarins can be obtained at lower temperatures [3]. Secondly, combining high temperature and low pH might induce hydrolysis of the polysaccharides, not least alginates [9]. Likewise, moderate to mild acid concentration and high temperature causes chain scission of fucoidan [39,40], likely induced by the sulfate groups on C2, which are in ideal position to protonate the oxygen on the glycosidic bond [41]. Still, acids are expected to enhance the extraction of soluble polysaccharides due to protons interfering with hydrogen bonds within the cell wall [25]. Therefore, an additional warm water extraction at moderately low pH (3.5–5.5) was introduced prior to the acid wash at pH < 2 performed in room temperature.

Previous research has demonstrated that the yield of fucoidan [26,33,42,43] as well as the MW of consecutively extracted alginates, are affected by the applied conditions (i.e. time, temperature and/or pH) during fucoidan extraction [33]. To examine this relationship and maximize the yield of Fuc/Lam while simultaneously obtaining alginates with satisfactory MW, RSM (Response Surface Methodology) was applied. To reduce the numbers of experiments, fractional factorial BBD (Box-Behnken Design) was used [44]. Extraction conditions that would gain high MW alginates and provide high yields of Fuc/Lam, was hypothesized to be found within the following condition ranges: T (50–70 °C), t (1–3 h) and pH (3.5–5.5). To limit the number of variables the alginate extraction was performed with a pre-defined set of processing conditions (Section 2.2.1).

According to literature [15], cellulose is not susceptible to degradation by the conditions applied in the Fuc/Lam and alginate extraction and was not included in the analysis.

3.2. Effect of processing conditions on the yield of Fuc/Lam and MW of extracted alginates

The yields of Fuc/Lam as well as the MWs of the alginates for all treatment series are shown in Table 3. Monosaccharide analysis of the Fuc/Lam-fractions revealed that the samples still contained varying amounts of mannitol (0.4–6.1 %) after dialysis. This part was therefore subtracted from the yields. The crude fractions from *SL* contained higher amounts of fucose (74.2 ± 4.6 % of the monosaccharides, Table S2, Supplementary materials) compared to *AE* (19.1 ± 9.1 % Table S3), while the relationship was the opposite for glucose (9.7 ± 1.3 % and 45.4 ± 9.3 %, for *SL* and *AE* respectively).

To refine the regression models describing the relationships between the independent variables (pH, T (°C) and t (h)) and the response variable (Fuc/Lam-yield (% dw)), backward elimination of insignificant independent variables ($p > 0.1$) was performed. This resulted in increased explained (R^2-adj.) and predicted (R^2-pred.) variations as well as decreased P_s and increased P_L (Table 4), while all remaining independent variables obtained significance levels below $p < 0.05$. The same methodology was applied for the models describing MWs of the

Table 3
Yield of Fuc/Lam (% dw of entering biomass) and MW (kDa) of alginates from each individual experiment performed for the RSM according to BBD.

Experiments	pH-T (°C)-t (h)	Fuc/Lam-yield (% dw)		Alginate MW (kDa)	
		SL	*AE*	*SL*	*AE*
1	3.5-50-2	2.35	2.22	430	248
2	5.5-50-2	2.1	1.96	687	276
3	3.5-70-2	2.62	1.75	225	196
4	5.5-70-2	2.21	1.65	524	285
5	3.5-60-1	1.72	1.27	407	248
6	5.5-60-1	1.86	1.06	505	356
7	3.5-60-3	2.2	1.16	245	236
8	5.5-60-3	2.17	1.88	919	333
9	4.5-50-1	1.72	1.93	581	355
10	4.5-70-1	2.57	1.72	375	297
11	4.5-50-3	2.85	2.25	477	313
12	4.5-70-3	2.53	2.25	320	279
13	4.5-60-2	1.95	1.63	579	314
14	4.5-60-2	2.18	1.68	692	311
15	4.5-60-2	2.35	1.55	483	298

Table 4
Results from ANOVA, P_s and P_L, and coefficients of determination, R^2-adj. and R^2-pred., for the reduced models describing Fuc/Lam-yield (% dw) and alginate MW (kDa).

Response	Species	Model significance (P_S)	Lack-of-fit (P_L)	R^2-adj. (%)	R^2-pred. (%)	Experimental error (s2)
F/L-yield (% dw)	*SL*	0.001	0.31	80.68	67.41	0.14
	AE	<0.001	0.16	85.18	66.11	0.14
Alg. MW (kDa)	*SL*	0.001	0.86	74.57	68.2	70.54
	AE	0.001	0.13	79.39	58.24	20.24

alginates. The resulting regression models describing the correlation between the independent variables and the responses, and the significances of each of the independent variables, can be found in Supplementary materials, Table S1.

As expected, pH had a significant positive linear effect on the MW of the alginates within the examined conditions, while T had a significant negative linear effect [9]. The parameters showing the highest significant influence on the Fuc/Lam yield, both for *SL* and *AE*, was longer t (positive linear correlation), higher T (negative linear correlation) and pH which exerted a quadratic effect (displaying an optimum within the examined region). It has previously been reported that mild acidic conditions (around pH 4) [43] and longer extraction time results in higher Fuc/Lam yields [33,42]. To the contrary, Lorbeer et al. [33], who examined fucoidan extraction from the brown algae *Ecklonia radiata*, did not see any significant dependence ($p < 0.05$) for T (25–45 °C) or pH (2–5), neither linear, quadratic nor interacting.

The reduced Fuc/Lam model was used to find extraction conditions that would generate the highest possible yield. Favoring *SL*, pH 4.5, T = 50 °C and t = 3.0 h fulfilled 100 % composite desirability, while the same conditions applied for *AE* fulfilled 93 % composite desirability. The same conditions were then applied in the full quadratic models to predict the MWs of the alginates: 516 kDa (*SL*) and 328 kDa (*AE*). Commercial alginates typically have MWs around 200 kDa [45]. Since these conditions were predicted to provide both good yields of Fuc/Lam and satisfactory alginate MWs, these were applied in the pilot-scale extractions.

3.3. Sequential extraction from SL and AE at pilot-scale

3.3.1. Compositional analysis of the biomass

When developing a biorefinery process, it is important to have a good understanding of the chemical composition of the biomass since the yield of individual components can define the economics of an entire chemical process [46]. Consequently, to determine the extraction yield of each polysaccharide, the content of polysaccharides in the starting biomass was determined by combining monosaccharide analysis, CNS, and ash analysis.

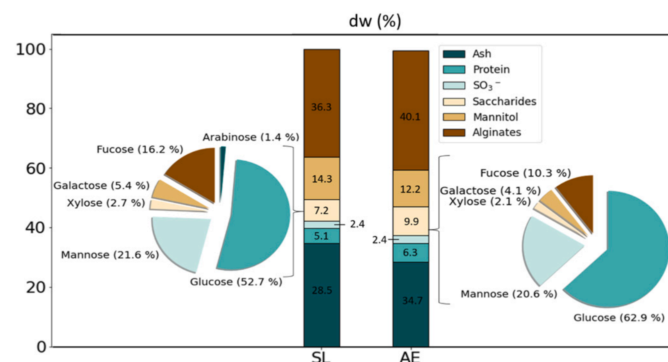

Fig. 3. Dry weight composition (% dw) of the biomass (*SL* and *AE*) used in the pilot scale extractions, determined by combining monosaccharide analysis, ash analysis, CNS and previously reported compositional data [8,11,47–50]. The alginate fraction also included polyphenols, lipids and pigments (<5 % dw). Detailed data of the chemical composition can be found in Supplementary materials, Table S6.

