



# Valorization of alginate-extracted seaweed biomass for the development of cellulose-based packaging films

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## ABSTRACT

Seaweed residues from *Alaria esculenta*, *Saccharina latissima* and *Ascophyllum nodosum* after alginate extraction have been valorized to produce cellulose-based fractions with different purification degrees. The residues were mainly composed of carbohydrates (35–57%) and proteins (12–37%), *Alaria* and *Saccharina* being richer in cellulose and *Ascophyllum* richer in fucoidan. The lower cellulose content in the latter made it unsuitable for the extraction of cellulosic fractions.

Self-supporting films were obtained from the cellulosic fractions from *Saccharina* and *Alaria* residues. While the higher cellulose purity films presented more desirable characteristics in terms of mechanical properties (with elastic moduli of ca. 5–7 GPa and elongation values of ca. 3–5%) and visual appearance, the presence of non-cellulosic components in the films from less purified fractions reduced their water sensitivity and promoted greater water barrier (with water permeability values of ca. 4–6 kg·m/s·m<sup>2</sup>·Pa). These results point towards the potential of a simple alkaline extraction to generate cellulose-based films from seaweed residuals with the best compromise between functional properties and economical and environmental efficiency.

## 1. Introduction

Since synthetic polymers began replacing natural materials more than half a century ago, the use of plastics has grown exponentially and is now an indispensable part of our lives [1]. The massive use of synthetic plastics is mostly due to their easy manufacturability, good insulating properties, low cost, high mechanical strength and durability [2,3]. However, it is precisely their extremely long durability that has led to the persistence of plastic waste, resulting in the accumulation of more than 25 million tons of plastic waste in the environment each year [4]. In this regard, the replacement of conventional plastics with biodegradable polymers made from renewable natural resources, i.e. biopolymers, are being considered lately as a more sustainable alternatives. However, the properties of biopolymers are still not comparable to those of reference synthetic polymers, especially in terms of barrier and mechanical properties, and their production costs are too high to compete in the market. Moreover, raw materials generally used for the production of biopolymers come from land-based crops and therefore

compete with their traditional use: the food and feed industries. In this context, aquatic biomass sources, such as seaweeds and aquatic plants, rich in cellulose, are being explored as an efficient alternative to land-based biomass for the production of biopolymers. Of special interest may be the use of seaweed industrial waste streams, such as those generated during alginate production. Alginate is an anionic polysaccharide found in brown seaweeds (*Phaeophyta*), widely used in the food industry, mainly as stabilizing, emulsifying, gelling, and thickening agent [5,6]. The alginate-producing industry typically extracts this phycocolloid from brown seaweeds by applying the following main steps: dilute formaldehyde treatment, dilute acid treatment, alkaline extraction, solid-liquid separation, precipitation and drying. During alkaline extraction the acidified seaweeds are immersed in a sodium carbonate or sodium hydroxide solution to convert the insoluble alginic acid into soluble sodium alginate [7,8]. Depending on the seaweed species, the season and extraction parameters used, the extraction yields of alginate have been reported to range from 17% to 40% [9–11]. Therefore, large amounts of seaweed residues are generated at industrial

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scale after its extraction, which are generally discarded as waste. However, part of the components present in the native seaweeds, which include cellulose, laminarin, fucoidans and to some extent alginate depending on extraction efficiency and starting materials, may remain in the residue [12,13] depending on the procedure. In particular, cellulose is expected to remain unaffected by the alginate extraction treatments; thus, its exploitation for the production of cellulosic materials would be an opportunity to add value to this industrial waste stream.

Cellulose presents a high potential for the development of bio-based food packaging and it has been widely used as a filler to improve the properties of other biopolymers [14,15] due to its high resistance and rigidity, among other aspects [16,17]. Although it is traditionally obtained from terrestrial biomass, the extraction of cellulose from marine biomass is of particular interest due to the abundance, great compositional diversity and interesting functional properties of marine resources. In fact, several studies have already reported on the possibility of developing bio-based food packaging materials based on cellulosic fractions extracted from marine biomass [18–21]. Although most of the available studies focus on the production of pure cellulose [22,23], a recent study has reported on the application of simplified extraction protocols yielding less purified cellulosic fractions with better mechanical and barrier performance [18,19]. Additionally, the presence of bioactive components may confer these cellulosic fractions interesting functionalities, such as antioxidant capacity [24]. Thus, the application of simplified extraction protocols for the production of cellulose-based fractions can be beneficial not only from an environmental and economic perspective, but also in terms of material properties.

Therefore, the aim of this work was the valorization of the waste generated after a typical extraction of alginate from three different brown seaweed species (*Alaria esculenta*, *Saccharina latissima* and *Ascophyllum nodosum*) for the extraction of less purified cellulose-based fractions by means of simplified extraction protocols. Furthermore, the suitability of these fractions to produce biopolymeric films for food packaging applications is evaluated, investigating the effect of the distinct fractions' composition on the performance of the films.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Raw materials

The seaweeds *Alaria esculenta* and *Saccharina latissima* (referred to as *Alaria* and *Saccharina*) were cultivated at the site of Seaweed Energy Solutions AS, Frøya, Norway harvested, rinsed in seawater and dried in May 2017. *Ascophyllum nodosum* (referred to as *Ascophyllum*) was collected in Kerry, Ireland in November 2018. The dry seaweeds and residues were ground into powder and stored at 0%RH cabinets until further use. The materials represent typical samples of common dried brown seaweeds available in Europe. All chemical reagents were obtained from Sigma-Aldrich (Spain).

#### 2.1.2. Alginate extraction and recovery of residuals

All steps regarding the alginate extraction aiming to generate non-extractable seaweed residuals were performed in a 1 L centrifugation bottle at ambient temperature. Sodium alginate was extracted from the raw seaweeds according to the following procedure 20 g dried seaweed (previously powdered in a Retch hammer mill (<0,5 mm)) was suspended in 500 mL 0.2 M HCl and subjected to gentle shaking for 12 h in an ion exchange procedure to replace all carboxylic counter ions with protons. After one centrifugation cycle (15 min at 3500 rpm), the supernatant was discharged and the residual was again homogeneously suspended in water, shaken for 1 h and subjected to a new centrifugation cycle. The supernatant was discharged and the residual, now containing alginic acid, was re-suspended in 900 mL 0,1 M NaHCO<sub>3</sub> to facilitate solubilization of sodium alginate. pH was adjusted to 8 with NaOH (pH

paper) and the mixture was vigorously shaken for 2 h. After a new centrifugation step (45 min at 4000 rpm), the now viscous supernatant was removed and subjected to alginate recovery (see below), otherwise this extract was discharged. The residual fraction was again suspended in water at pH > 7 and subjected to shaking for another 2 h. The centrifugation step was then repeated, the viscous supernatant was discharged/recovered and the insoluble residual was re-extracted. In short, this extraction procedure in water (pH > 7,5) was repeated 3 times, producing 4 supernatants containing sodium alginate and one non-extractable residual. These, being the target product of the current experiments, were frozen and freeze-dried. This extract production was done 8 times for each seaweed. When alginate was to be recovered, NaCl (s) was suspended in a small volume of water and added to the 4 individual extracts assuring 0.2% (w/v), and equal volumes of isopropanol was added by slowly stirring with a glass rod, leaving the mixture to precipitate. The fibrous alginate formed was recovered by a weak centrifugation cycle (2000 rpm 10 min). Then, the fibrous alginate precipitate was re-suspended in 250 mL 50% isopropanol by homogenization to a completely homogeneous paste and subjected to centrifugation. Washing and recovery was repeated with another portion of 50% isopropanol and finally pure isopropanol. The homogenized light-yellow alginate was drained on a filter paper to remove excess isopropanol and subjected to drying at 65 °C overnight. The recovery and washings of alginate from each extraction procedure required the consumption of 4 L of isopropanol for each series and were therefore not executed for all 24 extractions.

#### 2.1.3. Preparation of cellulosic fractions

Three different extraction protocols were carried out to obtain cellulosic fractions with different levels of purification, using the residues generated after extraction of alginate from *Alaria*, *Saccharina* and *Ascophyllum* seaweeds. For the production of the fractions coded as F2A, a treatment which is aimed to remove lignin, pigments and some non-cellulosic carbohydrates [25] was applied. Briefly, 8 g of dry residue were added under stirring to 700 mL of 1.4% NaClO<sub>2</sub> solution, having the pH adjusted to 3 with acetic acid. The extraction took place at 70 °C for 5 h and after that, the excess liquid was decanted. The solid fraction was collected and repeatedly washed with distilled water until neutral pH was reached. To obtain cellulosic fractions with a greater degree of purity, coded as F3A, a subsequent alkaline treatment step was applied to remove non-cellulosic carbohydrates. In that case, 8 g of sample (dry basis) were added to 400 mL of 5% KOH solution and the material was stirred at room temperature for 24 h. Afterwards, the mixture was heated up to 90 °C for 2 h. The resulting solid fraction was separated by filtration and thoroughly washed with distilled water until reaching neutral pH. As an alternative to the two-step purification protocol, the fractions coded as F3B were produced by subjecting the seaweed residues directly to the alkaline treatment. All the obtained fractions, in the form of a partially hydrated material, were stored in the fridge until further use. A small amount of each fraction was subjected to freeze-drying for further analyses.

#### 2.1.4. Production of cellulosic films

Cellulosic films were prepared by dispersing 0.25 g of the extracted fractions (dry weight) in 50 mL of distilled water. Homogenization was achieved through stirring with an ultra-turrax for approximately 1 min. The dispersions were then vacuum filtered using PTFE filters with 0.2 µm pore size to remove water. The solid material remaining in the filter was then dried at room temperature overnight. The formed films were peeled off the filters and stored in a desiccator at 0%RH. The thickness of the films, which was measured using a Palmer electronic digital micrometer, was within the range of 25–60 µm.

## 2.2. Compositional analysis

### 2.2.1. Carbohydrate analysis

The carbohydrate composition and amount in all samples were estimated after acid methanolysis, a technique which although not being able to cleave crystalline polysaccharides, has been reported as a good compromise between the lability of guluronic acid and scission of most glycosidic linkages [26]. As cellulose is present in the tested brown algae, a two-step sulphuric acid hydrolysis was also performed [27] as to determine the total glucose and the difference was ascribed to the crystalline cellulose content [28]. The samples were then analysed using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) with a ICS-3000 system (Dionex) equipped with a CarboPac PA1 column (4 × 250 mm, Dionex) at 30 °C and a flow rate of 1 mL min<sup>-1</sup>. Neutral sugars were eluted in water for 16 min with post-column addition of 0.5 mL min<sup>-1</sup> of 300 mM sodium hydroxide after a preconditioning isocratic step with 260 mM sodium hydroxide and 68 mM sodium acetate (7 min) and 5 min equilibration time in water prior to injection. Uronic acids were eluted in a gradient of 100 to 200 mM sodium acetate in 10 mM sodium hydroxide over 20 min. Fucose, glucose, galactose, arabinose, xylose, mannose, mannitol (Merck), guluronic acid, mannanuronic acid (Carbosynth, UK) and glucuronic acid were used for calibration and commercial microcrystalline cellulose and sodium alginate were used as positive controls. All experiments were carried out in triplicate.

### 2.2.2. Protein content

Samples were analysed for total nitrogen content using an Elemental Analyser Rapid N Exceed (Paralab S.L., Spain). About 100 mg of each of the powdered samples were pressed to form a pellet which was then analysed using the Dumas method, which is based on the combustion of the sample and subsequent detection of the released N<sub>2</sub> [29]. The total protein content was estimated from the nitrogen content multiplied by a factor of 6.25. This multiplication factor was verified by estimation of protein from total amino acid content (not including tryptophan or cysteine) following hydrolysis of the samples in 6 M HCl, 110 °C, 24 h, and subsequent analysis via HPLC quantification [30].

### 2.2.3. Ash content

The ash content (measure of mineral content) was determined by dry biomass calcination, according to the standard TAPPI T211 om-07 method. Approximately 0.25 g of dried material were added to a pre-weighed crucible and weighed. Combustion took place at 550 °C for 24 h in a muffle furnace. The ash content was gravimetrically quantified after combustion.

### 2.2.4. Lipid content

The lipid content was estimated using a Soxhlet extractor according to AOAC method 933.06 with slight modifications. Approximately 4 g of dry sample was extracted using a Soxhlet apparatus with 200 mL of hexane over 6 h. The lipid content was then gravimetrically quantified [31].

### 2.2.5. Lignin analysis

The Klason lignin content in the seaweed residues was determined according to the TAPPI T222 om-06 method, with slight modifications according to [32,33]. Approximately 300 mg of dry sample were weighed into pressure tubes and 3 mL of 72% H<sub>2</sub>SO<sub>4</sub> were added. The sample was mixed thoroughly and transferred to a water bath at 30 °C for 1 h. The tube was stirred every 10 min and afterwards neutralized with 84 mL water. The mixed tubes were placed in an autoclave for 1 h at 121 °C and then cooled down to room temperature. The resulting material was filtered, washed and dried overnight. The lignin content was determined gravimetrically.

