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Wahlström, N., Harrysson, H., Undeland, I. et al (2018). A Strategy for the Sequential Recovery of Biomacromolecules from Red Macroalgae Porphyra
umbilicalis Kützing. Industrial & Engineering Chemistry Research, 57(1): 42-53.
http://dx.doi.org/10.1021/acs.iecr.7b03768

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Cite This: Ind. Eng. Chem. Res. 2018, 57, 42−53

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A Strategy for the Sequential Recovery of Biomacromolecules from Red Macroalgae *Porphyra umbilicalis* Kützing

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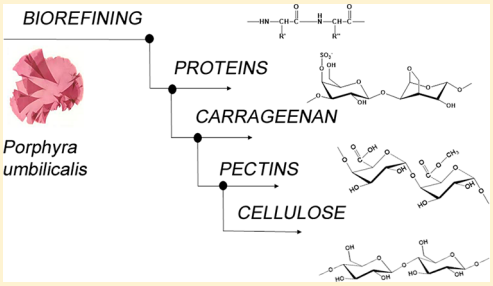
Supporting Information

ABSTRACT: A nondestructive, multicomponent fractionation strategy has been developed to extract proteins and polysaccharides from the red macroalgae *Porphyra umbilicalis* collected along the west coast of Sweden and cultivated indoors under controlled conditions. First, a protein-rich fraction was extracted in an ice-cold alkaline solution. The overall protein content in *Porphyra umbilicalis* was estimated to be 30.6% of the dry weight, and out of that, 15.0% could be recovered. Water-soluble polysaccharides were then extracted from the insoluble residual fraction using sequential alkaline and acidic treatments at 90 °C for 4 h. Spectroscopic and chromatographic analyses of the polysaccharide fractions show that high-molecular-weight carrageenans were obtained from the alkaline extraction and a galactose-rich pectin substance was obtained from the acidic extraction. The insoluble fraction remaining after all extraction steps was rich in cellulose. An elemental analysis of *Porphyra umbilicalis* via scanning electron microscopy with energy-dispersive X-ray spectrometry (SEM-EDS) showed the presence of C, O, Na, Ca, Mg, Al, Cl, and S. However, no heavy metals or other toxic elements, such as Pb, Hg, and As, were found.



1. INTRODUCTION

Macroalgae farming is a fast-growing area of aquaculture worldwide; however, so far, it has been largely undeveloped in Sweden, even though great potential exists for this industry there, because of Sweden's long coast line. Macroalgae farming offers several benefits, compared to land-based biomass production. It does not require irrigation, compete for valuable land area, or contribute to the negative effects associated with fertilizers. Therefore, macroalgae farming has been suggested as a possible way to mitigate global warming.[1] Other advantages of macroalgae farming are that the biomass grows quickly and it does not contribute to coastal eutrophication.[2] Macroalgae is a potential source for bioethanol production, and recent computational studies have shown that a biorefinery process can be designed such that biodiesel can be produced from alga with zero overall CO_2 emission.[3]

Some species of macroalgae are important in food, medical, and nutritional applications, because of their high levels of polysaccharides, proteins, vitamins, and trace minerals.[4] In particular, some of the water-soluble polysaccharides found in macroalgae often serve as thickening agents in the food industry, because of their excellent gelling abilities.[4] A commercially valuable maricultured macroalgae is the red seaweed *Porphyra* (also known as Nori), which belongs to a family of red algae called Bangiaceae. In addition to its direct use in food and as a source of ingredients, it could be an attractive biomass base for biorefining. The main polysaccharides present in the cell walls of *Porphyra* are carrageenan, pectin, and cellulose.