At the time of harvest, the dry weights of the biomasses were 9.4 and 12.4 % for *SL* and *AE* respectively, corresponding to a total of 1604 g (*SL*) and 2350 g (*AE*) dry biomass entering the system. Ash contents represented respectively 28.5 and 34.7 % of the dry weights (% dw).

The total dry weight content of sugars determined by monosaccharide analysis (excluding the content of mannitol, 14.3 and 12.2 % dw) was measured at 7.2 % dw (*SL*) 9.7 % dw (*AE*) of the dw (Fig. 3). Here, glucose was the most abundant monosaccharide at 3.9 % (*SL*) and 6.1 % (*AE*) the dw. Since the monosaccharide analysis used 12 M sulfuric acid leading to complete hydrolysis regardless of the morphology of the sugar (crystalline or non-crystalline), it was not possible to distinguish between glucose originating from laminarin or cellulose.

Estimating the content of fucoidans in brown algae also poses challenges related to the diverse and complex structure of these sulfated polysaccharides. According to structural elucidation of fucoidan in *SL* performed by Bilan et al. [38] four different groups of sulfated polysaccharides with slightly different structure, composition and molecular weights appear in the so-called fucoidan preparation. Bilan et al. states, that the main fraction consists mainly of fucose and galactose in a molar ratio of 5:1, with approximately 1.2 $-SO_3Na$ per sugar unit. Minor amounts of sugars such as xylose, mannose, and glucose are also present, in addition to a significant content of uronic acids. However, while some of the monosaccharides found in the biomass can be part of other polysaccharides, for example hemicelluloses [14], it has not yet been established whether the uronic acids are in fact part of the fucoidan structure or if it is contaminating alginates [38]. Hence, for simplicity, the extraction yield of fucoidan (see Section 3.3.2) was estimated only by the amount of available fucose: 1.2 % dw and 1.0 % dw in *SL* and *AE*, respectively. To our knowledge, no detailed structural analysis of fucoidan from *AE* has yet been published.

CNS was applied to determine the sulfur and protein content in the biomass. Most of the sulfur is expected to be part of the fucoidan and has been accounted as sulfite ($-SO_3^-$), measuring 2.4 % dw in *SL* and 2.4 % dw in *AE* (Fig. 3). Sulfur is also found in the amino acids methionine and cysteine, but their contribution was disregarded as they are only found in minor amounts (<0.5 % dw) in brown algae [8,47,48]. The protein content was determined to 5.1 % dw in *SL* and 6.3 % dw in *AE*, using a nitrogen-to-protein factor of 3.8 [8]. However, the nitrogen-to-protein factor fluctuates over the year due to fluctuations in the chemical composition of the seaweeds [11] and varies between species [11,47]. Furthermore, the total nitrogen contents fluctuate more between samples than the actual protein content [47]. Hence, determination of protein contents using a nitrogen-to-protein factor only gives an approximative value.

After summarizing the dry weights from the analyses described above, 36.3 % of the dw in *SL* and 40.1 % in *AE* were designated as “alginates” (Fig. 3). Alginates are expected to be the main contributor [11] to these fractions, but they further include polyphenols, lipids and pigments. The polyphenol content in *SL* and *AE* is reported to account for 0.4–1.4 % dw [11,48], while the lipid contents in late spring/early summer are in the range of 1–2.5 % dw [49,50]. The extractable pigment content in *SL* has been reported as below 1 % dw [51]. Consequently, polyphenols (0.9 % dw), lipids (1.8 % dw), and pigments (0.6 % dw) were subtracted from the unidentified fraction to get the alginate content: 33 % dw and 36.8 % dw in *SL* and *AE*, respectively.

Finally, an estimation of the total polysaccharide content in the biomasses was achieved by combining the analysis of the dry matter with previously reported compositional data [8,11,47–50]. It was assumed that all neutral sugars and -SO_3^- were part of the polysaccharides leading to a maximum polysaccharide content of 42.7 % dw available for extraction in *SL* and 49.5 % dw in *AE* (Section S3.2, Supplementary materials). Possible counterions contributing to the dry weight of the charged polysaccharides were disregarded.

3.3.2. Extraction of fucoidan, laminarin, alginate and cellulose from SL and AE

A flow diagram of the sequential process, applying the optimized conditions, to extract fucoidan, laminarin, alginate and cellulose from SL and AE is found in Fig. 2. The extraction process resulted in a polysaccharide yield of 23.4 % dw from *SL* (Table 5) compared to the estimated total yield of 42.7 %. However, while the latter value excludes ash the former includes ash contents, ranging from 0.6 to 20.3 % in the polysaccharide fractions (Table 6). The corresponding yield from *AE* was 26.3 % dw (Table 5), including ash contents ranging from 3.0 to 23.5 % (Table 6), compared to the maximal estimated yield of 49.0 %, excluding ash.

The yields of Fuc/Lam were considerably higher than predicted by the RSM: 2.8 % dw (*SL*) and 2.16 % dw (*AE*). For *SL*, the yield was almost twice (+176 %) as high than expected (5.0 % dw) while the yield were over four times (+440 %) higher for *AE* (9.5 % dw). The high yield from *AE* was attributed to the higher laminarin content available in the biomass in late spring. Laminarin contents up to 15 % dw have been recorded in wild *AE* harvested outside the Scottish coast in early summer [11]. The laminarin content in *SL* appears to peak later (early autumn), hence leading to a more modest increase in yield [11].

When analyzing the sugar content of the Fuc/Lam extracts from *AE* it was clear that it was indeed laminarin, and not fucoidan that accounted for the increased yield (Table 6). Less than 1 % of the neutral sugars in the Fuc/Lam fraction consisted of fucose, compared to 19.1 % in the BBD pre-trials. This translates to only 7.4 % of the fucose being extracted out of the available amount in the biomass. In Fuc/Lam from *SL* the fucose content was determined to 20.9 % dw, representing an extraction efficiency of 84.8 %. It has been suggested by Kloareg et al. [52] that fucoidans exists either as “free” molecules and/or bound in acid-labile supramolecular complexes within the cell walls of brown algae. Furthermore, fucose-containing sulfated polysaccharides (FCSPs) act as cross-linking bridges between alginates and cellulose. This model is supported by the work performed by Deniaud-Bouët et al. [53]. However, the term fucoidans includes a wide range of sulfated fucose-rich polysaccharides in brown algae [16], which consequently can include cross-linking FCSPs. Hence, it could be suitable to distinguish between water-extractable fucoidans and those bound in cell walls when analyzing the structural compositions and optimizing extraction conditions.

As the analyzed components in the Fuc/Lam fraction from *SL* only accounted for 84 % of the fraction’s dry weight (Table 6), it was assumed that the remaining unresolved fraction at least partly constituted of uronic acids [38]. It is possible that also the extract from *AE* contained fractions of uronic acids, although this was less apparent with the much lower fucoidan:laminarin ratio and the sum of the estimated dry weight reaching 108 % (ash, CNS, and monosaccharide analysis combined). Additionally, both Fuc/Lam fraction contained co-extracted proteins (Table 6).