### 2.2.6. Total phenolic content

The total phenolic content of the dried seaweeds and the residues was determined by the Folin-Ciocalteu method [34]. This colorimetric assay was carried out by dissolving the dry samples in water (for the residues) and ethanol (for the raw seaweed) at a concentration of 5 mg/mL. The Folin-Ciocalteu reagent was diluted 1:10 with distilled water and 125 µL of the final dilution was mixed with 20 µL of sample. Finally, 100 µL of sodium carbonate (75 mg/mL) were added and the samples were heated up to 40 °C during 10 min. Absorbance values were read at 750 nm wavelength. The calibration curve was built using gallic acid as a standard. The total phenolic content was expressed as mg of gallic acid (GA)/g extract. Determinations were carried out in triplicate.

### 2.3. ABTS<sup>•+</sup> radical cation scavenging activity

The ABTS<sup>•+</sup> radical cation scavenging activity of the seaweeds and the residues was determined according to [35]. Briefly, 0.192 g of ABTS were dissolved in 50 mL of PBS at pH 7.4 and mixed with 0.033 g of potassium persulfate overnight in the dark to yield the ABTS<sup>•+</sup> radical cation. Prior to use in the assay, the ABTS<sup>•+</sup> was diluted with PBS for an initial absorbance of ~0.700 ± 0.02 (1:50 ratio) at 734 nm, at room temperature. Free radical scavenging activity was assessed by mixing 1.0 mL diluted ABTS<sup>•+</sup> with 10 µL of sample (5 mg/mL of seaweeds in ethanol or 5 mg/mL of residues in water) and monitoring the change in absorbance at 6 min. A calibration curve was developed by using 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox).

The ABTS<sup>•+</sup> radical scavenging activity of the samples was expressed as a percentage of inhibition as follows:

$$\% \text{Inhibition} = (\text{Acontrol} - \text{Asample}) / \text{Acontrol} \times 100$$

where Acontrol is the absorbance of the control (ABTS<sup>•+</sup> without added sample) and Asample is the absorbance of the test sample. On the basis of a Trolox calibration curve, results were then expressed as mg Trolox equivalents (TE)/g extract. All determinations were carried out in triplicate.

### 2.4. Fourier transform infrared spectroscopy (FT-IR)

The dry seaweeds, residues and the extracted cellulosic fractions were analysed by FT-IR in attenuated total reflectance (ATR) mode using a Thermo Nicolet Nexus (GMI, USA) equipment. The spectra were taken at 4 cm<sup>-1</sup> resolutions in a wavelength range between 400 and 4000 cm<sup>-1</sup> and averaging a minimum of 32 scans.

### 2.5. Thermogravimetric analyses (TGA)

Thermogravimetric curves (TG) were recorded with a Setaram TG/DTA92 (SETARAM Instrumentation, France). The samples (ca. 10 mg) were heated from 30 to 800 °C with a heating rate of 10 °C/min under argon atmosphere. Derivative TG curves (DTG) express the weight loss rate as a function of temperature.

### 2.6. Scanning electron microscopy (SEM)

SEM was conducted on a Hitachi microscope (Hitachi S-4800) at an accelerating voltage of 10 kV and a working distance of 8–16 mm. Small pieces of the cellulosic films were sputtered with a gold-palladium mixture under vacuum before their morphology was examined.

### 2.7. Optical microscopy

Dispersions of the native seaweeds (5 mg/mL in ethanol) and the residues (5 mg/mL in water), as well as the extracted cellulosic fractions (in their partially hydrated form) were analysed by optical microscopy. Digital images were taken using an Eclipse 90i Nikon microscope (Nikon

corporation, Japan) equipped with 5-megapixels cooled digital colour microphotography camera Nikon Digital Sight DS-5Mc. A fluorescent filter UV-2A (Excitation 330–380 nm, Dichroic Mirror 400, LongPass 420 nm for emission) was additionally used to acquire images from the samples. Acquired images were analysed and processed by using Nis-Elements Br 3.2 Software (Nikon corporation, Japan).

## 2.8. X-ray diffraction (XRD)

XRD measurements were carried out on a D5005 Bruker diffractometer. The instrument was equipped with a Cu tube and a secondary monochromator. The configuration of the equipment was  $\theta$ – $2\theta$ , and the samples were examined over the angular range of  $3^\circ$ – $60^\circ$  with a step size of  $0.02^\circ$  and a count time of 200 s per step. Peak fitting was carried out using the Igor software package (Wavemetrics, Lake Oswego, Oregon) as described in a previous work [36]. The crystallinity index was determined by the method reported by Wang et al. [37]:

$$X_C(\%) = \frac{\sum A_{Crystall}}{A_{Total}} \times 100$$

where  $A_{Total}$  is the sum of the areas under all the diffraction peaks and  $\sum A_{Crystall}$  is the sum of the areas corresponding to the three crystalline peaks from cellulose I.

## 2.9. Water vapor permeability (WVP)

Direct permeability to water was determined from the slope of the weight gain versus time curves at  $24^\circ\text{C}$ . The films were sandwiched between the aluminum top (open O-ring) and bottom (deposit for the silica) parts of a specifically designed permeability cell with screws. A Viton rubber O-ring was placed between the film and bottom part of the cell to enhance sealability. These permeability cells containing silica were then placed in an equilibrated relative humidity cabinet at 75% RH and  $25^\circ\text{C}$ . The weight gain through a film area of  $10\text{ cm}^2$  was monitored and plotted as a function of time. Cells with aluminum films (with thickness of ca.  $11\ \mu\text{m}$ ) were used as control samples to estimate weight gain through the sealing. The WVP was calculated according to the following equation:

$$WVP (\text{kg}\cdot\text{m}/\text{Pa}\cdot\text{s}\cdot\text{m}^2) = \frac{WVTR \times L}{\Delta P}$$

where WVTR is the water vapor transmission rate ( $\text{kg}/\text{s}\cdot\text{m}^2$ ) (calculated from the slope of the linear region of the weight gain vs. time, divided by the exposed film area), L is the mean film thickness (m), and  $\Delta P$  is the difference of vapor pressure between the two sides of the film (Pa). The tests were done at least in triplicate.

## 2.10. Water uptake

The water swelling capacity of the cellulosic films was evaluated by soaking samples in sealed containers with 15 mL of distilled water at  $25^\circ\text{C}$ . Square specimens with a total surface area of  $6.25\text{ cm}^2$  were cut from the films and their initial weight as well as the weight gain after equilibration (24 h) were registered using a Precisa Gravimetrics AG SERIES 320XB analytical balance (Dietikon, Switzerland). Water uptake was calculated according to the following equation:

$$\text{Water uptake (\%)} = \frac{m_2 - m_1}{m_2} \times 100$$

where,  $m_1$  is the initial weight of the film, and  $m_2$  is the equilibrium weight of the film after immersion in water. The assays were carried out at least in triplicate.

## 2.11. Mechanical properties

Tensile tests were carried out at ambient conditions of typically  $24^\circ\text{C}$  and 50%RH on a Mecmesin MultiTest 1-i (1 kN) machine (Virginia, USA) with the Emperor™ software. Pre-conditioned rectangular-shaped specimens with initial gauge length of 8 cm and 1 cm in width were cut directly from the films. A fixed crosshead rate of 25 mm/min was utilized in all cases. The elastic modulus (E), tensile strength (TS), and elongation at break ( $\epsilon_B$ ) were determined from the stress-strain curves, estimated from force–distance data obtained for the different films. At least, two specimens of each film were tensile tested as to obtain statistically meaningful results.

## 2.12. UV and visible transmittance

Spectral transmittance of film samples was recorded on n 8453 Agilent UV–Vis spectrophotometer. A suitable size of rectangle film sample was directly inserted in a quartz cuvette and scanned in the UV–visible range 200–700 nm with an empty cuvette as a reference. UV and visible transmittance factor  $T_F$  (transmittance of a film sample per unit thickness) is defined as the following expression:

$$T_F = \frac{T_{300}}{x}, \frac{T_{350}}{x}, \frac{T_{450}}{x}$$

where  $T_{300}$ ,  $T_{350}$  or  $T_{450}$  is the value of transmittance at 300 nm (UVB), 350 nm (UVA) or 450 nm (visible), and x is the film thickness (in  $\mu\text{m}$ ).

## 2.13. Statistics

All data have been represented as the average  $\pm$  standard deviation. Different letters show significant differences both in tables and graphs ( $p \leq 0.05$ ). Analysis of variance (ANOVA) followed by a Tukey-test were used.

## 3. Results and discussion

### 3.1. Chemical composition of the raw seaweeds and the alginate-extracted residues

Compositional and structural analyses were firstly carried out on the residues generated after alginate extraction to evaluate their potential for the production of cellulose-based fractions, since, to the best of our knowledge, no previous works have reported on this. Moreover, to get a better understanding on the compositional and structural differences, the native dried seaweeds were also characterized. Table 1 summarizes the results from the compositional analysis.

When comparing the three dried seaweed preparations, it was clearly seen that they were mainly composed of carbohydrates (representing ca. 34–55% of the total dry mass), as recently reported for brown seaweeds [38], but they also contained significant amounts of proteins (ca. 7–14%) and minerals (ca. 19–47%). The macronutrient composition of *Alaria* and *Saccharina* (both belonging to the *Laminariales* order) was very similar, while *Ascophyllum* (belonging to the *Fucales* order) slightly differed, presenting the greatest amount of carbohydrates and the lowest protein content. When considering the seasonal variation of constituents, the estimated carbohydrate contents are within the estimated range, consistent with previous studies [38–41]. The protein content for brown seaweeds has been reported to be generally low (3–15%) compared to red and green seaweeds (10–47%) [42]. Thus, the determined values are in good agreement with the literature. As seen in Table 1, *Saccharina* contained slightly more protein than the other two seaweeds. The protein content is not only determined by the seaweed species, but it is also seasonal dependent, being the highest during the winter season (with values around 10% for brown seaweeds) and the lowest during the summer season (around 6%) [43,44]. This is mirroring

**Table 1**

(A) Macronutrient composition and (B) antioxidant capacity of the native dry seaweeds and the residues generated after alginate extraction.

A	<i>Alaria</i>		<i>Saccharina</i>		<i>Ascophyllum</i>	
	Seaweed	Residue	Seaweed	Residue	Seaweed	Residue
Carbohydrate (%) <sup>*</sup>	46.2 ± 13.7 <sup>a</sup>	35.5 ± 2.2 <sup>a</sup>	34.1 ± 2.8 <sup>a</sup>	48.7 ± 7.4 <sup>a</sup>	55.2 ± 4.9 <sup>a</sup>	57.2 ± 6.4 <sup>a</sup>
Protein (%)	10.2 ± 0.2 <sup>ab</sup>	29.9 ± 2.9 <sup>c</sup>	13.5 ± 0.1 <sup>b</sup>	37.5 ± 0.8 <sup>d</sup>	7.1 ± 0.3 <sup>a</sup>	12.1 ± 1.5 <sup>ab</sup>
Minerals (%)	37.6 ± 1.0 <sup>d</sup>	2.9 ± 1.5 <sup>a</sup>	47.6 ± 0.6 <sup>e</sup>	4.2 ± 1.6 <sup>a</sup>	19.8 ± 1.0 <sup>c</sup>	9.87 ± 0.8 <sup>b</sup>
Lipid (%)	1.94 ± 0.5 <sup>a</sup>	6.5 ± 3.4 <sup>ab</sup>	2.2 ± 0.1 <sup>a</sup>	7.5 ± 3.6 <sup>b</sup>	4.6 ± 1.5 <sup>ab</sup>	4.4 ± 0.04 <sup>ab</sup>
Sum (%)	96	75	97	98	87	84

B	<i>Alaria</i>		<i>Saccharina</i>		<i>Ascophyllum</i>	
	Seaweed	Residue	Seaweed	Residue	Seaweed	Residue
Polyphenols (mg GAE/g sample)	21.7 ± 1.9 <sup>b</sup>	39.4 ± 2.2 <sup>c</sup>	5.6 ± 1.9 <sup>a</sup>	19.5 ± 3.6 <sup>b</sup>	31.9 ± 4.6 <sup>c</sup>	51.6 ± 1.0 <sup>d</sup>
Antioxidant capacity ABTS (μmol TE/g sample)	248.8 ± 8.7 <sup>c</sup>	309.4 ± 3.1 <sup>d</sup>	40.6 ± 3.0 <sup>a</sup>	72.0 ± 4.5 <sup>b</sup>	328.5 ± 4.9 <sup>e</sup>	377.5 ± 3.0 <sup>f</sup>

Data shown as mean ± SD,  $n = 3$ . Values within the same row with different letters are significantly different ( $p \leq 0.05$ ).<sup>\*</sup> Total carbohydrate content calculated as the sum of all monosaccharide units analysed by HPAEC.

the huge seasonal variation in the storage polysaccharide laminaran with a peak in early autumn and accumulation of minerals in the spring [41,45]. The values reported in the literature for the mineral content in brown seaweeds are highly variable due to seasonal variations, but minerals can make up to 50% of the dry weight [46]. For instance, mineral contents ranging between 14 and 46% for *Saccharina latissima* [46,47], 25–32% for *Alaria esculenta* [41] and up to 26% for *Ascophyllum nodosum* [48] have been reported. The mineral contents determined in this study were reasonable given the large variability in the literature values. It should be considered that since this work focuses on the valorization of the residuals, the seaweeds were processed as received, i. e. no further washing in freshwater was carried out; thus, any residual sea salt remaining in the samples would have been concentrated in the seaweed biomass during drying and would have significantly contributed to the mineral content, although these residual salts were expected to be removed in the liquid effluents generated during the alginate extraction process. The low lipid content in the native seaweeds (2–5%), is in agreement with values previously reported for *Alaria*, *Saccharina* and *Ascophyllum*, ranging from 1% to 4% [49–51].