Carrageenan is a family of water-soluble polysaccharides composed of repeating units of partially sulfated disaccharides. Carrageenan exists as different isomers, of which the three most common isomers are κ-, ι-, and λ-carrageenan (Figure 1), where each monomer unit contains one, two, and three sulfate groups, respectively.[5] The degree of sulfation of the carrageenan strongly affects its properties. The ability of carrageenans to form cross-linked gels in the presence of K^+ or Ca^{2+} ions is one reason why these biomacromolecules are gaining increasing attention[6] and why they have been investigated as potential hydrogel scaffolds in drug delivery and biomedical applications.[7,8] κ-Carrageenan forms firm and elastic cross-linked gels in aqueous solutions in the presence of K^+ ions and more brittle gels in the presence of Ca^{2+} ions; ι-carrageenan forms soft gels in aqueous solution in the presence of Ca^{2+} ions. However, λ-carrageenan does not form any gels in the presence of metal ions.[9]

The conventional and straightforward method for the extraction of carrageenan from red seaweed is the use of an alkaline aqueous solution (pH 8−10) at high temperature (T = 80−95 °C) for 2−4 h. The dissolved carrageenan is

Received: September 11, 2017
Revised: November 18, 2017
Accepted: November 28, 2017
Published: November 28, 2017

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DOI: 10.1021/acs.iecr.7b03768
Ind. Eng. Chem. Res. 2018, 57, 42−53

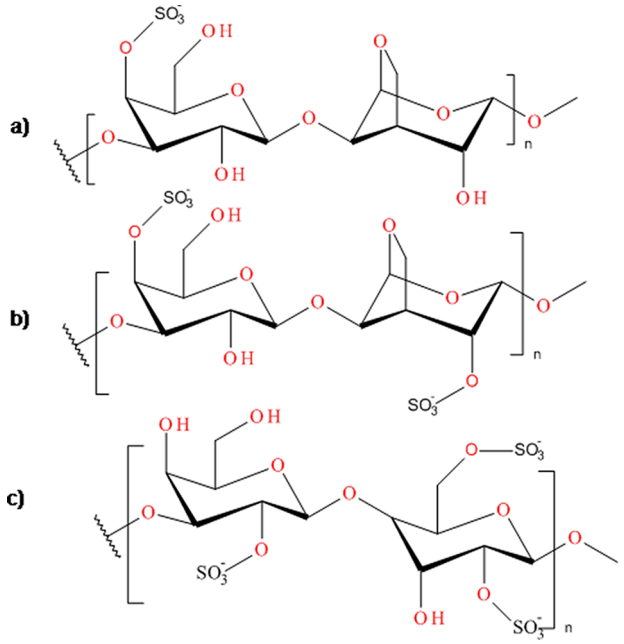

Figure 1. Representative repeating unit motifs of (a) κ, (b) ι, and (c) λ-carrageenan.

precipitated by adding ethanol to the supernatant.[6] However, another extraction method has also been suggested involving microwave-assisted extraction, which reduces the extraction time to 10−20 min and reduces the consumption of solvents and energy required for the extraction process, compared with extraction in an aqueous alkaline solution.[7] It is worth mentioning that commercial and conventional extraction techniques, such as the above-mentioned procedures, are designed to isolate carrageenan but they disregard other valuable components (such as proteins and lipids). The insoluble fraction remaining after extracting carrageenan is usually discarded, and no attempt is made to isolate the other valuable components. Hence, more than half of this potentially valuable biomass is wasted, which is in direct contradiction to the rapidly emerging need to use nature's resources in a more efficient and sustainable way. From a biorefinery point of view, it is essential to develop and implement biomass treatment techniques that allow us to recover as much of the valuable components in the biomass as possible. Unlike a traditional "one-component" approach, such techniques would help mitigate greenhouse gas emissions and the accumulation of waste fractions from industrial production.[10] Biorefinery processes are widely used to recover valuable components such as levulinic acid, succinic acid, and bioethanol from different types of biomasses.[11,12] Developing and applying a biorefinery type of process to red seaweed is important to use the biomass more effectively and steering away from the current industrial approach to isolate only the carrageenan while discarding the rest of the biomass. So far, only little progress has been made toward developing sequential processes in the field of macromolecule extractions from seaweed biomass. Recently, a procedure for the recovery of proteins from brown seaweed (*Saccharina latissima*) was reported, and that procedure is built upon cold alkaline extraction, followed by isoelectric precipitation, often called pH-shift processing.[13] The method has since been adapted to red and green seaweed species.[14] In addition, similar procedures have been reported for seaweed proteins that use ammonium sulfate in the protein precipitation step; however, these methods require a subsequent dialysis step to desalt the product. In all of these methods, as in traditional carrageenan extractions, a large portion of the biomass ends up in waste fractions. One such fraction is the non-alkali-soluble fraction that is removed during the first centrifugation step. We hypothesize that (i) this fraction contains a large amount of the cell wall-derived polysaccharides, which, in fact, decrease the extractability of the proteins,[15,16] and (ii) this fraction could therefore be a promising substrate for, e.g., carrageenan extractions.