The extracted alginates from *SL* and *AE* had only low concentrations of impurities (<2 % of the dry weight) originating from fucoidan and proteins, while the ash in the samples is attributed primarily to counterions. Depending on the desired use of the alginates, further purification processes, such as filtration, dialysis and SEC (size exclusion chromatography), could be performed to gain ultra-pure alginates suitable for more specialized uses, e.g. biomedical or pharmaceutical applications [54]. Further characterization of the chemical composition and the gel forming qualities of the alginates is described in Section 3.4.

The yield of cellulose from *SL* (6.9 % dw), was higher than the measured total glucose content in the biomass (3.9 % dw, Fig. 3). Since the cellulose fraction extracted from *SL* was almost 97 % pure, having only minor amounts of proteins, sulfite, ash, and other monosaccharides (Table 6), the glucose content was seemingly underestimated in the crude *SL* biomass. Likewise, the glucose content appeared to be underestimated in *AE* when combining the yield of glucose from laminarin and cellulose (extracted glucose, 12.7 % dw, compared to estimated 6.1 % dw). The discrepancy could be explained by a combination of bound water in the extracted cellulose [55], and/or incomplete hydrolysis of crystalline cellulose prior to monosaccharide analysis of the crude biomass. Furthermore, as described in Section 2.3.3, a partial epimerization from glucose to mannose occurred when performing monosaccharide analysis on some samples, including the biomass. This would also lead to glucose being underestimated in *SL* and *AE*. Yet, it cannot explain the whole discrepancy, since the total dry weight of glucose and mannose was still lower than the combined yield of the extracted glucose in cellulose and laminarin (Table S6).

The cellulose extracted from *AE* contained higher concentrations of all contaminants (Table 6). Presumably, alginates were present since the sum of the analyzed components only accounted for 57.47 % of the fraction’s dry weight. When bleaching the cellulose fraction from *AE* it was hypothesized that the extended H_2O_2-bleaching would degrade remaining alginates, hence, the additional alkali wash to further remove alginates (performed on *SL*) was excluded. Consequently, the actual yield of cellulose from *AE* (described in Table 5) was lower than the yield from *SL*, as only 40.89 % of the cellulose fraction is in fact glucose. Additional washing procedures could be applied to remove more of the water-soluble alginates from the insoluble cellulose fraction.

The harsher conditions needed to gain pure cellulose from *AE*, combined with a lower yield of water-soluble fucoidans, implied that the components in the cell wall and extracellular matrix were more strongly associated within *AE* compared to *SL*.

Table 5
Polysaccharide yields (individual and total) extracted from *SL* and *AE*. Described as total yield in g and as % dw of entering dry biomass in the pilot-scale extraction.

	Fuc/Lam		Alginate		Cellulose		Total	
	(g)	(% dw)	(g)	(% dw)	(g)	(% dw)	(g)	(% dw)
SL	80	5.0	172.6	10.8	111.2	6.9	363.8	22.7
AE	223.5	9.5	166.5	7.1	228.9	9.7	618.9	26.3

3.3.3. Fractionation of fucoidan and laminarin from co-extracted Fuc/Lam

To separate the co-extracted fucoidan and laminarin in FL1 (see Fig. 2), precipitation of laminarin followed by TFF (tangential-flow filtration) was applied. TFF has been described as a simple and economical process to obtain fucoidan and laminarin of commercial quantities and purity levels. TFF separates solutes based on their size (i.e., MW). As a result, the MWCO of the membranes chosen for filtration should be based on the MW of the polysaccharides, and the desired separation level of those. Membranes ranging from 10 to 100 kDa are appropriate considering typical MWs of fucoidan and laminarin, since such a separation will result in fucoidan being retained and laminarin permeating the membrane [56]. However, the MWCOs are assigned considering globular proteins, and not polysaccharides having diverse conformations and most often behaves like random coils in aqueous solutions. Further, the choice of membrane material is important to achieve effective separation of polysaccharides by TFF [57]. The expected amount of fucoidan in purified fractions is reported being in the range of 70–90 %, where the remaining part includes protein, inorganic salts and water [56].

The purity and degree of separation were determined using

Table 6
Chemical composition of the extracted polysaccharides expressed as percent dry weight (% dw) determined using ash analysis, CNS, and monosaccharide analysis. The “Total (% dw)” describes the combined analyzed dry weight in each polysaccharide when summarizing all analytical tools.

		Fuc/Lam		Alginate		Cellulose	
		SL	*AE*	*SL*	*AE*	*SL*	*AE*
Ash (% dw)		20.35 ± 0.35	2.95 ± 0.03	16.9 ± 0.05	23.5 ± 2.11	0.62 ± 0.07	4.74 ± 0.03
CNS (% dw)	Protein	11.29 ± 0.07	3.54 ± 0.18	0.40 ± 0.06	0.64 ± 0.15	1.34 ± 0.33	7.40 ± 1.11
	-(SO_3^-)-	16.25 ± 0.10	0.88 ± 0.10	0.68 ± 0.33	0.4 ± 0.05	0.28 ± 0.05	1.35 ± 0.08
Monosaccharide analysis (% dw)	Mannitol	0.66 ± 0.05	3.08 ± 0.14	n.d.	n.d.	0.29 ± 0.03	0.09 ± 0.00
	Fucose	20.88 ± 0.96	0.78 ± 0.08	0.32 ± 0.01	0.36 ± 0.00	0.14 ± 0.01	1.04 ± 0.05
	Arabinose	0.21 ± 0.01	0.11 ± 0.00	n.d.	0.02 ± 0.00	0.17 ± 0.01	0.1 ± 0.01
	Galactose	2.60 ± 0.13	0.48 ± 0.04	0.03 ± 0.01	0.07 ± 0.00	0.14 ± 0.01	0.77 ± 0.03
	Rhamnose	0.52 ± 0.02	0.13 ± 0.00	n.d.	n.d.	n.d.	n.d.
	Glucose	5.55 ± 0.26	91.69 ± 3.04	0.06 ± 0.01	0.27 ± 0.00	114.32 ± 1.63	40.89 ± 0.29
	Xylose	0.98 ± 0.07	0.12 ± 0.02	0.09 ± 0.01	0.09 ± 0.01	0.05 ± 0.00	0.14 ± 0.01
	Mannose	4.19 ± 0.20	1.16 ± 0.04	0.20 ± 0.03	0.33 ± 0.02	0.61 ± 0.04	0.95 ± 0.11
Total (% dw)		83.48 ± 1.06	104.92 ± 3.05	18.68 ± 0.34	25.68 ± 2.12	117.96 ± 1.68	57.47 ± 1.16