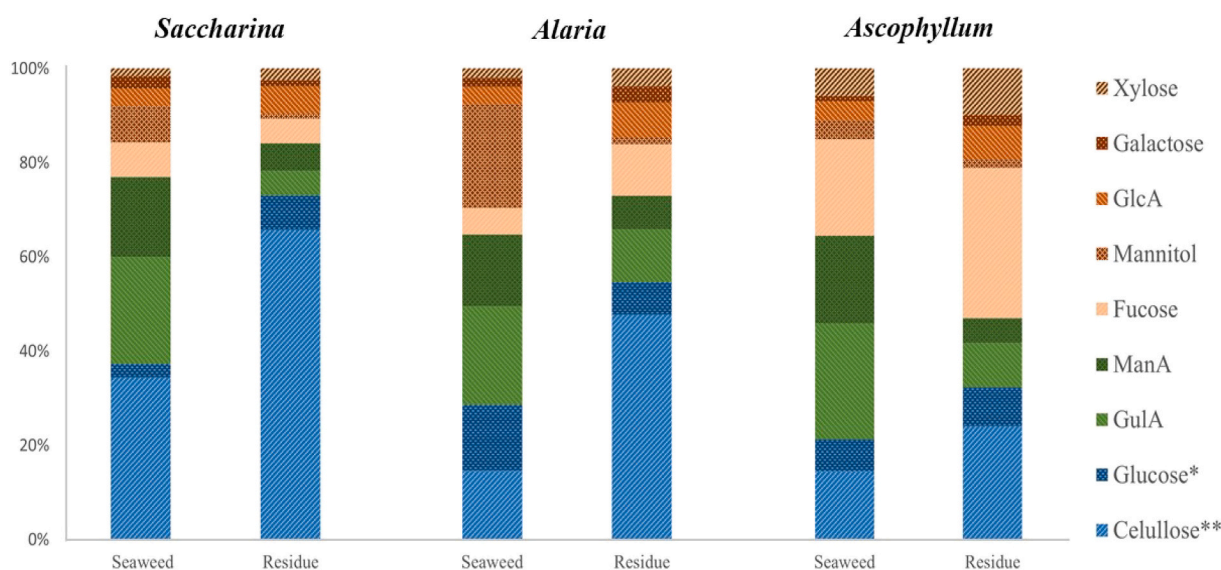
As deduced from the results in Table 1, after alginate extraction the protein content increased significantly in the three residues, while the mineral content was reduced, as expected. Furthermore, the amount of lipids increased in *Alaria* and *Saccharina*. On the other hand, the total amount of carbohydrates was not significantly affected. These results suggest that most proteins and lipids were unaffected by the alginate extraction process, while a significant proportion of minerals were washed off or degraded during the different steps applied for alginate extraction. The alginate extraction yields were estimated to be around 10% for *Alaria*, 15% for *Saccharina* and 27% for *Ascophyllum*. On the other hand, the amount of residue generated after the extraction of alginate was ca. 35% for *Alaria*, 31% for *Saccharina* and 45% for *Ascophyllum*, which means that approximately 50% of the water soluble low molecular weight constituents were removed within the liquid effluents generated during the extraction process from *Alaria* and *Saccharina*, while ca. 30% from *Ascophyllum* was lost upon extraction. As deduced from the results summarized in Table 1, the mass balance of macronutrient content was greater than 84% for most of the samples, which is within an acceptable margin of experimental error, thus confirming the reliability of the obtained values. The lower mass balance obtained for the residue from *Alaria* is most likely due to an underestimation of the amount of carbohydrates and indicates that the hydrolysis protocol applied for the monosaccharide analyses should be optimized for this type of samples in the future.

It should be noted that, as expected, due to the inherent low lignin content in brown seaweeds [41], very minor amounts were detected in the residues (ca. 1–2%). This confirms that the first step in the typical cellulose extraction protocol, which consists of a NaClO<sub>2</sub> treatment and aims to remove mainly lignin, pigments and polyphenols, may be

skipped in this case.

Brown seaweeds are generally rich in phenolic compounds, which have been reported to have antioxidant properties [52]. While some data about the phenolic content of brown seaweeds are available in the literature (around 3% for *Alaria* [41,53], 1% for *Saccharina* [38,41,44,53] and 2.5% for *Ascophyllum* [54]), it is hard to obtain reliable and consistent values for the antioxidant capacity. The polyphenol content and the antioxidant capacity of the three seaweeds and corresponding residues were determined using a simplified crude colorimetric approach and the results are shown in Table 1. Regarding the polyphenol content, *Ascophyllum* seaweed showed the highest one (ca. 32 mg GAE/g sample), while *Saccharina* showed the lowest content (ca. 6 mg GAE/g sample). For the three seaweeds, the amount of polyphenols increased in the residues after alginate extraction, reaching values of around 44 mg GAE/g sample for *Alaria* residue, 20 mg GAE/g sample for *Saccharina* residue and 52 mg GAE/g sample for *Ascophyllum* residue. Even though the alginate extraction treatment produced the breakage of the seaweed cell wall, the polyphenols did not seem to be released into the liquid effluents generated during the extraction process, suggesting that they were still linked to the polysaccharides remaining in the solid residues, such as cellulose and fucoidans (cf. Fig. 1). This is typical for alginate extractions at around neutral pH [10] as used in our study. Values of ca. 30–150 mg GAE/g sample, 44–95 mg GAE/g sample and 9–59 mg GAE/g sample have been reported for various extracts obtained from *Ascophyllum* [55], *Alaria* and *Saccharina* extracts [56], respectively. Thus, the polyphenol contents obtained in this study are within reasonable ranges. The fact that the polyphenols remain in the residues is the main reason we chose not to use a conventional formaldehyde pre-treatment prior to alginate extraction, since for food applications the residuals should be free of formaldehyde that would otherwise react with and cross-link the polyphenols. Formaldehyde is principally used in alginate production to improve alginate quality as a preservative and by reducing polyphenol catalysed depolymerisation and decolouration especially if using an alkali-based extraction [10].

In line with the results from the polyphenol content, the antioxidant capacity of the residues was greater than for the native seaweeds, being the highest in both the *Ascophyllum* seaweed and residue. The values increased from 329 μmol Trolox/g sample to 378 μmol Trolox/g sample for *Ascophyllum*, from 249 μmol Trolox/g sample to 309 μmol Trolox/g sample for *Alaria* and from 41 μmol Trolox/g sample to 72 μmol Trolox/g sample for *Saccharina*, after the alginate extraction. The high antioxidant capacity of *Ascophyllum* seaweed is not only linked to its greater polyphenol content, but also to its greater content in fucoidan (as later demonstrated by the monosaccharide analysis, cf. Table 2), a sulphated polysaccharide with attributed high antioxidant capacity [57]. These results highlight the potential of the residues generated after the alginate extraction, in particular from *Ascophyllum* seaweed, as antioxidants.



**Fig. 1.** Relative carbohydrate composition of the native seaweeds and the residues generated after alginate extraction. The results from the sugar constituents are expressed as g polysaccharide per 100 g total carbohydrate. Data correspond to the mean calculated values,  $n = 3$ .

\*\*The crystalline cellulose content was determined as the difference between the typical Saeman sulphuric hydrolysis [27] and the non-crystalline glucose determined after acid methanolysis [28,84].

\*Glucose contribution from the non-crystalline fraction.

Mannose was detected in only very minor amounts.

**Table 2**

Extraction yields and carbohydrate composition of the cellulosic fractions extracted from the residues of *Alaria* and *Saccharina*. The results from the sugar constituents are expressed as g polysaccharide per 100 g dry weight sample.

	<i>Alaria</i>			<i>Saccharina</i>		
	F2A	F3A	F3B	F2A	F3A	F3B
Extraction yield (%)	20.6 ± 0.4 <sup>b</sup>	14.6 ± 0.2 <sup>a</sup>	18.5 ± 0.3 <sup>b</sup>	38.6 ± 2.0 <sup>d</sup>	25.8 ± 0.5 <sup>c</sup>	37.5 ± 1.1 <sup>d</sup>
Fucose	3.0 ± 0.8 <sup>b</sup>	<0.5	0.7 ± 0.2 <sup>a</sup>	2.9 ± 0.3 <sup>b</sup>	<0.5	<0.5
Galactose	2.5 ± 0.4 <sup>a</sup>	<0.5	1.8 ± 0.2 <sup>a</sup>	2.2 ± 0.3 <sup>a</sup>	<0.5	<0.5
Glucose	34.1 ± 1.6 <sup>a</sup>	90.1 ± 9.8 <sup>b</sup>	83.6 ± 7.2 <sup>b</sup>	37.6 ± 10.0 <sup>a</sup>	85.9 ± 10.1 <sup>b</sup>	78.0 ± 7.4 <sup>b</sup>
Mannose	2.8 ± 0.6 <sup>a</sup>	tr	tr	4.2 ± 0.7 <sup>a</sup>	tr	tr
Xylose	1.0 ± 0.3 <sup>a</sup>	1.2 ± 0.4 <sup>a</sup>	0.8 ± 0.3 <sup>a</sup>	2.8 ± 1.0 <sup>b</sup>	<0.5	<0.5
GlcA	3.9 ± 0.4 <sup>a</sup>	<0.5	<0.5	6.1 ± 1.1 <sup>a</sup>	<0.5	<0.5
GulA	9.2 ± 0.4 <sup>c</sup>	<0.5	<0.5	6.7 ± 0.4 <sup>b</sup>	0.6 ± 0.3 <sup>a</sup>	<0.5
ManA	8.6 ± 0.2 <sup>c</sup>	<0.5	<0.5	6.5 ± 0.2 <sup>b</sup>	0.5 ± 0.3 <sup>a</sup>	<0.5

Data shown as mean ± SD,  $n = 3$ . Values within the same row with different letters are significantly different ( $p \leq 0.05$ ).

This may be of interest for the development of bioactive packaging films [39,52], as well as for nutraceutical applications [58].

### 3.1.1. Carbohydrate composition of the raw seaweeds and the alginate-extracted residues

Since carbohydrates were the main component in both the native seaweeds and the residues, a more detailed analysis of the carbohydrate composition was carried out and the results are shown in Fig. 1.

Alginate is the main carbohydrate in brown seaweeds, accounting for up to 40% of the dry matter [59], and it consists of (1 → 4)-linked β-D-mannuronic acid and α-L-guluronic acid residues. Thus, the relative amount of alginate in the samples was estimated from the mannuronic

acid (ManA) and guluronic acid (GulA) contents (cf. Fig. 1 and Table S1). As expected, the alginate content in the seaweeds was significantly reduced in the residues after the applied extraction treatments, decreasing from ca. 16% to 8% of the total dry weight for *Alaria*, from ca. 13% to 5% for *Saccharina* and from ca. 23% to 8% for *Ascophyllum*. The inability of conventional alginate extraction processes to quantitatively extract all the alginate present in brown seaweeds and consequently, the presence of significant quantities of alginate in the residual biomass might actually be positive, as this polysaccharide might complement the mechanical properties of cellulose and in the extracted fractions for the production of packaging films. Apart from mannuronic acid and guluronic acid, other constituent sugars such as glucose, mannitol and fucose were abundant in the seaweed biomass. Glucose can be mainly associated to the presence of cellulose. On the time when *Alaria* and *Saccharina* were harvested the content of laminaran is typically at its minimum, and if present in the dried and finely milled seaweed, the major part of laminaran will be efficiently removed during acid pre-extraction and the initial water washings. Glucose was more abundant in the *Saccharina* biomass, representing ca. 36% of the total carbohydrate fraction in the native seaweed and ca. 72% in the residue. Although the relative amount of glucose increased in the three seaweeds after alginate extraction, this was less obvious in the case of *Ascophyllum*, suggesting that the amount of crystalline cellulose, resistant to the extraction treatments, was lower in that case. Mannitol, which represented ca. 21% of the total carbohydrate fraction in *Alaria*, was almost completely removed upon alginate extraction, as expected [60]. On the other hand, fucose, mainly attributed to the presence of fucoidan, represented ca. 20% of the total carbohydrate in *Ascophyllum*, in line with previous work [61]. Fucoidans have been reported to remain in the residue generated after alginate extraction, being subsequently extracted by acidic treatments [62]; thus the increased amount of fucose in the residue, accounting for ca. 31% of the total carbohydrate, was not surprising. The greater fucoidan content in the *Ascophyllum* biomass can be in fact linked to its greater antioxidant capacity (cf. Table 1). These results indicate that while *Saccharina* residue might be the most appropriate for the extraction of cellulose, *Ascophyllum* residue may have potential for the extraction of bioactive fucoidan-rich fractions.