Based on this hypothesis, our aim is to develop a strategy for the multicomponent fractionation of biomacromolecules (food-grade proteins and polysaccharides) from the red algae *Porphyra umbilicalis* collected along the west coast of Sweden to allow for a more-efficient use of the algal polymeric components. The goal is that each extraction step can be carried out without causing unwanted degradation of the other components; for example, proteins require cold conditions while polysaccharides are more heat-resistant. Unwanted modification or degradation of the proteins (e.g., denaturation or losses of essential amino acids) and polysaccharides (e.g., molecular weight reduction) may lead to losses in the technofunctional properties, nutritional value, and material properties. The exact polysaccharide composition of *Porphyra umbilicalis* is not fully established, so another objective of this study is to structurally map and characterize the different recovered polysaccharide fractions from this algal species. Regarding the fractions recovered, the protein contents and the isomeric composition of the carrageenan-, cellulose-, and the pectin-enriched extracts will be investigated.

2. EXPERIMENTAL SECTION

2.1. Materials. *Porphyra umbilicalis* were collected on November 22, 2015, from Inre Vattenholmen (58°52′37.4″N 11°6′52.1″E) and brought back to the laboratory within 2 h of collection. Collected *Porphyra umbilicalis* tufts were rinsed several times in natural seawater to remove grazers and loose epiphytes. Tufts were then placed into cultivation tanks (60 L) and cultivated at a temperature of 10 °C in a neutral light cycle (12 h daylight, 12 h darkness) at a light intensity of 140 μE m^{-2} s^{-1}. The light source was an OSRAM Lumilux Warm White 58 W bulb. The seaweeds continuously received filtered seawater that was passed through 1 μm filters. No additional medium or chemicals were added to the water. The natural seawater used in the flow-through system was pumped in from outside the bay. Thus, the salinity may have fluctuated somewhat, depending on the freshwater coming in from the coast. No salinity measurements were performed, but the salinity in this region is typically ca. 25 ppt.

Sodium carbonate (≥99.5% purity), sodium hydroxide (≥99.98% purity), potassium chloride (KCl), calcium chloride ($CaCl_2$), 37% hydrochloric acid, ethanol (96% purity), reference carrageenan (type II, which contains mostly κ-carrageenan and a minor amount of λ-carrageenan, Mw = 950 000 Dg/mol), methanol (≥99.98% purity), acetyl chloride (≥99% purity), pyridine (≥98% purity), trifluoroacetic acid (99.5% purity), toluene (≥99.99% purity), deuterium oxide (≥99.8% purity), L-(+)-arabinose (≥99% purity), D-(+)-glucose (≥99% purity), D-(+)-mannose (≥99% purity), D-(+)-galactose (≥99% purity), D-(+)-xylose (≥99% purity), D-(+) rhamnose (≥99% purity), D-(+)-glucuronic acid (≥98% purity), D-(+) galacturonic acid, and α-cellulose (from spruce wood) were all