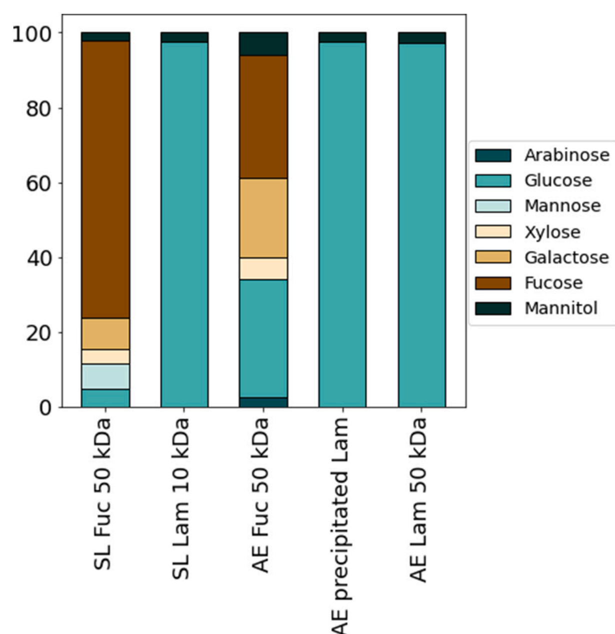

Fig. 4. Weight ratio (wt%) of neutral sugars and mannitol in fucoidan and laminarin separated from FL1 from *SL* and *AE* using precipitation and TFF, MWCO 10 or 50 kDa (% dw of the monosaccharides can be found in Table S12 in Supplementary materials).

monosaccharide analysis, ash measurement, CNS, and NMR. The monosaccharide analysis showed that the fucoidan fraction from *SL* (Fig. 4) had high concentration of fucose (≈74 wt% of the monosaccharides and mannitol) and a lower concentration of galactose, xylose, mannose, glucose, and mannitol. The laminarin fraction from *SL*, just like the two analyzed laminarin fractions from *AE* (Figs. 4, S3), appeared to only contain glucose and mannitol, which is consistent with known laminarin structures which occasionally contain mannitol at the reducing end [17]. Of note, preliminary work (results not presented) has shown traces of fucose, arabinose, rhamnose, xylose and mannose in laminarin samples fractionated by the same methods. Compared to the fucoidan fraction from *SL*, the fraction from *AE* (Fig. 4), had a lower degree of fucose in relation to the degree of glucose, galactose, and xylose. While part of the glucose appears to originate from laminarin (Fig. S2) the monosaccharide analysis also suggests that fucoidan from *AE* has a more heterogenous composition than fucoidan from *SL*. More anomeric signals in the NMR spectrum, which indicate different sugar residues or more diversified fucoidan structures, provide additional support (Fig. S2).

Table 7
Results from ash, CNS and monosaccharide analysis of fractionated fucoidan and laminarin. The sulfur content has been converted to SO_3^- while the nitrogen content has been converted to proteins. Composition of the total sugars is given in Fig. 4.

	Ash (% dw)	-(SO_3)- (% dw)	Protein (% dw)	Sugars (% dw)
SL Fuc 50 kDa	22.29 ± 0.70	19.60 ± 0.22	9.27 ± 0.07	30.44 ± 1.26
SL Lam 10 kDa	9.51 ± 0.35	7.64[a]	21.62[a]	33.32 ± 1.78
AE Fuc 50 kDa	10.95 ± 0.34	7.88 ± 0.04	8.45 ± 0.17	23.05 ± 0.46
AE precip. Lam	3.82 ± 0.76	0.61 ± 0.13	3.95 ± 0.07	97.00 ± 4.73
AE Lam 50 kDa	2.71 ± 0.68	1.08 ± 0.1	1.78 ± 0.23	94.88 ± 2.15

[a] Due to limited material one replicate was analyzed.

Ash analysis revealed that the fucoidans extracted from *SL* and *AE* contained 22 % and 11 % ash, while CNS analysis estimated 9 % and 8 % protein, respectively (Table 7). The majority of the ash was presumably counterions accompanying the sulfate groups. The laminarin fraction separated from *SL* had high concentrations of both sulfates and proteins, in total around 28 % and 10 % ash. The high concentration of impurities can likely be explained by the low actual yield of laminarin (in total 89 mg from 1 L of crude FL1). The laminarin fractions from *AE* contained only low (0.6–4 % dw) concentrations of ash, sulfates, and proteins.

The filtration appeared to decrease the yields of fucoidan and laminarin (Table S13). The fractionation of FL1 from *SL* yielded only 38.6 % of the initial fucoidan and laminarin, while the corresponding yield was 42.5 % for *AE*. Parts of the losses can be explained by either cake formation or fouling of the membranes, as particles larger than the MWCO accumulates on the membrane surface in a growing cake layer [58].

Overall, precipitation of laminarin followed by TFF represents a simple method to fractionate co-extracted fucoidan and laminarin. However, all fractions contained varying degrees of impurities (Table 7). Prolonged filtration time as well as further purification steps could be included to gain purer fucoidans. There are not yet standardized purification procedures for fucoidans [59], but common techniques involve precipitation in EtOH and ion-exchange chromatography (IEX) [14,16]. IEX has been reported as the most efficient method to gain fucoidans of high purity levels [16].

3.3.4. Identification of valuable waste streams

The MB demonstrated that it was possible to recover more of the extracted polysaccharides by careful handling of waste streams and washing procedures. Generally, material balances are fundamental in process design and essential to obtain high-quality data from laboratory or pilot-plant experiments when assessing the potential for up-scaling

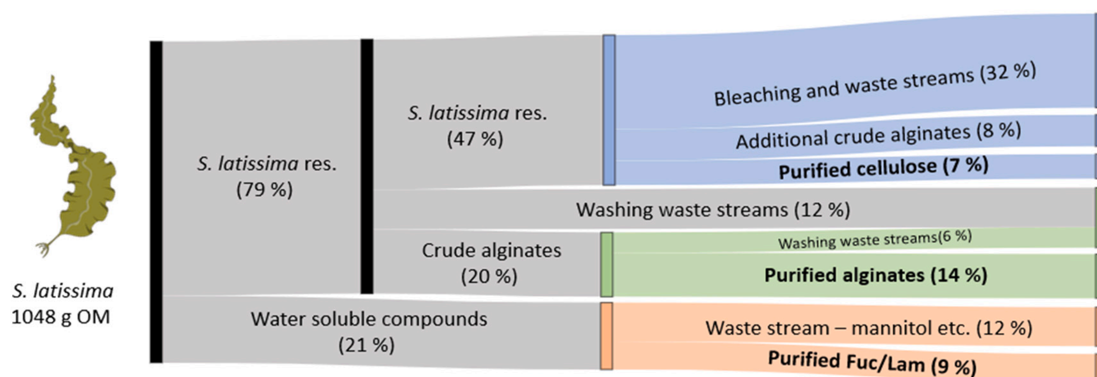

Fig. 5. Sankey diagram covering the flow of OM (g) in the sequential extraction process on *SL*. The colors indicate the different system boundaries established in Fig. 2. It was indicated that several of the waste streams had high concentration of alginates and to a lesser extent also contained fucoidans. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

[60]. Focus of the MB determined in this study was only the flow of organic material (OM) within four different system boundaries, visualized in Fig. 2 together with all sampling points: FL1-FL3, A1-A8 and C1-C4. An extensive description of the mass flow, and specifically the flow of OM, can be found in the Supplementary materials, Table S9.