### 3.1.2. Structural characterization by FT-IR

To better understand the compositional differences between the native seaweeds and their residues, they were characterized by means FT-IR and the obtained spectra can be seen in Fig. 2.

In general, all the samples presented spectra characteristic from materials rich in polysaccharides and proteins, showing the broad band at  $3600\text{--}3200\text{ cm}^{-1}$  (characteristic from O—H and N—H stretching), the bands at  $3000\text{--}2900\text{ cm}^{-1}$  (associated to C—H stretching), as well as several pronounced bands within the range of  $1300\text{--}1100\text{ cm}^{-1}$  (corresponding to C—O—C stretching vibrations of polysaccharides). Two alginate-characteristic bands, located at ca.  $787\text{--}808\text{ cm}^{-1}$  and ca.  $1030\text{--}1080\text{ cm}^{-1}$ , assigned to mannuronic and guluronic acids, respectively [63], were detected in all the samples. Additionally, the band at ca.  $1620\text{ cm}^{-1}$ , which has been linked to the symmetric COO- stretching vibration in alginate [64], was also visible. The appearance of these bands in the residues confirms the presence of residual alginate, as suggested by the monosaccharide analyses. Moreover, the band at ca.  $1200\text{--}1260\text{ cm}^{-1}$ , associated to S=O stretching vibration of sulphate groups [65], was more evident in the *Ascophyllum* seaweed and residue and was correlated with the greater abundance of sulphated fucoidan in these seaweeds. It should be noted that the bands at ca.  $1160$ ,  $1105$  and  $1060\text{ cm}^{-1}$ , which are typically linked to C—C stretching and C—O stretching from cellulose [24,64,66], became more intense in the residues, confirming the concentration of cellulose in the biomass generated after alginate extraction. The presence of proteins was also confirmed by the appearance of the bands located at  $1640\text{ cm}^{-1}$  (amide I) (overlapped with the band at  $1620\text{ cm}^{-1}$ ) and  $1540\text{ cm}^{-1}$  (amide II). The greater intensity of the  $1510\text{ cm}^{-1}$  band in the residues confirms that the proteins were not affected by the alginate extraction and were more concentrated in the residues. Interestingly, the peak at ca.  $1730\text{ cm}^{-1}$ , corresponding to C=O stretching, was much sharper and intense in *Ascophyllum* (both seaweed and residue). This band has been detected in fucoidans extracted from several seaweed species and has been suggested to arise from acetylation of glucuronic moieties [39,64,65]. According to the literature, the band at ca.  $1410\text{ cm}^{-1}$ , which can only be seen in the raw seaweeds, may be related to C—OH deformation vibration with contribution of O—C—O symmetric stretching vibration of carboxylate group [64,66]. This band has been previously attributed to the presence of soluble alginate [39], which was removed upon the applied extraction treatments.

### 3.1.3. Morphological characterization

The morphology of the different seaweeds and residues was studied by means of optical microscopy. Fig. 3 shows representative images taken with bright light and ultraviolet filters, in which fluorescent characteristics of material components can be observed.

The images evidence a great difference in the cell structure of *Ascophyllum* as compared with *Alaria* and *Saccharina*. While *Alaria* and *Saccharina* showed well-defined rectangular-shaped cells, *Ascophyllum* presented cells with a less defined shape which appeared to be more round-shaped. This can be related to the lower cellulose content in *Ascophyllum*. After the extraction of alginate, the cell walls from the three seaweeds did not lose their integrity, which is reasonable since cellulose (one of the major structural components in cell walls) [67,68] was not affected by the extraction treatments. However, instead of being tightly packed, the individual cells were away from each other, indicating that the components removed upon the applied treatments were acting as gluing agents. Upon observation of the samples with the ultraviolet filter, it was evident that while the images from the native seaweeds were mostly dominated by the appearance of bright blue fluorescent regions, the images of the corresponding residues showed very faint to no light blue fluorescence and instead showed red fluorescent regions, which were much brighter and intense in the case of the *Ascophyllum* residue. According to the compositional analyses, it is likely that the blue fluorescent areas correspond to alginate-rich regions, which were almost completely removed after the applied treatments. On the other hand, based merely on the compositional analyses, the bright red areas may correspond to protein- or polyphenol-rich domains, mainly located intracellularly.

### 3.2. Characterization of the extracted cellulosic fractions

Different extraction protocols were applied to the residues to obtain cellulosic fractions with different levels of purification, being F3A the most purified one, while F2A and F3B were expected to contain other components other than cellulose. When using the residue from *Ascophyllum*, very low extraction yields (ca. 0.2–3%) were obtained. This supports the hypothesis of *Ascophyllum* having a lower cellulose content, as deduced from the monosaccharide analyses (cf. Fig. 1). As expected, the extraction yield decreased with the purity of the fractions, going from 21 to 15% for *Alaria* residue and from 39 to 26% for *Saccharina* residue (cf. Table 2). The higher extraction yields for *Saccharina* residue

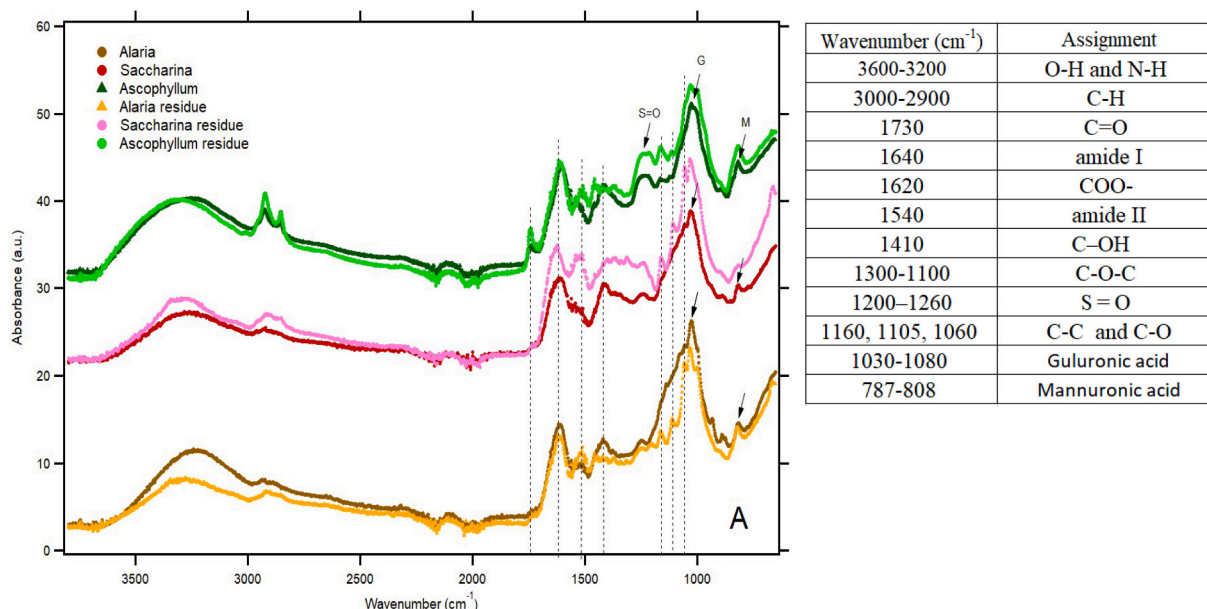
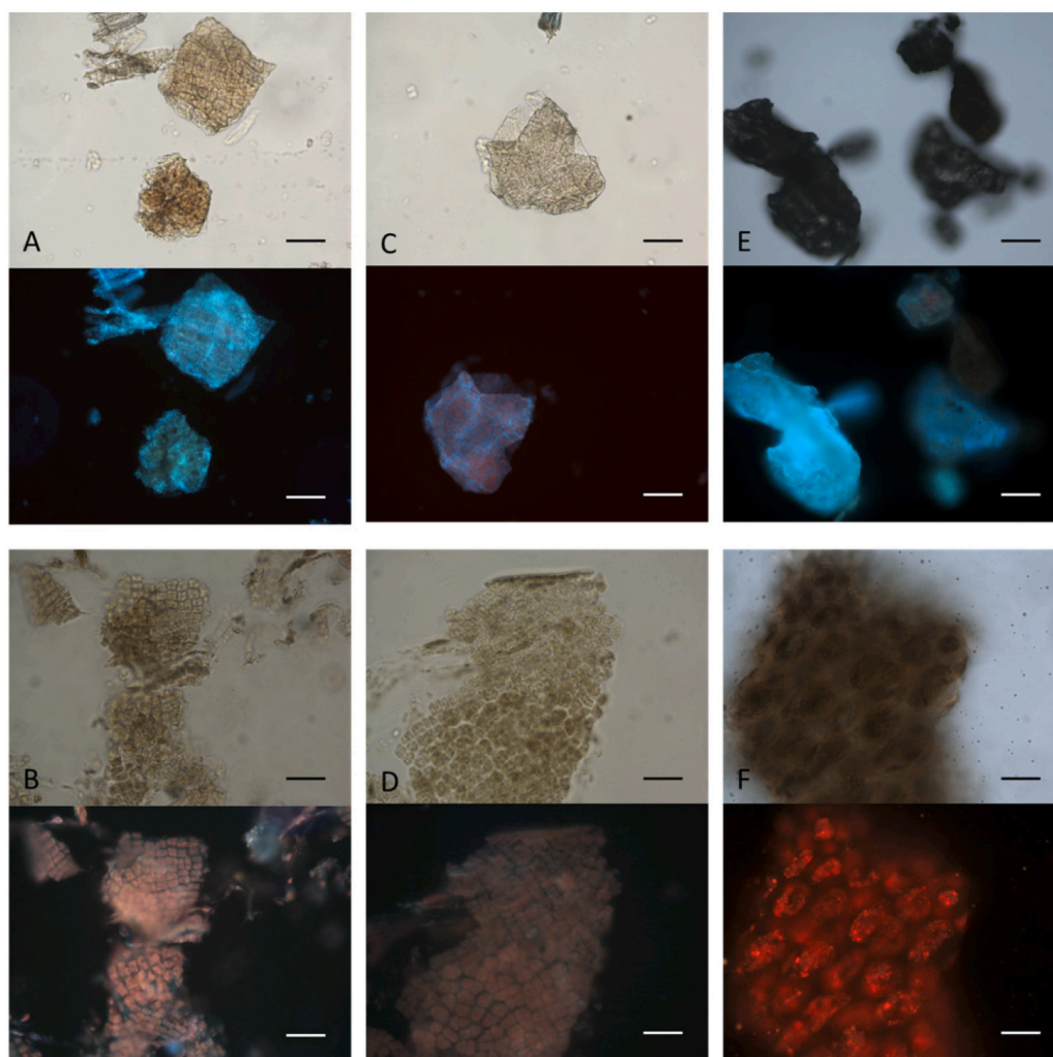


Fig. 2. FT-IR spectra of the native seaweeds and the residues generated after alginate extraction. G: guluronic acid; M: mannuronic acid.



**Fig. 3.** Optical microscopy images of the native seaweeds and the residues generated after alginate extraction. (A) *Alaria* seaweed, (B) *Alaria* residue, (C) *Saccharina* seaweed, (D) *Saccharina* residue, (E) *Ascophyllum* seaweed and (F) *Ascophyllum* residue. Scale bars correspond to 50  $\mu\text{m}$ . Top images were taken with bright light while bottom images were taken using a fluorescent filter UV-2A.

are reasonable given its higher cellulose content (cf. Fig. 1).

### 3.2.1. Carbohydrate composition

Monosaccharide analyses of the fractions extracted from *Alaria* and *Saccharina* were carried out to investigate their carbohydrate composition and the results are summarized in Table 2. It is worth noting that the residual alginate was not removed upon the bleaching treatment with  $\text{NaClO}_2$ . Thus, the F2A fractions were mainly composed of cellulose and residual alginate, being *Alaria* richer in alginate and *Saccharina* richer in cellulose. In contrast, the F3A and F3B fractions were almost pure cellulose (>95%).

### 3.2.2. Structural characterization by FT-IR

FT-IR characterization of the obtained fractions was carried out to identify changes in the composition and molecular structure of their main components after the different extraction procedures. Fig. 4 shows the spectra for the different fractions extracted from the residues.