DOI: 10.1021/acs.iecr.7b03768
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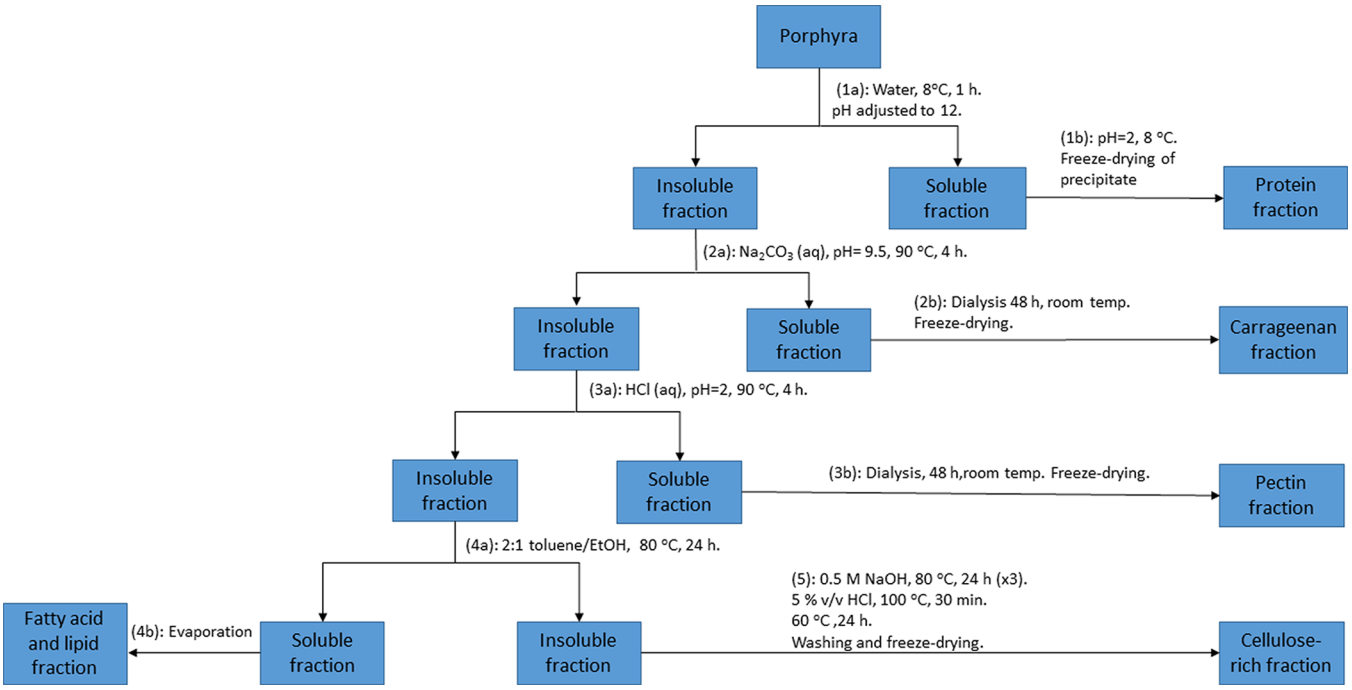

Figure 2. Schematic illustration of the sequential fractionation protocol for protein and polysaccharides recovery from *Porphyra umbilicalis*. The numbered labels (1)−(5) correspond to the individual isolation steps.

purchased from Sigma−Aldrich. Sulfuric acid (72%) was purchased from Labservice AB, Sweden, and lemon pectin ≥90% (≥74% galacturonic acid, with trace amounts of arabinose, glucose, galactose, rhamnose, mannose, xylose, and glucuronic acid; Mw = 100 000 g/mol) was purchased from Alfa Aesar. Hydrochloric acid (37%) for protein extraction was purchased from Acros (Gothenburg, Sweden). Dialysis membranes were purchased from Spectrum Laboratories, Inc. (MWCO = 1.0 kg/mol, 6.4 mL/cm).

2.2. Fractionation of Biomacromolecules. The fractionation of the macromolecular components in *Porphyra umbilicalis* was carried out as outlined in the schematic in Figure 2. The numbered labels (1)−(5) correspond to the individual extraction steps that are further explained in Sections 2.2.1−2.2.5.