Out of the total 17,160 gram wet biomass (*SL*) entering the system OM represented 1048 g (Table S9, Fig. 5). When assessing the crude polysaccharide extraction (black system boundary, Fig. 2), only 0.4 % of the OM entering the system was not recorded in the outflows (Table S10). Likewise, the discrepancy in the sequence covering the alginate precipitation and purification (green system boundary) was small, showing 2.3 % more OM leaving the system compared with entering OM. To get a rough indication of the composition of all waste streams accumulating during alginate extraction (A1-A7), ICP-MS was applied (Table S11). Except from high concentrations of mono- and divalent ions, 0.4–0.6 % of sulfur was present in each fraction. Assuming that all sulfur originates from fucoidan and applying the structure suggested by Bilan et al. (5:1, fucose:galactose and 1.2 NaSO3 per sugar unit) [38], this corresponded to 9.3 g of fucoidan being rinsed away when washing the residues after Fuc/Lam-extraction, and additionally 12.0 g escaping when the crude alginate was extracted. No additional analysis than ICP-MS was performed on these waste fractions due to limited amount of material.

When purifying the crude Fuc/Lam fractions (orange system boundary, Fig. 2), the waste streams produced during dialysis were not collected. Neither were the waste fractions following bleaching of the cellulose fraction (blue system boundary). This led to high discrepancy in the in- and out-flows recorded in these processes; 63.3 and 50.0 % more mass was recorded in the inflows, than in the outflows of each system. Considering the high concentration of water-soluble mannitol (14.3 % dw) in the starting biomass, this represented presumably the main material loss when dialyzing FL1 and FL2 (see Fig. 5). The extraction process could easily be adapted to further include collection of mannitol. Ultra-filtration (UF) membranes with MWCO from 0.5 to 1 kDa could be used to retain the laminarin while permeating mannitol and minerals [56,61]. Subsequently, the mannitol fraction could be demineralized using e.g. reverse osmosis, ion-exchange or nanofiltration [61]. Losses accumulating when extracting cellulose are mainly ascribed unwanted components degrading during bleaching, yet parts of the cellulose can degrade as well [62]. Additionally, materials (including cellulose) escaped during filtration and washing.

Lastly, it was observed that the water waste streams prior to cellulose bleaching (C2 and C3, Fig. 2) had high viscosities and high dry weights, implying that these still contained alginates. This was confirmed by monosaccharide analysis and NMR, revealing that only a minor fraction (<4 % dw) accounted for neutral sugars and displaying spectra characteristic for alginates (results not shown). Consequently, additional 112 g of crude alginates (representing around 80 g of OM) could have been extracted from the biomass. Moreover, only 68 % of the OM in the crude alginate fraction was recovered as purified alginates. While <12 g of those is ascribed fucoidan (determined by ICP-MS, see section above), a large part of the remaining losses, comprising >50 g OM is likely alginates lost during purification. Hence, around 75 % more alginates could have been collected by excessive and careful washing, than presented earlier in this study (Table 5).

3.4. Alginate and alginate hydrogel characterization

One of the objectives when expanding the traditional alginate extraction was to ensure that the MW and gel-forming properties of the alginates would not be compromised. The physical and mechanical properties of alginate hydrogels is governed by structural characteristics of the alginates, such as chemical composition and sequence, MW and MW distribution. Some important technological properties of hydrogels are mechanical strength, porosity of the gel network, swelling/shrinking (here represented by syneresis) [10,63] and elasticity [63]. Gelation is based on the affinity of alginates towards certain divalent ions, such as Ca^{2+}. The selective binding is strictly restricted to the G residues, which arranges according to the so-called egg-box model [64]. There is a direct dependence between gel strength, porosity, and elasticity, which in turn is related to the MG-distribution. Hydrogels enriched in G residues, having long stiff G-blocks and less elastic M-sequences, adopts a stiffer, more open and static network compared to the more dynamic, entangled networks having high M-contents [10]. Alginates extracted from the stipe of *Laminaria hyperborea* (*LH*) exhibit particularly high G-content ($F_G \sim 0.67$) while alginates originating from the fronds of *LH* have a higher ratio of M ($F_G \sim 0.45$) [65], comparable to alginates from *SL* and *AE*, as seen in Table 8. These alginates and respective hydrogel properties were therefore used as comparison when evaluating the quality of the alginates extracted from *SL* and *AE*.

The chemical composition of the alginates (*SL*, *AE*, and *LH*) and their MWs are presented in Table 8. The G content in the alginates from *SL* and *AE* were similar, yet slightly higher, than the G content in the alginates from *LH* fronds. The MWs of the alginates from *SL*, 396 kDa, and *AE*, 230 kDa, were lower than the values predicted by the regression models developed in the pre-trials of 516 kDa (*SL*) and 328 kDa (*AE*) (see Section 3.2). The decrease in MW was likely caused by the additional acid precipitation included during the pilot-scale extraction, intended to enhance the purification of the alginates. Since both alginates and fucoidans precipitate in EtOH [16] it was assumed that acid precipitation would increase the purity of the alginates. Since a great part of the sulfates on fucoidan remain charged at pH ≈ 2, fucoidan does not precipitate and gets washed away to a higher extent. This was demonstrated by measuring the concentration of sulfur (% dw) in alginate extracts

Table 8
MW, chemical composition (M- and G-residues), fractions (F) of the residues and different diads and triads, together with the average length of the G-blocks ($N_{G>1}$), for alginates extracted from SL, AE and fronds of LH. NMR spectra can be found in Supplementary materials, Fig. S4.

	M_w (kDa)	F_G	F_M	F_{GG}	$F_{GM,MG}$	F_{MM}	$F_{GGM,MGG}$	F_{MGM}	F_{GGG}	$N_{G>1}$
SL	396	0.48	0.52	0.31	0.17	0.35	0.040	0.13	0.27	9
AE	230	0.49	0.51	0.33	0.16	0.35	0.043	0.12	0.29	9
LH	272	0.46	0.54	0.29	0.17	0.38	0.041	0.15	0.25	7

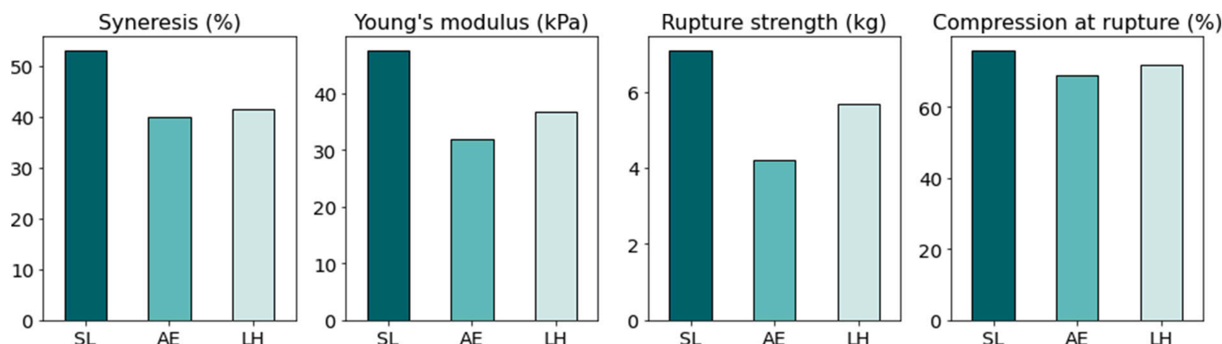

Fig. 6. Physical properties of hydrogels produced from alginates extracted from cultivated SL and AE and commercial alginates from LH fronds. Details in Supplementary materials, Table S14.

from *SL* after each purification step (Fig. 7). Acid precipitation decreased the sulfur content by 59 % while the EtOH precipitation decreased the sulfur content by 38 %. According to NMR (Fig. S4, Supplementary materials) the purified alginate from *SL* contained approximately 1.5 % dw fucoidan/laminarin, while the corresponding value for *AE* was 0.6 % dw.