As observed, the F3A and F3B fractions presented very similar spectra, while F2A fractions differed significantly, showing spectra more similar to those from the corresponding residues. The bands related to mannuronic and guluronic acid, located at ca.  $787\text{--}808\text{ cm}^{-1}$  and ca.  $1030\text{--}1080\text{ cm}^{-1}$  respectively, were only detected in the residues and in F2A fractions. Furthermore, these fractions showed a relatively intense

band at  $1620\text{ cm}^{-1}$ , also visible in the residue, which is associated to carbonyl groups of uronic acid [62,64]. In the case of the F3A and F3B fractions this band was not visible and instead a weak band at  $1630\text{ cm}^{-1}$  (corresponding to bound water) was detected. This indicates that the residual alginate remaining in the residue was only removed when the alkaline treatment was applied. The band located at  $1200\text{--}1260\text{ cm}^{-1}$ , indicative of the presence of sulphate, which may arise from the presence of fucoidan [64], was detected in all the F2A fractions, but it appeared much more intense in the case of *Ascophyllum*. In that case, the band was visible for the three fractions, but it appeared much fainter in the case of F3A, confirming its greater degree of cellulose purity. Furthermore, the  $1540\text{ cm}^{-1}$  band, characteristic of proteins (amide II), was only visible in the F2A fractions. In fact, the protein content in the F2A fractions was determined to be approximately 31% for *Alaria*, 33% for *Saccharina* and 15% for *Ascophyllum*. On the other hand, protein contents lower than 2% were determined for the F3A and F3B fractions from the three seaweed residues. In agreement with the monosaccharide analyses, several of the cellulose characteristic bands, such as those located at a.  $1160$ ,  $1105$  and  $895\text{ cm}^{-1}$  [24,64,66], showed higher relative intensity in the F3A and F3B fractions, confirming their greater amount of cellulose.



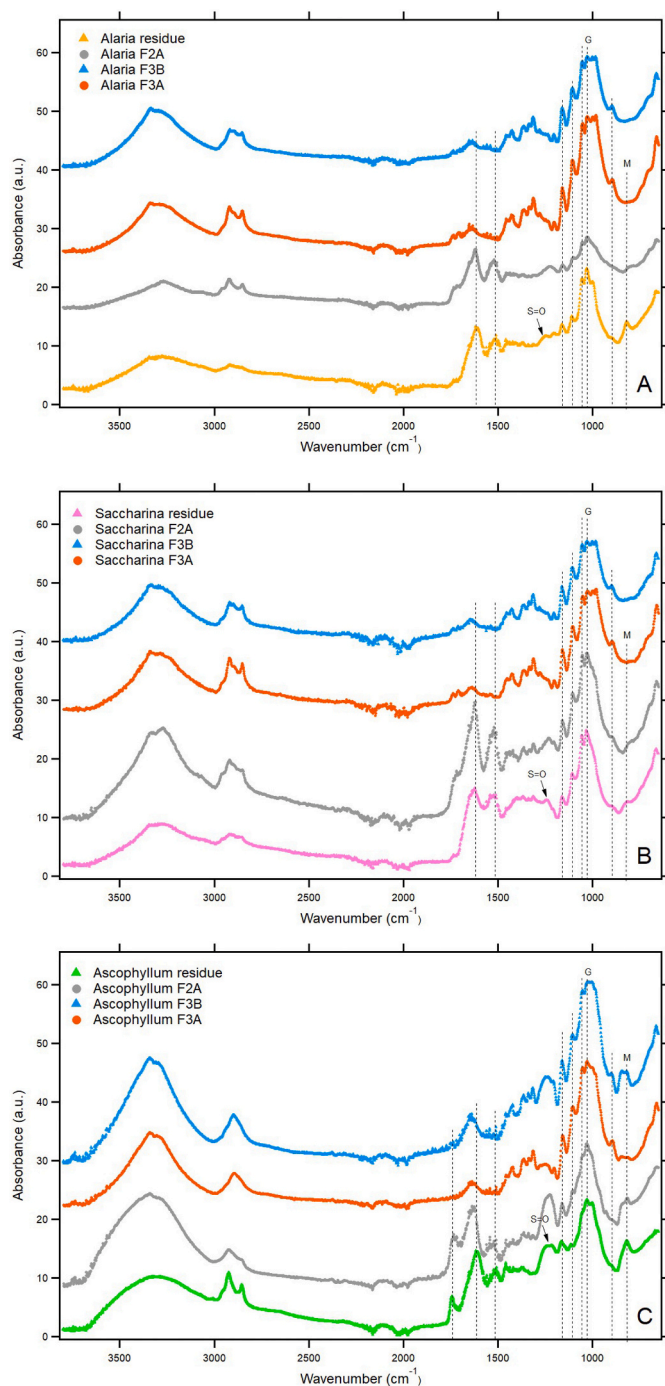


Fig. 4. FT-IR spectra of the cellulose fractions extracted from (A) *Alaria* residue, (B) *Saccharina* residue and (C) *Ascophyllum* residue.

### 3.2.3. Morphological characterization

Similar to the raw seaweeds and residues, the extracted fractions were analysed by optical microscopy. The images, shown in Fig. S1, illustrate the changes in the cell structure with the different extraction methods and purification levels. An obvious difference in the cell morphology of the fractions extracted from *Ascophyllum* residue can be observed. As mentioned before, the cell structure of *Ascophyllum* is very different from that of the two other seaweed species, which is reasonable given the fact that they belong to different seaweed families. Notably, unlike the cell clusters observed in the case of the residues, the individual cells could be clearly identified in the F2A fractions from the three seaweed species; however, the cell wall structure must have not

been completely disrupted with the  $\text{NaClO}_2$  treatment since the boundaries of the cells could still be seen. For the F3A and F3B fractions cell tissue could still be seen, but the cell walls appeared to be completely destroyed. Furthermore, the blue regions observed with the fluorescent filter, attributed to alginate-rich regions, were visible in the F2A fractions (composed of cellulose and alginate) while the F3A and F3B fractions (almost pure cellulose) showed no fluorescence. It should also be noted that F3A and F3B fractions showed almost identical morphologies, suggesting that the alkaline treatment alone was sufficient to disrupt the cell wall structure of the residues.

### 3.2.4. Thermal stability

TGA analyses were also carried out to determine the thermal stability of the extracted fractions and link their degradation profiles to their composition. The derivative of the weight loss with the temperature was plotted and the results are shown in Fig. 5.

The different levels of cellulose purification in the extracted fractions clearly influenced their degradation profiles. While the *Alaria* and *Saccharina* F3A and F3B fractions showed a degradation mechanism clearly dominated by one strong peak at around 300–330 °C, attributed to the degradation of cellulose [69], the F2A fractions showed a multi-step degradation profile. The degradation step centred around 230 °C for *Alaria* and *Ascophyllum* F2A can be attributed to the presence of proteins and other polysaccharides in these fractions. Notably, the fractions extracted from *Saccharina* showed an additional degradation peak at around 460 °C, similarly to what has been reported for *Laminaria* seaweed [70]; this peak may originate from the presence of secondary cellulose degradation products, such as cellulose esters or complexes formed with residual cations remaining in the material [71,72]. The different degradation behaviour of the fractions extracted from *Ascophyllum* could be explained by lower cellulose content in these samples, which in turn were expected to be richer in other polysaccharides such as fucoidan. In fact, the F2A fraction extracted from *Ascophyllum* residue presents a very similar degradation profile to that previously reported for pure fucoidan [73].

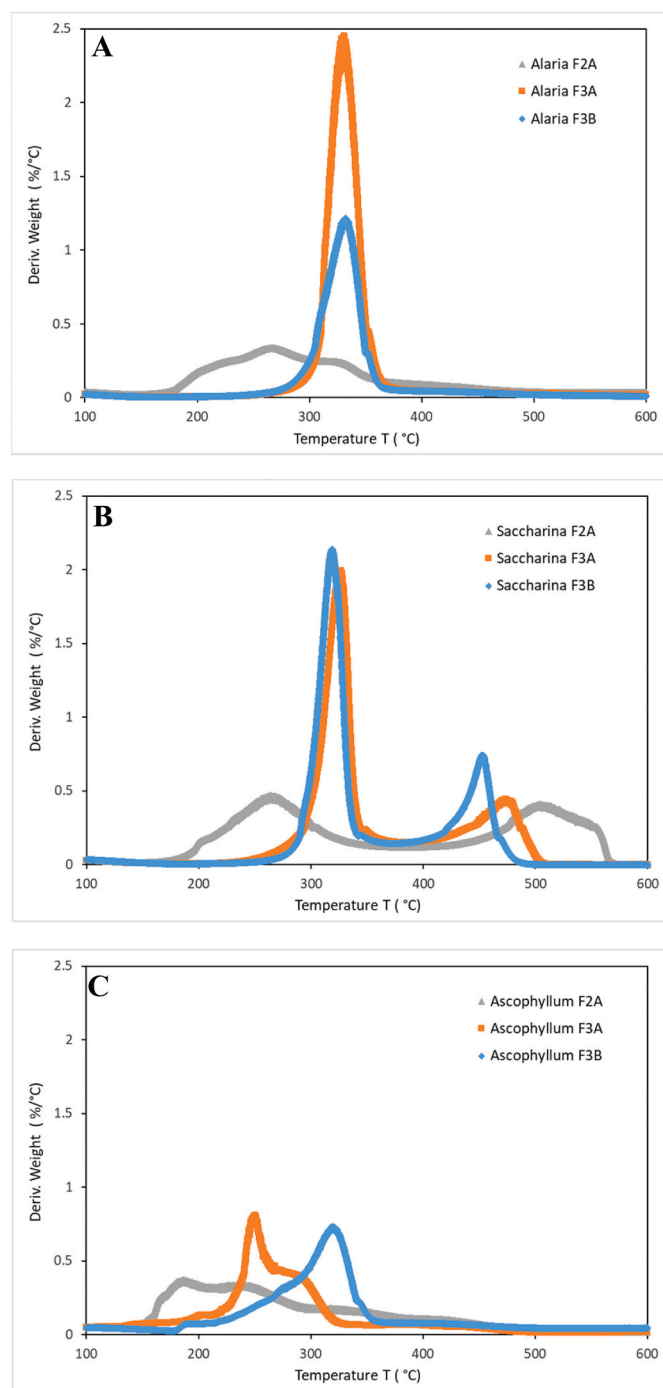
### 3.3. Production and characterization of cellulosic films

The cellulosic fractions extracted from *Alaria* and *Saccharina* were used to produce films and their structural and functional properties were characterized to evaluate their potential as food packaging materials.

#### 3.3.1. Transparency and microstructure of the films

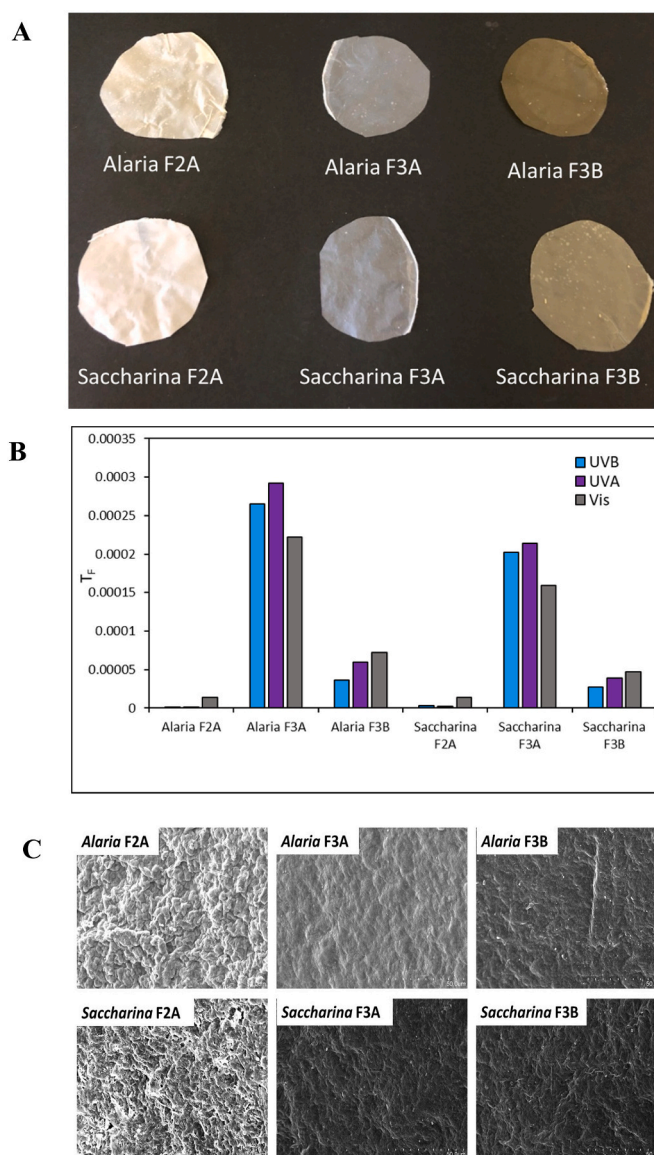
As seen in Fig. 6A, the visual appearance, i.e. colour and transparency, of the films was significantly affected by the purification level. While the F3A and F3B films were translucent, the F2A films were completely opaque, which may be due to the presence of non-cellulosic carbohydrates. In fact, the transparency of the films was assessed by internal transmittance (Ti) measurements and the results (cf. Fig. 6B) evidenced that the F3A films, i.e. the ones with the greatest cellulose content, were the most transparent. It was also noted that while the fractions subjected to the bleaching treatment (F2A and F3A) yielded films with a white coloration, the F3B films showed a greenish colouration, which could arise from the presence of seaweed pigments. Considering that for food packaging applications transparent materials are preferable, the F3B and F3A films would be more interesting. It should also be noted that the appearance of small aggregates in all the developed films, which most likely arise from an insufficient dispersion of cellulose in the aqueous dispersions prepared prior to the vacuum filtration step, could be avoided by applying an additional homogenization step such as sonication. A better dispersion of cellulose is expected to improve further the functional properties of the films and thus, this will be optimized in the future.