2.2.1. Step (1): Extraction of Proteins. Protein from *Porphyra* was extracted as described by Harrysson et al.[14] In short, dry-milled *Porphyra* was homogenized in distilled water in a 1:43.6 ratio, which corresponded to a 1:6 ratio, based on the algal wet weight, using a Turrax IKA T18 basic for 2 min at 18 000/min, followed by milling with beads using a Retsch MM 400 at a speed of 1/30 s for 2 min. Finally, this process was followed by stirring for 1 h at 8 °C. While keeping the homogenate on ice, the pH was adjusted to 12 with 1 M NaOH and the mixture was centrifuged at 8000*g* for 10 min (4 °C). The supernatant was decanted and kept on ice while the pH was adjusted to 2 with 1 M HCl. The sediment (insoluble fraction) was collected, frozen at −80 °C, and then freeze-dried for the subsequent extraction of carrageenan (see Section 2.2.2). The supernatant was frozen overnight at −20 °C, then thawed and centrifuged at 8000*g* for 10 min (4 °C), The pellet (protein fraction) was collected, freeze-dried and stored at −80 °C until further analysis. The extraction was done in duplicate. The protein yield was calculated according to the following formula:

$$\text{protein yield (\%)} = \frac{(\text{total protein in the isolate}) \times (\text{amount of isolate achieved})}{(\text{total protein in the crude seaweed}) \times (\text{amount of seaweed})} \times 100$$

and the deviation was calculated as

$$\text{deviation} = \frac{(\text{maximum yield}) - (\text{minimum yield})}{2}$$

The weight yield was calculated as the mass of the freeze-dried fractions divided by the mass of the dried starting algae material.

To determine whether there were significant differences between data from the crude seaweed and the protein-rich fraction, a *t*-test was carried out using SPSS (IBM Corp., Armonk, NY, USA). The equality of variances between the two groups was calculated using Levene's test.

2.2.2. Step (2): Extraction of Carrageenan. Carrageenan was extracted by the method suggested by Hilliou et al.,[6] with some modifications. A freeze-dried subsample (2 g) of the insoluble fraction from step (1) was stirred in 1.0 M Na_2CO_3 at room temperature for 24 h. The sample was washed several times with water and suspended in 200 mL of Na_2CO_3 solution (pH 9.5). The mixture was stirred at 90 °C for 4 h (Figure 2, step (2a)). After cooling to room temperature, the mixture was centrifuged at 4000 rpm for 10 min and the supernatant was collected, dialyzed for 48 h, and then freeze-dried (Figure 2, step (2b)). In one trial, an alternative workup procedure was used in which the supernatant was concentrated by evaporation and then precipitated in an excess of cold ethanol. The precipitate was recovered by centrifugation and dried. The insoluble fraction from the carrageenan extraction was kept for further extractions.

2.2.3. Step (3): Extraction of Pectin. The insoluble fraction remaining after the carrageenan extraction in step (2) was washed several times with water in order to remove any traces from the previous extraction. The washed sample was then

DOI: 10.1021/acs.iecr.7b03768
Ind. Eng. Chem. Res. 2018, 57, 42−53

suspended in 200 mL of 0.01 M HCl (pH 2) and heated to 90 °C for 4 h (Figure 2, step (3a)). After cooling to room temperature, the mixture was centrifuged at 4000 rpm for 10 min and the supernatant was collected and freeze-dried (Figure 2, step (3b)). The insoluble fraction was kept for further extractions.

2.2.4. Step (4): Removal of Lipids, Fatty Acids, and Other Water-Insoluble Components. Fatty acids, lipids, and other water-insoluble components were removed from the insoluble fraction in step (4a) to produce the final cellulose-rich fraction. To make the process comparably more environmentally friendly, a method using toluene and ethanol[11] was chosen in favor of the more traditional method by Lee et al., which uses chloroform and methanol.[17] The insoluble fraction remaining from step (3) was stirred in 200 mL of a 2:1 toluene/ethanol mixture for 24 h at 80 °C (Figure 2, step (4a)). After cooling to room temperature, the mixture was centrifuged at 4000 rpm for 10 min and the supernatant was removed (Figure 2, step (4b)). The insoluble fraction was kept for further isolation of the cellulose. No further analysis of the fatty acids and lipids in the supernatant was performed in this study.