Hydrogels formed by alginates extracted from *SL* and *AE*, had similar mechanical properties: Young's modulus (E (kPa)), rupture strength (kg) and compression at rupture (%) (results conveyed in Fig. 6), as hydrogels produced by *LH*-alginates. The slightly higher elasticity (*SL*: 47.6 kPa compared to *AE*: 32.0 kPa and *LH*: 36.7 kPa), rupture strength (*SL*: 7.1 kg compared to *AE*: 4.2 kg and *LH*: 5.7 kg) and compression at rupture (*SL*: 76 % compared to *AE*: 69 % and *LH*: 72 %) of the *SL*-gels can be explained by the higher MW of those alginates. The opposite was true for hydrogels from *AE*, lower MW led to slightly lower elasticity and strength. It's generally agreed that the degree of syneresis correlates to the amount and length of MG-blocks [63]. Yet, the syneresis of *SL*-alginate hydrogels were around 10 % higher than the others, although the fraction of MG-blocks were in a similar range for all species ($F_{GM,MG}$ = 0.16–0.17). All in all, alginates extracted from cultivated *SL* and *AE* represent an alternative source of alginates and have comparable properties to alginates extracted from *LH* fronds.

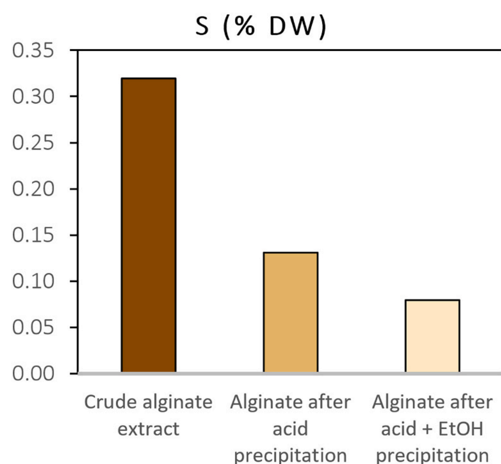

Fig. 7. Sulfur content (% dw) in crude alginate fraction, alginates after acid precipitation and alginates after sequential acid and EtOH precipitation. Details in Supplementary materials, Table S15.

4. Conclusions

Mild chemical treatments were applied to expand conventional alginate extraction, to further include extraction of fucoidan, laminarin and cellulose from cultivated *SL* and *AE*. Introducing Fuc/Lam extraction prior to alginate extraction did not severely affect the MWs of the alginates. MWs were either in a similar range (*AE*, ca. 200 kDa) or higher (*SL*, ca. 350 kDa) compared to most commercial alginates. Further, hydrogels produced from these alginates had properties comparable to gels formed by alginates from the fronds of *LH*.

Seasonal variations determine when it is most suitable to extract which components from the seaweed, since the chemical composition varies over the year. Compared to *AE*, *SL* used in this study had only low concentrations of laminarin, which agrees with previous findings, stating that laminarin content peaks earlier in *AE* than in *SL*. Laminarin accumulates in *SL* over the summer and peak in the beginning of fall. Hence, later harvesting should be aimed for if co-extraction of laminarin is desired from *SL*. Further, it was noticed that at the time both biomasses had high concentrations of mannitol, up to 14 % of the dry weight. The described process could be modified to further include collection of mannitol by applying ultrafiltration on the crude laminarin fraction after TFF. Contrary to the laminarin content, higher yields of water soluble fucoidan were obtained from *SL* than from *AE*. The developed method for recovery of fucoidans from brown algae is therefore deemed more applicable to *SL*.

This study demonstrates that cultivated *SL* and *AE* are good candidates for transitioning from single- to multicomponent biorefineries based on brown algae. While cultivated brown algae can contribute to meeting increasing demands of high value compounds, inclusion of cultivated seaweeds will be necessary to avoid over-exploitation of wild resources with a growing market for seaweed-based products. Additionally, the developed process is equally relevant for seaweed biorefineries based on wild harvested brown algae.

CRediT authorship contribution statement

P.S.B. designed experiments, performed research and analyzed data of pre-trials, screening experiments and pilot-scale trials, and wrote the first draft of the manuscript. M.O., G.I.S., and O.A.A., designed experiments, performed research, and analyzed data for pre-trials of the sequential extraction, and reviewed the manuscript. W.I.S. designed experiments, performed research, and analyzed data, for the alginate gel experiments and reviewed the manuscript. Ø.A. analyzed data, carried out supervision, and edited the manuscript. H.S. analyzed data, edited the manuscript, and acquired funding. F.L.A. initiated the research, conceptualization, analyzed data, edited the manuscript, carried out supervision, and acquired funding.

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2022.102928.