The morphology of the different films was studied by SEM and representative images are shown in Fig. 6C. As observed, the more purified films (F3A and F3B) presented a more compact and uniform



**Fig. 5.** TGA derivative curves of the cellulosic fractions extracted from (A) *Alaria* residue, (B) *Saccharina* residue and (C) *Ascophyllum* residue.

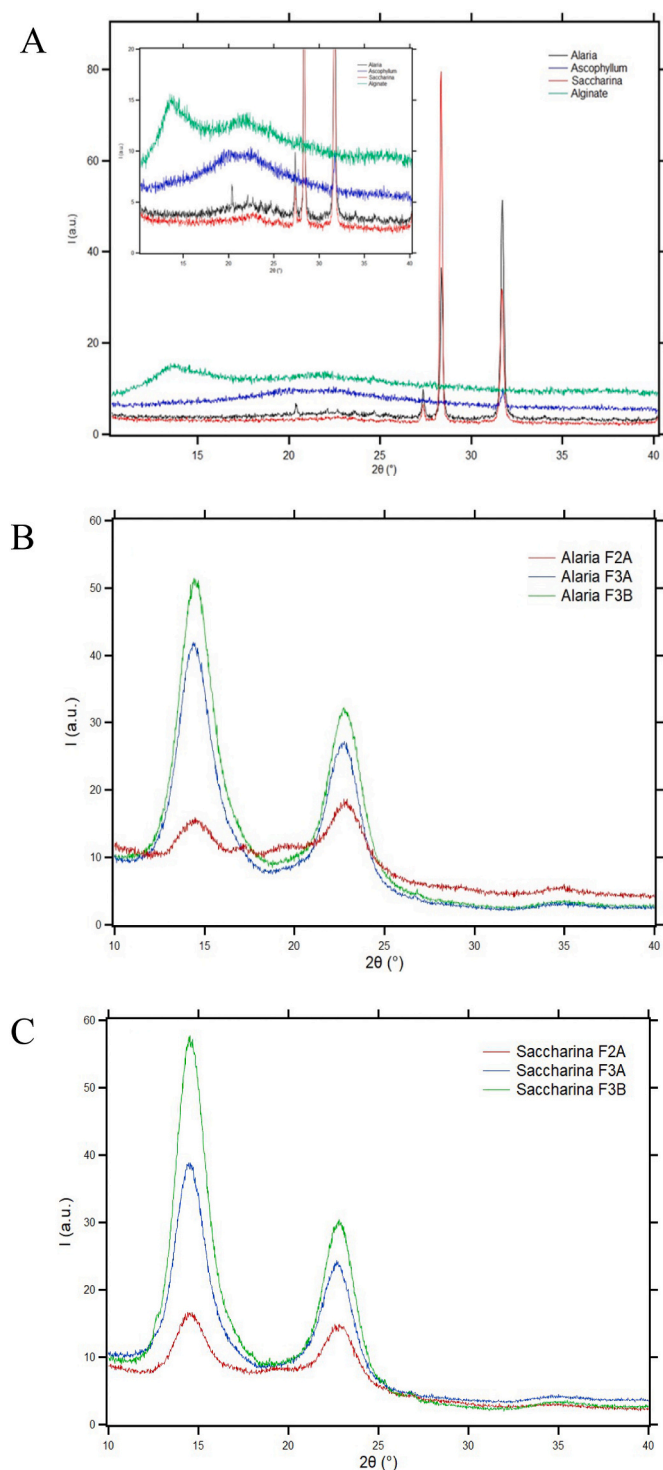
surface than the less purified F2A films. The greater heterogeneity of the F2A films can be attributed to the presence of amorphous components, such as proteins and non-cellulosic carbohydrates. The fibrillar structure characteristic from cellulose can be identified in the F3A and F3B films; however, instead of showing pure cellulose fibers, the images evidence the presence of cellulose embedded in an amorphous matrix. A similar surface morphology was reported for cellulosic films containing residual agar [24]. It is suspected that the residual alginate remaining in the fractions, acted as an amorphous matrix in which the cellulose fibrils were embedded.



**Fig. 6.** (A) Visual appearance and (B) transmittance factor of the cellulosic films obtained from the seaweed residues in the UVB, UVA and visible wavelength regions (C) SEM images from the surface of the cellulosic films obtained from the seaweed residues. Scale bars correspond to 50  $\mu$ m.

### 3.3.2. Characterization of the crystalline structure of the cellulosic films by XRD

XRD analyses were carried out to investigate the crystalline structure of cellulose in the produced films. For reference, the XRD patterns from the native seaweeds, as well as an alginate standard, were also acquired and the results are shown in Fig. 7A. As expected, the alginate standard showed a spectrum with very broad and weak peaks, located at  $13.6^\circ$  and  $21.6^\circ$ , indicating its highly amorphous character. In the case of *Ascophyllum*, two overlapping weak shoulders at  $20^\circ$  and  $22.5^\circ$  (corresponding to the alginate), as well as one small peak at  $31.7^\circ$  were detected. In contrast, the patterns from the native *Alaria* and *Saccharina* seaweeds were dominated by several sharp and intense peaks at  $27.4^\circ$ ,  $28.3^\circ$  and  $31.7^\circ$ , arising from crystalline components such as minerals and salts. None of these peaks were visible in the spectra from the cellulosic films, as shown in Fig. 7B-C, suggesting that these components were removed upon the applied purification treatments. All the films presented three main diffraction peaks located at  $14.5^\circ$ ,  $17.0^\circ$  (being those two peaks overlapped in the F3A and F3B films) and  $22.7^\circ$ , which



**Fig. 7.** XRD patterns from (A) the native seaweeds and an alginate standard, (B) the cellulosic films obtained from the residues of *Alaria* and (C) the cellulosic films obtained from the residues of *Saccharina*.

can be assigned to crystalline cellulose I [74]. One additional broad shoulder at 19° was visible in the F2A films, being more evident in the case of *Alaria*. This shoulder may correspond to the residual alginate remaining in the F2A fractions, which was in fact more abundant in *Alaria* (cf. Table 2). Interestingly, the relative intensity of the cellulose crystalline peaks in the F3A and F3B was not as expected. A preferential orientation in the (200) crystalline plane has been typically reported for native cellulose regardless of its origin [69,75]; therefore the diffraction

peak associated with this crystalline peak, appearing at 22.7°, is the most intense. In contrast, the relative intensity of peak at 14.5° (corresponding to the (1-10) crystalline plane) was much more intense in the F3A and F3B fractions. This suggests a preferential orientation of the (1-10) crystal plane parallel to the film surface. This effect has been previously reported to occur in regenerated cellulose films [76] and thus, may be indicative of an alteration in the crystalline arrangement of cellulose as a result of the alkaline treatment applied for the production of F3A and F3B fractions. The crystallinity of the films was estimated by fitting the areas under the diffraction patterns, resulting in values of ca. 80% for *Alaria* F2A, 89% for *Alaria* F3A and 89% for *Alaria* F3B, 88% for *Saccharina* F2A, 91% for *Saccharina* F3A and 96% for *Saccharina* F3B. These results confirm the high cellulose purity in the F3A and F3B fractions and evidence a higher amount of crystalline cellulose in the *Saccharina* biomass.

### 3.3.3. Mechanical properties and water barrier performance

The mechanical properties of the cellulosic films were evaluated through tensile testing. The results, summarized in Table 3, evidence an improvement in the mechanical performance of the films with the purification of cellulose, which is in agreement with previous works [25]. In fact, the F2A films had the least desirable mechanical properties, i.e. the lowest Young's moduli and tensile strength. This can be attributed to the presence of other components such as proteins and amorphous non-cellulosic polysaccharides [77], making it difficult to obtain homogeneous aqueous suspensions and yielding heterogeneous structures, such as those observed by SEM (cf. Fig. 6C). In contrast, the F3B and F3A films presented higher rigidity and strength, while also showing a reasonably good ductility when compared to other cellulosic films [18,19,25,78]. These films show superior mechanical performance in terms of strength than nanocomposite brown seaweed films [79]. They perform even better than commercial biopolymers such as thermoplastic starch or poly(lactic) acid in terms of rigidity and strength [18,19,80]. It should also be mentioned that the slightly greater degree of cellulose purity in F3A did not produce any additional advantage in terms of mechanical performance.

The water vapor permeability (WVP) of the films (cf. Table 3) was also affected by the purification degree of the fractions, with the most purified F3A films showing less barrier (i.e. higher permeability) than

**Table 3**

Crystallinity, mechanical and water barrier properties of the cellulosic films obtained from the residues of *Alaria* and *Saccharina*.

	$X_C$ (%)	E (GPa)	TS (MPa)	$\epsilon_b$ (%)	$P_{H_2O} \cdot 10^{-14}$ (kg·m/ s·m <sup>2</sup> ·Pa)	Maximum water swelling (%)
<i>Alaria</i> F2A	80	2.3 ± 1.1 <sup>a</sup>	39.2 ± 9.2 <sup>a</sup>	2.2 ± 1.2 <sup>a</sup>	5.6 ± 0.9 <sup>ab</sup>	335 ± 69 <sup>a</sup>
<i>Alaria</i> F3A	89	4.8 ± 1.3 <sup>ab</sup>	68.4 ± 20.7 <sup>a</sup>	4.6 ± 1.7 <sup>a</sup>	7.8 ± 0.6 <sup>b</sup>	627 ± 75 <sup>c</sup>
<i>Alaria</i> F3B	89	4.6 ± 1.1 <sup>ab</sup>	74.9 ± 25.2 <sup>a</sup>	4.6 ± 1.1 <sup>a</sup>	6.1 ± 0.1 <sup>ab</sup>	550 ± 53 <sup>c</sup>
<i>Saccharina</i> F2A	88	4.8 ± 0.6 <sup>ab</sup>	51.9 ± 13.2 <sup>a</sup>	4.2 ± 1.1 <sup>a</sup>	4.3 ± 0.6 <sup>a</sup>	350 ± 32 <sup>a</sup>
<i>Saccharina</i> F3A	91	5.1 ± 0.5 <sup>ab</sup>	64.0 ± 18.9 <sup>a</sup>	3.2 ± 1.2 <sup>a</sup>	10.7 ± 0.7 <sup>c</sup>	445 ± 7 <sup>abc</sup>
<i>Saccharina</i> F3B	96	6.8 ± 2.2 <sup>b</sup>	69.0 ± 42.3 <sup>a</sup>	2.9 ± 1.1 <sup>a</sup>	3.9 ± 0.6 <sup>a</sup>	362 ± 37 <sup>a</sup>

Data shown as mean ± SD,  $n = 3$ . Values within the same column with different letters are significantly different ( $p \leq 0.05$ ).

$X_C$ : Crystallinity index determined from the XRD patterns; E: Young's modulus; TS: tensile strength;  $\epsilon_b$ : elongation at break;  $P_{H_2O}$ : water permeability.

the F2A and F3B films. This might be contrary to what expected, considering the greater crystallinity of the F3A fractions and the higher permeability values reported for pure alginate films [81]; however, a previous study showed a similar trend for cellulose-based films containing residual agar and attributed this effect, among other factors, to the reduced amount of free hydroxyl groups due to the interactions established between cellulose and agar [24]. Furthermore, the high protein content in the F2A films may have also contributed to reduce their WVP, since some studies have reported on the good water resistance for protein films [82,83]. The water swelling capacity of the films was also measured to evaluate their moisture sensitivity. The results (cf. Table 3) indicate that, in general, *Alaria* films showed a higher water swelling capacity than *Saccharina* films. This is in line with the XRD results and could be explained by the greater amount of amorphous polysaccharides, such as residual alginate, in the fractions extracted from *Alaria* residue. Furthermore, maximum water swelling values were obtained for the F3A films. The greater swelling of the most purified fractions supports the hypothesis of a higher amount of free hydroxyl groups in F3A fractions after removal of other components, such as residual alginate and proteins. It is expected that the cellulose and the residual alginate establish interactions through hydrogen bonding, thus reducing the amount of free hydroxyl groups able to bind to water.