2.2.5. Step (5): Isolation of the Cellulose-Rich Fraction. A modified version of the method suggested by Siddhanta et al.[18] was used in an attempt to obtain a cellulose-rich insoluble fraction. The insoluble fraction that remained from step (4a) was suspended in 0.5 M NaOH and heated to 80 °C for 24 h (Figure 2, step (5)). The mixture was centrifuged at 4000 rpm for 10 min. The supernatant was removed and the insoluble fraction was washed several times with water until the washing water reached a neutral pH. The procedure was repeated twice. After washing, the insoluble fraction was suspended in 5% HCl and heated to boiling for 30 min. The resulting slurry was then stirred at 60 °C for 24 h. The sample was centrifuged at 4000 rpm for 10 min and the insoluble fraction was washed several times with water to remove excess acid. The final insoluble fraction was freeze-dried for further characterization.

2.3. Gel Formation from the Carrageenan Fraction. The carrageenan-rich fraction recovered by precipitation with ethanol was used to prepare gels. A commercial carrageenan (a high-purified κ-carrageenan) was used as a reference. Carrageenan samples were first dissolved in deionized water at a concentration of 1% (w/w); however, the concentration of the commercial carrageenan was 0.8% (w/w), because of its high viscosity. To each sample, 0.5 mL of either a $CaCl_2$ or a KCl solution (1% w/w in water) was added under stirring. The samples were heated to 80 °C and then allowed to cool to room temperature.

2.4. Characterization of Crude Biomass and Fractions.
2.4.1. Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR). The carrageenan and pectin fractions (i.e., fractions recovered in steps (2) and (3)) were analyzed by ^{1}H NMR. Freeze-dried samples (25 mg) were dissolved in 1.0 mL of D_2O, and NMR spectra were recorded on a Bruker DMX-500 NMR spectrometer at 500 MHz. The spectra were calculated as an average of 128 scans, and the data were analyzed using MestReNova software.

2.4.2. Fourier Transform Infrared (FTIR) Spectroscopy. FTIR spectra of dried *Porphyra umbilicalis* and the dried fractions recovered in steps (2), (3), and (5) were recorded between 4000 cm^{-1} and 600 cm^{-1} at room temperature, using a PerkinElmer Spectrum 2000 FTIR with an attenuated total reflectance crystal accessory (ATR Golden Gate). Corrections were made for atmospheric water and carbon dioxide in all of the measurements, and the spectra were calculated as an average of 64 scans. The data were analyzed using PerkinElmer Spectrum software.

2.4.3. Size-Exclusion Chromatography (SEC). The molecular weights and dispersities of the extracted water-soluble polysaccharides (fractions recovered in steps (2b) and (3b)) were estimated using a Dionex Ultimate-3000 HPLC system (Dionex Sunnyvale, CA, USA). Dried samples (20 mg) were completely dissolved in 10 mL of 10 mM NaOH. Before injection, the samples were filtered through 0.20 μm polytetrafluoroethylene (PTFE) filters. The HPLC system was equipped with a WPS-3000SL autosampler, an LPG-3400SD gradient pump, three PSS Suprema columns with pore sizes of 30, 1000, and 1000 Å in series (300 mm × 8 mm, 10 μm particle size) together with a guard column (50 mm × 8 mm, 10 μm particle size) and a Waters-410 Refractive Index Detector (Wafers, Milford, CA, USA). The mobile phase was 10 mM NaOH and the temperature was kept at 40 °C. Pullulan samples with molecular weights ranging from 342 g/mol to 708 000 g/mol were used as reference samples.