References

[1] C. Fiset, A.J. Irwin, Z.V. Finkel, The macromolecular composition of noncalcified marine macroalgae, J. Phycol. 55 (6) (2019) 1361–1369, https://doi.org/10.1111/jpy.12913.
[2] R. Araújo, et al., Current status of the algae production industry in Europe: an emerging sector of the blue bioeconomy, Front. Mar. Sci. 7 (626389) (2021), https://doi.org/10.3389/fmars.2020.626389.
[3] G.Ó. Hreggviðsson, et al., Biocatalytic refining of polysaccharides from brown seaweeds, in: M.D. Torres, S. Kraan, H. Dominguez (Eds.), Sustainable Seaweed Technologies, 1st ed., Elsevier, 2020, pp. 447–504.
[4] N. Wahlström, et al., Cellulose from the green macroalgae Ulva lactuca: isolation, characterization, optotracing, and production of cellulose nanofibrils, Cellulose 27 (7) (2020) 3707–3725, https://doi.org/10.1007/s10570-020-03029-5.
[5] M. Zollmann, et al., Green technology in green macroalgal biorefineries, Phycologia 58 (5) (2019) 516–534, https://doi.org/10.1080/00318884.2019.1640516.
[6] K.A. Jung, S.R. Lim, Y. Kim, J.M. Park, Potentials of macroalgae as feedstocks for biorefinery, Bioresour. Technol. 135 (2013) 182–190, https://doi.org/10.1016/j.biortech.2012.10.025.
[7] M. Mac Monagail, L. Cornish, L. Morrison, R. Araújo, A.T. Critchley, Sustainable harvesting of wild seaweed resources, Eur. J. Phycol. 52 (4) (2017) 371–390, https://doi.org/10.1080/09670262.2017.1365273.
[8] S. Forbord, et al., 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 (4) (2020) 2215–2232, https://doi.org/10.1007/s10811-020-02038-y.
[9] A. Haug, Composition and Properties of Alginates, Norwegian University of Science and Technology, Trondheim, 1964. Doctoral dissertation.
[10] K.I. Draget, G. Skjåk-Bræek, O. Smidsrød, Alginate based new materials, Int. J. Biol. Macromol. 21 (1–2) (1997) 47–55, https://doi.org/10.1016/S0141-8130(97)00040-8.
[11] P. Schiener, K.D. Black, M.S. Stanley, D.H. Green, The seasonal variation in the chemical composition of the kelp species Laminaria digitata, Laminaria hyperborea, Saccharina latissima and Alaria esculenta, J. Appl. Phycol. 27 (1) (2015) 363–373, https://doi.org/10.1007/s10811-014-0327-1.
[12] R.C. Rajak, S. Jacob, B.S. Kim, A holistic zero waste biorefinery approach for macroalgal biomass utilization: a review, Sci. Total Environ. 716 (137067) (May 2020), https://doi.org/10.1016/j.scitotenv.2020.137067.
[13] F. Cherubini, The biorefinery concept: using biomass instead of oil for producing energy and chemicals, Energy Convers. Manag. 51 (7) (Jul. 2010) 1412–1421, https://doi.org/10.1016/j.enconman.2010.01.015.
[14] E. Deniaud-Bouët, K. Hardouin, P. Potin, B. Kloareg, C. Hervé, A review about brown algal cell walls and fucose-containing sulfated polysaccharides: cell wall context, biomedical properties and key research challenges, Carbohydr. Polym. 175 (2017) 395–408, https://doi.org/10.1016/j.carbpol.2017.07.082.
[15] Yuan-Zong Lai, Chemical degradation, in: D.N.-S. Hon, N. Shiraishi (Eds.), Wood and Cellulosic Chemistry, 2nd revis ed., Marcel Dekker, Inc., New York, 2001, pp. 443–512.
[16] N.M.A. Ponce, C.A. Stortz, A comprehensive and comparative analysis of the fucoidan compositional data across the Phaeophyceae, Front. Plant Sci. 11 (556312) (2020), https://doi.org/10.3389/fpls.2020.556312.
[17] S.U. Kadam, B.K. Tiwari, C.P. O'Donnell, Extraction, structure and biofunctional activities of laminarin from brown algae, Int. J. Food Sci. Technol. 50 (1) (Jan. 2015) 24–31, https://doi.org/10.1111/ijfs.12692.
[18] Q. He, Q. Wang, H. Zhou, D. Ren, Y. He, Highly crystalline cellulose from brown seaweed Saccharina japonica: isolation, characterization and microcrystallization, Cellulose 25 (10) (2018) 5523–5533, https://doi.org/10.1007/s10570-018-1966-1.
[19] A.J. Onyianata, 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 (14) (2020) 7997–8010, https://doi.org/10.1007/s10570-020-03223-5.
[20] S.U. Kadam, et al., Laminarin from Irish brown seaweeds Ascophyllum nodosum and Laminaria hyperborea: ultrasound assisted extraction, characterization and bioactivity, Mar. Drugs 13 (7) (2015) 4270–4280, https://doi.org/10.3390/md13074270.
[21] M. Garcia-Vaquero, J.V. O'Doherty, B.K. Tiwari, T. Sweeney, G. Rajauria, Enhancing the extraction of polysaccharides and antioxidants from macroalgae using sequential hydrothermal-assisted extraction followed by ultrasound and thermal technologies, Mar. Drugs 17 (8) (Aug. 2019), https://doi.org/10.3390/md17080457.
[22] M.T. Ale, J.D. Mikkelsen, A.S. Meyer, Important determinants for fucoidan bioactivity: a critical review of structure-function relations and extraction methods for fucose-containing sulfated polysaccharides from brown seaweeds, Mar. Drugs 9 (10) (2011) 2106–2130, https://doi.org/10.3390/md9102106. MDPI AG.
[23] E.C.C. Stanford, On algin: a new substance obtained from some of the commoner species of marine algae, Chem. News 47 (1883) 254–257.
[24] M. Indergaard, A. Jensen, Produkter fra norske makroalger, in: Utnyttelse av marin biomasse, NTH-Trykk, Ås/Trondheim, 1991, pp. 68–76.
[25] T. Hahn, S. Lang, R. Ulber, K. Muffler, Novel procedures for the extraction of fucoidan from brown algae, Process Biochem. 47 (12) (2012) 1691–1698, https://doi.org/10.1016/j.procbio.2012.06.016.
[26] W.A.P. Black, E.T. Dewar, F.N. Woodward, MANUFACTURE OF ALGAL CHEMICALS. IV. - Laboratory-scale isolation of fucoidin from brown marine algae, J. Sci. Food Agric. 3 (1952) 122–129.
[27] K. Bogolitsyn, A. Parshina, L. Aleshina, Structural features of brown algae cellulose, Cellulose 27 (2020) 9787–9800, https://doi.org/10.1007/s10570-020-03485-z.
[28] A.J. Mian, E. Percival, Carbohydrates of the brown seaweeds Himanthalia lorea, Bifucaria bifurcata, and Padina pavonita. Part I. Extraction and fractionation, Carbohydr. Res. 26 (1973) 133–146, https://doi.org/10.1016/S0008-6215(00)85030-2.
[29] H. Doh, W.S. Whiteside, Isolation of cellulose nanocrystals from brown seaweed, Sargassum fluitans, for development of alginate nanocomposite film, Polym. Cryst. 3 (4) (2020), https://doi.org/10.1002/pcr2.10133.
[30] N. Wahlström, H. Harrysson, I. Undeland, U. Edlund, A strategy for the sequential recovery of biomacromolecules from red macroalgae Porphyra umbilicalis Kützing, Ind. Eng. Chem. Res. 57 (1) (2018) 42–53, https://doi.org/10.1021/acs.iecr.7b03768.
[31] S.L.C. Ferreira, et al., Box-Behnken design: an alternative for the optimization of analytical methods, Anal. Chim. Acta 597 (2) (2007) 179–186, https://doi.org/10.1016/j.aca.2007.07.011.
[32] M. Cavazzuti, Responce surface modelling, in: Optimization Methods: From Theory to Design Scientific and Technological Aspects in Mechanics, Springer-Verlag, Berlin Heidelberg, 2013, pp. 43–76.
[33] A.J. Lorbeer, J. Lahnstein, V. Bulone, T. Nguyen, W. Zhang, Multiple-response optimization of the acidic treatment of the brown alga Ecklonia radiata for the sequential extraction of fucoidan and alginate, Bioresour. Technol. 197 (2015) 302–309, https://doi.org/10.1016/j.biortech.2015.08.103.
[34] T. Lundstedt, et al., Experimental design and optimization, Chemom. Intell. Lab. Syst. 42 (1998) 3–40, https://doi.org/10.1007/978-3-540-49148-4_3.
[35] K. Ingar Draget, K. Østgaard, O. Smidsrød, Homogeneous alginate gels: a technical approach, Carbohydr. Polym. 14 (2) (1991) 159–178, https://doi.org/10.1016/0144-8617(90)90028-Q.
[36] O. Smidsrød, A. Haug, Properties of poly(1,4-hexuronates) in the gel state - II. Comparison of gels of different chemical composition, Acta Chem. Scand. 26 (1972) 79–88.
[37] H. Ertesvåg, G. Skjåk-Bræk, Modification of alginate using mannuronan C-5-epimerases, in: Christopher Bucke (Ed.), Methods in Biotechnology, 1st ed. Carbohydrate Biotechnology Protocols, vol. 10, Springer Protocols, 1997, pp. 71–78.