#### 4. Conclusions

Cellulose-based fractions with different levels of purification were extracted from the residues generated after alginate extraction from three brown seaweeds, namely *Alaria esculenta*, *Saccharina latissima* and *Ascophyllum nodosum*. The biomass was mainly composed of carbohydrates (34–55%), but significant amounts of proteins and minerals were also detected. After the alginate extraction, proteins and polyphenols were concentrated in the residues, increasing their antioxidant potential. Furthermore, although some residual alginate remained in the residues (5–8%), the carbohydrate fraction from *Alaria* and *Saccharina* residues was enriched in cellulose, while fucoidan concentration increased in *Ascophyllum*. The lower cellulose content in the latter resulted in very low extraction yields (0.2–3%) for the cellulose-based fractions as compared with *Alaria* (15–21%) and *Saccharina* (26–39%) residues. This indicates that while *Saccharina* and *Alaria* residues are suitable for the extraction of cellulosic fractions, *Ascophyllum* residue may have potential for the extraction of bioactive fucoidan-rich fractions.

The less purified F2A fractions from *Saccharina* and *Alaria* were mainly composed of cellulose and residual alginate, while the F3A and F3B fractions were almost pure cellulose (>95%). These fractions were used to produce films, which were subsequently characterized to evaluate their potential as food packaging materials. Overall, the films with higher cellulose purity (F3A and F3B), presented more desirable characteristics in terms of mechanical properties and visual appearance. On the other hand, the reduced amount of free hydroxyl groups in the F2A and F3B fractions as a consequence of interactions being established between cellulose and other components reduced their water sensitivity and permeability. The simple alkaline treatment applied for the extraction of F3B produces films with the best compromise between functional properties and economical and environmental efficiency. In particular, the greater content of crystalline cellulose in *Saccharina* residue, led to greater extraction yields and produced more rigid and less permeable films. These results confirm that the waste stream generated after alginate extraction without a formaldehyde pre-treatment is a valuable source for the extraction of carbohydrates with interest in food packaging applications.

#### CRedit authorship contribution statement

Vera Cebrián-Lloret: Data curation, Formal analysis, Investigation, Methodology, Writing - original draft.

Mandy Metz: Investigation, Methodology, Writing - original draft.

Antonio Martínez-Abad: Data curation, Formal analysis, Investigation, Methodology, Writing - review & editing.

Svein Halvor Knutsen: Data curation, Formal analysis, Investigation, Methodology, Resources, Writing - review & editing.

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Amparo López-Rubio: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Writing - review & editing.

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#### 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.

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#### Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.algal.2021.102576>.

#### References

- [1] A.A. Shah, F. Hasan, A. Hameed, S. Ahmed, Biological degradation of plastics: a comprehensive review, *Biotechnol. Adv.* 26 (3) (2008) 246–265.
- [2] R.C. Thompson, C.J. Moore, F.S. Vom Saal, S.H. Swan, Plastics, the environment and human health: current consensus and future trends, *Philos. Trans. R.Soc. B Biol.Sci.* 364 (1526) (2009) 2153–2166.
- [3] A. Turner, Black plastics: linear and circular economies, hazardous additives and marine pollution, *Environ. Int.* 117 (2018) 308–318.
- [4] A. Lee, M.S. Liew, Ecologically derived waste management of conventional plastics, *J.Mater.Cycles Waste Manag.* (2019) 1–10.
- [5] K.I. Draget, C. Taylor, Chemical, physical and biological properties of alginates and their biomedical implications, *Food Hydrocoll.* 25 (2) (2011) 251–256.
- [6] J.P. Fuenzalida, P.K. Nareddy, I. Moreno-Villoslada, B.M. Moerschbacher, M. J. Swamy, S. Pan, M. Ostermeier, F.M. Goycoolea, On the role of alginate structure in complexing with lysozyme and application for enzyme delivery, *Food Hydrocoll.* 53 (2016) 239–248.
- [7] A. Abraham, B. Afewerki, B. Tsegay, H. Ghebremedhin, B. Teklehaimanot, K. S. Reddy, Extraction of agar and alginate from marine seaweeds in red sea region, *Int. J.Mar. Biol. Res.* 3 (2) (2018) 1–8.
- [8] P. Vauchel, R. Kaas, A. Arhaliass, R. Baron, J. Legrand, A new process for extracting alginates from *Laminaria digitata*: reactive extrusion, *Food Bioprocess Technol.* 1 (3) (2008) 297–300, <https://doi.org/10.1007/s11947-008-0082-x>.
- [9] S.-Y. Chee, P.-K. Wong, C.-L. Wong, Extraction and characterisation of alginate from brown seaweeds (Fucales, Phaeophyceae) collected from Port Dickson, Peninsular Malaysia, *J. Appl. Phycol.* 23 (2) (2011) 191–196.
- [10] T.A. Davis, M. Ramirez, A. Mucci, B. Larsen, Extraction, isolation and cadmium binding of alginate from *Sargassum* spp, *J. Appl. Phycol.* 16 (4) (2004) 275–284.
- [11] C.G. Gomez, M.V.P. Lambrecht, J.E. Lozano, M. Rinaudo, M.A. Villar, Influence of the extraction-purification conditions on final properties of alginates obtained from brown algae (*Macrocystis pyrifera*), *Int. J. Biol. Macromol.* 44 (4) (2009) 365–371.
- [12] C. Bertagnolli, M.G.C. da Silva, E. Guibal, Chromium biosorption using the residue of alginate extraction from *Sargassum filipendula*, *Chem. Eng. J.* 237 (2014) 362–371.

- [13] S.L. Cardoso, B.P. Moino, C.S.D. Costa, M.G.C. da Silva, M.G.A. Vieira, Evaluation of metal affinity of Ag<sup>+</sup>, Cd<sup>2+</sup>, Cr<sup>3+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> and Pb<sup>2+</sup> in residue of double alginate extraction from *Sargassum filipendula* seaweed, *Chem. Eng. Trans.* 52 (2016) 1027–1032.
- [14] S.K. Ramamoorthy, M. Skrifvars, A. Persson, A review of natural fibers used in biocomposites: plant, animal and regenerated cellulose fibers, *Polym. Rev.* 55 (1) (2015) 107–162.
- [15] D. Trache, M.H. Hussin, C.T. Hui Chuin, S. Sabar, M.R.N. Fazita, O.F.A. Taiwo, T. M. Hassan, M.K.M. Haafiz, Microcrystalline cellulose: isolation, characterization and bio-composites application—a review, *Int. J. Biol. Macromol.* 93 (2016) 789–804, <https://doi.org/10.1016/j.ijbiomac.2016.09.056>.
- [16] H.P.S. Khalil, Y.Y. Tye, C.K. Saurabh, C.P. Leh, T.K. Lai, E.W.N. Chong, M.R. Fazita, J.M. Hafidz, A. Banerjee, M.I. Syakir, Biodegradable polymer films from seaweed polysaccharides: a review on cellulose as a reinforcement material, *Express Polym. Lett.* 11 (4) (2017).
- [17] N. Wahlström, U. Edlund, H. Pavia, G. Toth, A. Jaworski, A.J. Pell, F.X. Choong, H. Shirani, K.P.R. Nilsson, A. Richter-Dahlfors, Cellulose from the green macroalgae *Ulva lactuca*: isolation, characterization, optotracing, and production of cellulose nanofibrils, *Cellulose* (2020) 1–19.
- [18] I. Benito-González, A. López-Rubio, M. Martínez-Sanz, High-performance starch biocomposites with cellulose from waste biomass: film properties and retrogradation behaviour, *Carbohydr. Polym.* 216 (2019) 180–188.
- [19] I. Benito-González, A. Lopez-Rubio, G. Rafael, M. Martínez-Sanz, Cellulose nanocrystal-based films produced by more sustainable extraction protocols from *Posidonia oceanica* waste biomass, *Cellulose* 26 (13–14) (2019) 8007–8024, <https://doi.org/10.1007/s10570-019-02641-4>.
- [20] C. Fontes-Candia, E. Erboz, A. Martínez-Abad, A. López-Rubio, M. Martínez-Sanz, Superabsorbent food packaging bioactive cellulose-based aerogels from *Arundo donax* waste biomass, *Food Hydrocoll.* 96 (2019) 151–160.
- [21] M. Hasan, T.K. Lai, D.A. Gopakumar, M. Jawaid, F.A.T. Owolabi, E.M. Mistar, T. Alfatah, N.Z. Noriman, M.K.M. Haafiz, H.P.S.A. Khalil, Micro crystalline bamboo cellulose based seaweed biodegradable composite films for sustainable packaging material, *J. Polym. Environ.* 27 (7) (2019) 1602–1612.
- [22] A.K. Siddhanta, K. Prasad, R. Meena, G. Prasad, G.K. Mehta, M.U. Chhatbar, M. D. Oza, S. Kumar, N.D. Sanandya, Profiling of cellulose content in indian seaweed species, *Bioresour. Technol.* 100 (24) (2009) 6669–6673.
- [23] S. Singh, K.K. Gaikwad, S.-I. Park, Y.S. Lee, Microwave-assisted step reduced extraction of seaweed (*Gelidium aceroso*) cellulose nanocrystals, *Int. J. Biol. Macromol.* 99 (2017) 506–510.
- [24] M. Martínez-Sanz, V. Cebrián-Lloret, J. Mazarro-Ruiz, A. López-Rubio, Improved performance of less purified cellulosic films obtained from agar waste biomass, *Carbohydr. Polym.* 115887 (2020).
- [25] I. Benito-González, M. Martínez-Sanz, A. Lopez-Rubio, Potential of lignocellulosic fractions from *Posidonia oceanica* to improve barrier and mechanical properties of bio-based packaging materials, *Int. J. Biol. Macromol.* 118 (2018) 542–551, <https://doi.org/10.1016/j.ijbiomac.2018.06.052>.
- [26] M.G.E. Diepenmaat-Wolters, S.A.G.F. Angelino, C. Verbeek, A.C. Douma, High-performance anion-exchange chromatography method for analysis of propylene glycol alginate in beer, *J. Am. Soc. Brew. Chem.* 55 (4) (1997) 147–152.
- [27] J.F. Saeman, Kinetics of wood saccharification-hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature, *Ind.Eng.Chem.* 37 (1) (1945) 43–52.
- [28] S. Willför, A. Pranovich, T. Tamminen, J. Puls, C. Laine, A. Suurmäki, B. Saake, K. Uotila, H. Simolin, J. Hemming, Carbohydrate analysis of plant materials with uronic acid-containing polysaccharides—a comparison between different hydrolysis and subsequent chromatographic analytical techniques, *Ind. Crop. Prod.* 29 (2–3) (2009) 571–580.
- [29] Wileset al., 1998 P. G. Wiles I. K. Gray R. C. Kissling & International, C. D. C. E. J. G. K. G. H. M. K. K. L. H. M. K. M. A. P. M. R. F. V. M. %J J. of A. (1998). Routine analysis of proteins by Kjeldahl and Dumas methods: review and interlaboratory study using dairy products. 81(3), 620–632.
- [30] S.A. Cohen, D.P. Michaud, Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography, *Anal. Biochem.* 211 (2) (1993) 279–287.
- [31] AOAC, AOAC: Official Methods of Analytical Chemist, Assoc. of Official Anal. Chem, 1990.
- [32] A. Paz, S. da Silva Sabo, M. Vallejo, E. Marguet, Pinheiro de Souza, R. Oliveira, J. M. Domínguez, Using brewer's spent grain to formulate culture media for the production of bacteriocins using Patagonian strains, *LWT* 96 (2018) 166–174, <https://doi.org/10.1016/j.lwt.2018.05.027>.
- [33] J. Sameni, S. Krigstin, M. Sain, Characterization of lignins isolated from industrial residues and their beneficial uses, *Bioresources* 11 (4) (2016) 8435–8456.
- [34] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, [14] analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, in: *Methods in Enzymology*, vol. 299, Elsevier, 1999, pp. 152–178.
- [35] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.* 26 (9) (1999) 1231–1237.
- [36] M. Martínez-Sanz, P. Lopez-Sanchez, M.J. Gidley, E.P. Gilbert, Evidence for differential interaction mechanism of plant cell wall matrix polysaccharides in hierarchically-structured bacterial cellulose, *Cellulose* 22 (3) (2015) 1541–1563.
- [37] N. Wang, E. Ding, R. Cheng, Thermal degradation behaviors of spherical cellulose nanocrystals with sulfate groups, *Polymer* 48 (12) (2007) 3486–3493.
- [38] 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.
- [39] N. Blanco-Pascual, M.P. Montero, M.C. Gómez-Guillén, Antioxidant film development from unrefined extracts of brown seaweeds *Laminaria digitata* and *Ascophyllum nodosum*, *Food Hydrocoll.* 37 (2014) 100–110, <https://doi.org/10.1016/j.foodhyd.2013.10.021>.
- [40] L.E. Rioux, S.L. Turgeon, M. Beaulieu, Characterization of polysaccharides extracted from brown seaweeds, *Carbohydr. Polym.* 69 (3) (2007) 530–537, <https://doi.org/10.1016/j.carbpol.2007.01.009>.
- [41] 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.
- [42] S. Kumar, D. Sahoo, A comprehensive analysis of alginate content and biochemical composition of leftover pulp from brown seaweed *Sargassum wightii*, *Algal Res.* 23 (2017) 233–239, <https://doi.org/10.1016/j.algal.2017.02.003>.
- [43] J. Fleurence, M. Morancás, J. Dumay, Seaweed proteins, in: *Proteins in Food Processing: Second Edition* 10, 2018, pp. 245–262, <https://doi.org/10.1016/B978-0-08-100722-8.00010-3>.
- [44] J.V. Vilg, G.M. Nylund, T. Werner, L. Qvirist, J.J. Mayers, H. Pavia, I. Undeland, E. Albers, Seasonal and spatial variation in biochemical composition of *Saccharina latissima* during a potential harvesting season for Western Sweden, *Bot. Mar.* 58 (6) (2015) 435–447, <https://doi.org/10.1515/bot-2015-0034>.
- [45] D. Manns, A.L. Deuschle, B. Saake, A.S. Meyer, Methodology for quantitative determination of the carbohydrate composition of brown seaweeds (*Laminariaceae*), *RSC Adv.* 4 (49) (2014) 25736–25746.
- [46] J.P. Monteiro, T. Melo, J. Skjermo, S. Forbord, O.J. Broch, P. Domingues, R. Calado, M.R. Domingues, Effect of harvesting month and proximity to fish farm sea cages on the lipid profile of cultivated *Saccharina latissima*, *Algal Res.* 54 (2021), 102201.
- [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] W.A.P. Black, The seasonal variation in chemical composition of some of the littoral seaweeds common to Scotland. Part I. *Ascophyllum nodosum*, *J.Soc.Chem. Ind.* 67 (9) (1948) 355–357.
- [49] 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>.
- [50] J.M. Lorenzo, R. Agregán, P.E.S. Munekata, D. Franco, J. Carballo, S. Şahin, R. Lacombe, F.J. Barba, Proximate composition and nutritional value of three macroalgae: *Ascophyllum nodosum*, *Fucus vesiculosus* and *Bifurcaria bifurcata*, *Mar. Drugs* 15 (11) (2017), <https://doi.org/10.3390/md15110360>.
- [51] G.S. Marinho, S.L. Holdt, C. Jacobsen, I. Angelidakis, Marine drugs lipids and composition of fatty acids of *saccharina latissima* cultivated year-round in integrated multi-trophic aquaculture, *Mar. Drugs* 13 (2013) 4357–4374, <https://doi.org/10.3390/md13074357>.
- [52] S. Gupta, N. Abu-Ghannam, Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods, *Innov. Food Sci. Emerg. Technol.* 12 (4) (2011) 600–609, <https://doi.org/10.1016/j.ifset.2011.07.004>.
- [53] P. Stévant, H. Marfaing, T. Rustad, I. Sandbakken, J. Fleurence, A. Chapman, Nutritional value of the kelps *Alaria esculenta* and *Saccharina latissima* and effects of short-term storage on biomass quality, *J. Appl. Phycol.* 29 (5) (2017) 2417–2426, <https://doi.org/10.1007/s10811-017-1126-2>.
- [54] M.R. Tabassum, A. Xia, J.D. Murphy, Seasonal variation of chemical composition and biomethane production from the brown seaweed *Ascophyllum nodosum*, *Bioresour. Technol.* 216 (2016) 219–226, <https://doi.org/10.1016/j.biortech.2016.05.071>.
- [55] L. Audibert, M. Fauchon, N. Blanc, D. Hauchard, E. Ar Gall, Phenolic compounds in the brown seaweed *Ascophyllum nodosum*: distribution and radical-scavenging activities, *Phytochem. Anal.* 21 (5) (2010) 399–405, <https://doi.org/10.1002/pca.1210>.
- [56] C. Afonso, J. Matos, I. Guarda, A. Gomes-Bispo, R. Gomes, C. Cardoso, S. Gueifão, I. Delgado, I. Coelho, I. Castanheira, N.M. Bandarra, Bioactive and nutritional potential of *Alaria esculenta* and *saccharina latissima*, *J. Appl. Phycol.* (2020), <https://doi.org/10.1007/s10811-020-02298-8>.
- [57] S.J. Lim, W.M.W. Aida, M.Y. Maskat, S. Mamot, J. Ropien, D.M. Mohd, Isolation and antioxidant capacity of fucoidan from selected Malaysian seaweeds, *Food Hydrocoll.* 42 (2014) 280–288.
- [58] L. Cunha, A. Grenha, Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications, *Mar. Drugs* 14 (3) (2016), <https://doi.org/10.3390/md14030042>.
- [59] S.J. Horn, E. Moen, K. Østgaard, Direct determination of alginate content in brown algae by near infra-red (NIR) spectroscopy, *J. Appl. Phycol.* 11 (1) (1999) 9–13, <https://doi.org/10.1023/A:1008024009954>.
- [60] K.N. Kerner, J.F. Hanssen, T.A. Pedersen, Anaerobic digestion of waste sludges from the alginate extraction process, *Bioresour. Technol.* 37 (1) (1991) 17–24, [https://doi.org/10.1016/0960-8524\(91\)90107-U](https://doi.org/10.1016/0960-8524(91)90107-U).
- [61] K.-T. Kim, L.-E. Rioux, S.L. Turgeon, Alpha-amylase and alpha-glucosidase inhibition is differentially modulated by fucoidan obtained from *Fucus vesiculosus* and *Ascophyllum nodosum*, *Phytochemistry* 98 (2014) 27–33, <https://doi.org/10.1016/j.phytochem.2013.12.003>.