2.4.4. High-Performance Anion Exchange Chromatography with Pulsed Amperiometric Detection (HPAEC-PAD). The composition of the monosaccharides and uronic acids in the *Porphyra umbilicalis* biomass and in the polysaccharide fractions recovered in steps (2), (3), and (5) were assessed using high-performance anion exchange chromatography with pulsed amperiometric detection (HPAEC-PAD). Acid hydrolysis of *Porphyra* and the insoluble fraction to hydrolyze the polysaccharides into monosaccharides was performed by adding 0.8 mL of 72% H_2SO_4 to each 25 mg sample. The samples were left on a shaking board at room temperature for 90 min. Thereafter, 9.2 mL of water was added to obtain a final volume of 10 mL. The samples were placed on a shaking board and left for 70.5 h at 80 °C. One of the main components in pectin is galacturonic acid. Previous studies have shown that acid hydrolysis by a strong acid such as sulfuric acid causes partial degradation of galacturonic acid.[19] Therefore, an alternative mild hydrolysis method was used in combination with methanolysis for the pectin fraction. A dried pectin sample (1.0 mg) was placed in an oven-dried Pyrex tube and 1 mL of 2 M HCl solution in methanol was added to the tube. The tube was sealed and heated to 100 °C for 5 h in a heating block. The solution was then neutralized by adding 200 μL of pyridine to the reaction tube. The sample was cooled to room temperature and the solvent was evaporated under flowing N_2 gas. Hydrolysis was then performed by adding 1 mL of 2 M trifluoroacetic acid (TFA) to the dried sample, and the solution was then heated to 120 °C for 1 h in a heating block, followed by cooling to room temperature. All samples were diluted by a factor of 10 with distilled water, and the composition of carbohydrates and uronic acids was determined using a high-performance anion exchange chromatograph equipped with a pulsed amperiometric detector (HPAEC-PAD, ICS-3000 Dionex, Sunnyvale, CA, USA). The column was a CarboPac PA1 (4 mm × 250 mm) column, and Milli-Q water, 0.26 M NaOH, and 0.17 M NaOAc were used as the mobile phase. A mixture of arabinose, rhamnose, galactose, glucose, xylose, mannose, galacturonic acid, and glucuronic acid was used as the reference sample. The data were processed and analyzed using Chromelon 7.1.

2.4.5. Thermogravimetric Analysis (TGA). The moisture, char, and ash content of the *Porphyra umbilicalis* biomass and the dried fractions recovered in steps (1), (2), (3), and (5)

DOI: 10.1021/acs.iecr.7b03768
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were estimated using a Mettler−Toledo TGA/DSC system. A quantity of ∼4 mg of each sample was heated from 40 °C to 800 °C at a heating rate of 10 °C/min under an N_2 atmosphere. The flow rate was set to 50 mL/min. After being heated to 800 °C, O_2 was introduced instead of N_2 and the temperature was kept at 800 °C for 15 min. The moisture content was calculated as the weight lost by the samples between 40 °C and 110 °C. The char content was calculated as the weight remaining after the samples were heated to 800 °C in N_2, and the ash content was calculated as the mass remaining after the completed program.

2.4.6. Scanning Electron Microscopy (SEM) and Elemental Analysis via Energy-Dispersive Spectroscopy (EDS). The surfaces and internal morphologies of the algal parts before grinding, and during different stages of extraction, were observed by ultrahigh-resolution field emission scanning electron microscopy (FE-SEM), using a Hitachi S-4800 system. The samples were air-dried at room temperature overnight and then attached to the sample supports, using double-sided adhesive carbon tape, and sputter-coated with an 8-nm-thick Pt/Pd layer, using a Cressington 208HR under an inert atmosphere. The elemental composition of selected areas was assessed via SEM-EDS (energy-dispersive spectrum) using an X-Max^N from Oxford Instruments. Aztec 3.0 software was used for imaging SEM-EDS data.

2.4.7. Analysis of the Total Protein Content. The total nitrogen content in the protein extract was analyzed by a LECO Trumac nitrogen analyzer (USA). A conversion factor of 5 was used to convert total nitrogen to protein.[20]

3. RESULTS AND DISCUSSION

Consistent with the biorefinery concept, we developed a multicomponent fractionation strategy that allows for the recovery of proteins and polysaccharides from *Porphyra*, which is in contrast with the conventional methods that target only the carrageenan component or proteins. Chemical analyses of the polysaccharide fractions were also carried out to obtain a deeper understanding of the exact polysaccharide composition of *Porphyra umbilicalis* grown along the west coast of Sweden.