[38] M.I. Bilan, et al., 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.

[39] A. Pielesz, W. Binias, J. Paluch, Mild acid hydrolysis of fucoidan: characterization by electrophoresis and FT-Raman spectroscopy, Carbohydr. Res. 346 (2011) 1937–1944, https://doi.org/10.1016/j.carres.2011.05.016.

[40] V.H. Pomin, A.P. Valente, M.S. Pereira, P.A.S. Mourão, Mild acid hydrolysis of sulfated fucans: a selective 2-desulfation reaction and an alternative approach for preparing tailored sulfated oligosaccharides, Glycobiology 15 (12) (2005) 1376–1385, https://doi.org/10.1093/glycob/cwj030.

[41] L. Münster, B. Hanulíková, M. Machovský, F. Late, I. Ku, J. Vícha, Mechanism of sulfonation-induced chain scission of selectively oxidized polysaccharides, Carbohydr. Polym. 229 (115503) (2020), https://doi.org/10.1016/j.carbpol.2019.115503.

[42] M.T. Ale, J.D. Mikkelsen, A.S. Meyer, Designed optimization of a single-step extraction of fucose-containing sulfated polysaccharides from Sargassum sp, J. Appl. Phycol. 24 (4) (2012) 715–723, https://doi.org/10.1007/s10811-011-9690-3.

[43] A.F. Hifney, M.A. Fawzy, K.M. Abdel-Gawad, M. Gomaa, Industrial optimization of fucoidan extraction from Sargassum sp. and its potential antioxidant and emulsifying activities, Food Hydrocoll. 54 (2016) 77–88, https://doi.org/10.1016/j.foodhyd.2015.09.022.

[44] G.E.P. Box, K.B. Wilson, On the experimental attainment of optimum conditions [Online]. Available: R. Stat. Soc. Ser. B 13 (1) (1951) 1–45 https://www.jstor.org/stable/2983966.

[45] I. Donati, S. Paoletti, Material properties of alginates, in: B.H.A. Rehm (Ed.), Alginates: Biology and Applications, Springer-Verlag Berlin, Berlin Heidelberg, 2009, pp. 1–53.

[46] L.M.L. Laurens, et al., Strain, biochemistry, and cultivation-dependent measurement variability of algal biomass composition, Anal. Biochem. 452 (1) (2014) 86–95, https://doi.org/10.1016/j.ab.2014.02.009.

[47] D. Manns, M.M. Nielsen, A. Bruhn, B. Saake, A.S. Meyer, Compositional variations of brown seaweeds Laminaria digitata and Saccharina latissima in Danish waters, J. Appl. Phycol. 29 (3) (2017) 1493–1506, https://doi.org/10.1007/s10811-017-1056-z.

[48] S. Sharma, L. Neves, J. Funderud, L.T. Mydland, M. Øverland, S.J. Horn, Seasonal and depth variations in the chemical composition of cultivated Saccharina latissima, Algal Res. 32 (2018) 107–112, https://doi.org/10.1016/j.algal.2018.03.012.

[49] G.S. Marinho, S.L. Holdt, C. Jacobsen, I. Angelidaki, Lipids and composition of fatty acids of Saccharina latissima cultivated year-round in integrated multi-trophic aquaculture, Mar. Drugs 13 (7) (2015) 4357–4374, https://doi.org/10.3390/md13074357.

[50] L. Foseid, I. Natvik, H. Devle, D. Ekeberg, Identification of fatty acids in fractionated lipid extracts from Palmaria palmata, Alaria esculenta and Saccharina latissima by off-line SPE GC-MS, J. Appl. Phycol. 32 (6) (2020) 4251–4262, https://doi.org/10.1007/s10811-020-02193-2.

[51] P. Garcia-Perez, et al., Pigment composition of nine brown algae from the Iberian northwestern coastline: influence of the extraction solvent, Mar. Drugs 20 (2) (2022), https://doi.org/10.3390/md20020113.

[52] B. Kloareg, M. Demarty, S. Mabeau, Polyanionic characteristics of purified sulphated homofucans from brown algae, Int. J. Biol. Macromol. 8 (6) (1986) 380–386, https://doi.org/10.1016/0141-8130(86)90060-7.

[53] E. Deniaud-Bouët, N. Kervarec, G. Michel, T. Tonon, B. Kloareg, C. Hervé, Chemical and enzymatic fractionation of cell walls from Fucales: insights into the structure of the extracellular matrix of brown algae, Ann. Bot. 114 (6) (2014) 1203–1216, https://doi.org/10.1093/aob/mcu096.

[54] K. Nøkkling-Eide, A.-M. Langen, 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, 2022. Unpublished.

[55] K. Nakamura, T. Hatakeyama, H. Hatakeyama, Studies on bound water of cellulose by differential scanning calorimetry, Text. Res. J. 51 (9) (1981) 607–613.

[56] F. Hjelland, A.Henning Andersen, H.S. Yang, PROCESS FOR ISOLATING FUCOIDAN AND LAMINARIN FROM LIVE, HARVESTED SEAWEED, 2020. US 10.590,207 B2.

[57] S. Eder, et al., Effect of polysaccharide conformation on ultrafiltration separation performance, Carbohydr. Polym. 260 (117830) (2021), https://doi.org/10.1016/j.carbpol.2021.117830.

[58] M.R. Mackley, N.E. Sherman, Cross-flow cake filtration kinetics mechanisms and kinetics, Chem. Eng. Sci. 47 (12) (1992) 3067–3084, https://doi.org/10.1016/0009-2509(92)87007-D.

[59] M.T. Ale, A.S. Meyer, Fucoidans from brown seaweeds: an update on structures, extraction techniques and use of enzymes as tools for structural elucidation, RSC Adv. 3 (2013) 8131–8141, https://doi.org/10.1039/c3ra23373a.

[60] G.P. Towler, R.K. Sinnott, Fundamentals of material balances, in: Chemical Engineering Design: Principles, Practice and Economics of Plant and Process Design, 5th ed., Elsevier Inc., 2008, pp. 49–79.

[61] J.W.Van Hal, W.J.J. Huijgen, Process for Mannitol Extraction From Seaweed, 2014. NL C 2009482.

[62] Y. Wu, J. Wu, F. Yang, C. Tang, Q. Huang, Effect of H2O2 bleaching treatment on the properties of finished transparent wood, Polymers (Basel) 11 (5) (776) (2019), https://doi.org/10.3390/polym11050776.

[63] O. Aarstad, B.L. Strand, L.M. Klepp-Andersen, G. Skjaìšk-Bræk, Analysis of G-block distributions and their impact on gel properties of in vitro epimerized mannuronan, Biomacromolecules 14 (10) (2013) 3409–3416, https://doi.org/10.1021/bm400658k.

[64] G.T. Grant, E.R. Morris, D.A. Rees, P.J.C. Smith, D. Thom, Biological interactions between polysaccharides and divalent cations: the egg-box model, Fed. Eur. Biochem. Soc. Lett. 32 (1) (1973) 195–198, https://doi.org/10.1016/0014-5793(73)80770-7.

[65] A. Haug, B. Larsen, O. Smidsrød, Uronic acid sequence in alginate from different sources, Carbohydr. Res. 32 (2) (1974) 217–225, https://doi.org/10.1016/S0008-6215(00)82100-X.