- [62] Y. Yuan, D. Macquarrie, Microwave assisted extraction of sulfated polysaccharides (fucoidan) from *Ascophyllum nodosum* and its antioxidant activity, *Carbohydr. Polym.* 129 (2015) 101–107, <https://doi.org/10.1016/j.carbpol.2015.04.057>.
- [63] N.P. Chandía, B. Matsuhira, A.E. Vásquez, Alginic acids in *Lessonia trabeculata*: characterization by formic acid hydrolysis and FT-IR spectroscopy, *Carbohydr. Polym.* 46 (1) (2001) 81–87, [https://doi.org/10.1016/S0144-8617\(00\)00286-1](https://doi.org/10.1016/S0144-8617(00)00286-1).
- [64] E. Gómez-Ordóñez, P. Rupérez, FTIR-ATR spectroscopy as a tool for polysaccharide identification in edible brown and red seaweeds, *Food Hydrocoll.* 25 (6) (2011) 1514–1520, <https://doi.org/10.1016/j.foodhyd.2011.02.009>.
- [65] V. García-Ríos, E. Ríos-Leal, D. Robledo, Y. Freile-Pelegrin, Polysaccharides composition from tropical brown seaweeds, *Phycol. Res.* 60 (4) (2012) 305–315.
- [66] D. Leal, B. Matsuhira, M. Rossi, F. Caruso, FT-IR spectra of alginic acid block fractions in three species of brown seaweeds, *Carbohydr. Res.* 343 (2) (2008) 308–316, <https://doi.org/10.1016/j.carres.2007.10.016>.
- [67] E. Deniaud-Bouët, N. Kervarec, G. Michel, T. Tonon, B. Kloareg, C. Hervé, Chemical and enzymatic fractionation of cell walls from fuciales: 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>.
- [68] A.A. Salmeán, D. Duffieux, J. Harholt, F. Qin, G. Michel, M. Czjzek, W.G.T. Willats, C. Hervé, Insoluble (1 → 3), (1 → 4)-β-D-glucan is a component of cell walls in brown algae (Phaeophyceae) and is masked by alginates in tissues, *Sci. Rep.* 7 (1) (2017) 2880, <https://doi.org/10.1038/s41598-017-03081-5>.
- [69] K. Das, D. Ray, N.R. Bandyopadhyay, S. Sengupta, Study of the properties of microcrystalline cellulose particles from different renewable resources by XRD, FTIR, nanoindentation, TGA and SEM, *J. Polym. Environ.* 18 (3) (2010) 355–363.
- [70] E. Membrane, P. Sallis, Thermochemical characterization of brown seaweed, *Laminaria digitata* from UK shores, *J. Anal. Appl. Pyrolysis* 131 (2018) 42–51, <https://doi.org/10.1016/j.jaap.2018.02.011>.
- [71] P. Jandura, B. Riedl, B.V. Kokta, Thermal degradation behavior of cellulose fibers partially esterified with some long chain organic acids, *Polym. Degrad. Stab.* 70 (3) (2000) 387–394, [https://doi.org/10.1016/S0141-3910\(00\)00132-4](https://doi.org/10.1016/S0141-3910(00)00132-4).
- [72] E. Membrane, P. Sallis, Effect of temperature on kinetics of biogas production from macroalgae, *Bioresour. Technol.* 263 (2018) 410–417, <https://doi.org/10.1016/J.BIORTECH.2018.05.023>.
- [73] P.S. Saravana, Y.-J. Cho, Y.-B. Park, H.-C. Woo, B.-S. Chun, Structural, antioxidant, and emulsifying activities of fucoidan from *Saccharina japonica* using pressurized liquid extraction, *Carbohydr. Polym.* 153 (2016) 518–525, <https://doi.org/10.1016/j.carbpol.2016.08.014>.
- [74] L.H. Thomas, V.T. Forsyth, A. Šturcová, C.J. Kennedy, R.P. May, C.M. Altaner, D. C. Apperley, T.J. Wess, M.C. Jarvis, Structure of cellulose microfibrils in primary cell walls from collenchyma, *Plant Physiol.* 161 (1) (2013) 465–476.
- [75] U.P. Agarwal, S.A. Ralph, R.S. Reiner, C. Baez, New cellulose crystallinity estimation method that differentiates between organized and crystalline phases, *Carbohydr. Polym.* 190 (2018) 262–270.
- [76] C. Yamane, T. Aoyagi, M. Ago, K. Sato, K. Okajima, T. Takahashi, Two different surface properties of regenerated cellulose due to structural anisotropy, *Polym. J.* 38 (8) (2006) 819–826, <https://doi.org/10.1295/polymj.PJ2005187>.
- [77] M. Martínez-Sanz, E. Erboz, C. Fontes, A. López-Rubio, Valorization of *Arundo donax* for the production of high performance lignocellulosic films, *Carbohydr. Polym.* 199 (2018) 276–285.
- [78] I. Benito-González, C.M. Jaén-Cano, A. López-Rubio, A. Martínez-Abad, M. Martínez-Sanz, Valorisation of vine shoots for the development of cellulose-based biocomposite films with improved performance and bioactivity, *Int. J. Biol. Macromol.* 165 (2020) 1540–1551, <https://doi.org/10.1016/j.ijbiomac.2020.09.240>.
- [79] H. Doh, K.D. Dunno, W.S. Whiteside, Preparation of novel seaweed nanocomposite film from brown seaweeds *Laminaria japonica* and *Sargassum natans*, *Food Hydrocoll.* 105 (2020), 105744, <https://doi.org/10.1016/j.foodhyd.2020.105744>.
- [80] S.M. Bhasney, P. Bhagabati, A. Kumar, V. Katiyar, Morphology and crystalline characteristics of polylactic acid [PLA]/linear low density polyethylene [LLDPE]/microcrystalline cellulose [MCC] fiber composite, *Compos. Sci. Technol.* 171 (2019) 54–61.
- [81] J.W. Rhim, Physical and mechanical properties of water resistant sodium alginate films, *LWT Food Sci. Technol.* 37 (3) (2004) 323–330, <https://doi.org/10.1016/j.lwt.2003.09.008>.
- [82] H.J. Park, M.S. Chinnan, Gas and water vapor barrier properties of edible films from protein and cellulosic materials, *J. Food Eng.* 25 (4) (1995) 497–507, [https://doi.org/10.1016/0260-8774\(94\)00029-9](https://doi.org/10.1016/0260-8774(94)00029-9).
- [83] Y.C. Seung, C. Rhee, Mechanical properties and water vapor permeability of edible films made from fractionated soy proteins with ultrafiltration, *LWT Food Sci. Technol.* 37 (8) (2004) 833–839, <https://doi.org/10.1016/j.lwt.2004.03.009>.
- [84] F. Bertaud, A. Sundberg, B. Holmbom, Evaluation of acid methanolysis for analysis of wood hemicelluloses and pectins, *Carbohydr. Polym.* 48 (3) (2002) 319–324.