3.1. Extraction Yields for Proteins and Polysaccharides. The developed fractionation protocol yielded four individual macromolecular fractions (Figure 2, steps (1b), (2b), (3b), and (5)) and the weight yields of the proteins and polysaccharide-rich fractions are given in Table 1. The weight yields are calculated as the mass of the freeze-dried fractions divided by the mass of the dried starting algae material. In addition, we calculated the specific protein yield in the fraction recovered from step (1) (see Section 2.2.1) to 15% ± 4.8%. The specific yield of the *Porphyra* protein obtained in the fraction recovered from step (1) is in the same range as the value obtained when the pH-shift process was applied to *Saccharina latissimi* (16.01%).[13] However, the latter yield was calculated based on protein measurements of the supernatants obtained throughout the pH-shift process using the spectrophotometric Lowry analysis principle and not on analysis of total nitrogen in final extracts. Using a similar analysis and calculation procedure as that used by Vilg et al.[13] in the present study resulted in a protein yield of 26.1% for *Porphyra umbilicalis*. This higher yield could have been due in part to the freezing step during the isoelectric precipitation. Precipitation is when most seaweed proteins are lost; the alkaline solubilization step recovers up to 59.6% of the proteins. However, it was recently shown that freeze−thaw cycles increased the precipitation yield by 8.5%.[14] Our yield here was lower than protein yields (46.3% and 45.4%) reported from other red seaweeds (*Hypnea charoides* and *Hypnea japonica*, respectively) using alkali or solubilization in combination with 2-mercaptoethanol, followed by ammonium sulfate precipitation.[21] However, using 2-mercaptoethanol in the solubilization step prevents the protein concentrates from being food-grade. Carrageenan can be recovered as a solid by adding ethanol to the supernatant after conventional alkaline extraction.[6] Here, however, after adding ethanol to the supernatant in a 2:1 volume ratio after extraction of the carrageenan, a suspension was obtained, rather than a precipitate. This may have been due to the low concentration of the carrageenan in the supernatant, which may have required a larger volume of ethanol to fully recover the carrageenan as a solid. For this reason, the carrageenan was recovered by freeze-drying instead of precipitation, to limit the loss of product. The same behavior was observed after the extraction of pectin. No solid product was recovered after the addition of ethanol, which indicated a very low concentration of pectin. Therefore, the pectin fraction was also recovered by freeze-drying. As seen in Table 1, only 1.2% (w/w) of the initial material remained as an insoluble fraction after all extraction steps. However, product loss occurred during the last freeze-drying step due to difficulties in retrieving the samples from the round flask; hence, this value is less reliable than the values for the other fractions.

Table 1. Extraction Weight Yields for Proteins and Polysaccharides Enriched Fractions Extracted from *Porphyra umbilicalis*[a]

compound	weight yield (%)
protein-rich fraction	7.5 ± 2.4
carrageenan-rich fraction	19.9 ± 3.9
pectin-rich fraction	19.2 ± 4.3
cellulose-rich fraction	1.2[b]

[a]The values are given as the mass of the freeze-dried fractions divided by the mass of the dried starting algae material. [b]No standard deviation is given because the value is based on a single extraction.

The SEM analysis revealed the topography of the *Porphyra umbilicalis* blades before grinding and the effect of progressive fractionation (Figure 3). Before treatment, the dried blades had flat structures without holes or pores on their surfaces. After grinding and freeze-drying, and as the extraction proceeded, the surface becomes progressively rougher, more cavitated, and more eroded, and eventually it left behind an insoluble fibrous scaffold.

3.2. Analysis of the Composition of *Porphyra umbilicalis* before and after Fractionation. The FTIR spectrum of *Porphyra umbilicalis* shows many of the characteristic peaks for polysaccharides (Figure 4, left). A list of the identified peaks and peak designations is available in Table S2 in the Supporting Information. The broad band at 3500−3200 cm^{-1} corresponds to the O−H stretching of the hydroxyl groups, and the bands from 3000 cm^{-1} to 2800 cm^{-1} correspond to C−H stretching.[22] The band located between 1630 cm^{-1} and 1600 cm^{-1} corresponds to the O−H stretching of bonded water.[16] The sharp peaks at 1374 cm^{-1} and 1032 cm^{-1} correspond to C−H bending and C−O−C linkages, respectively.[16] The small peak at 898 cm^{-1} is characteristic of the β-glycoside bond, which confirms that sugar units linked together in the polysaccharide structures are present in the

DOI: 10.1021/acs.iecr.7b03768
Ind. Eng. Chem. Res. 2018, 57, 42−53

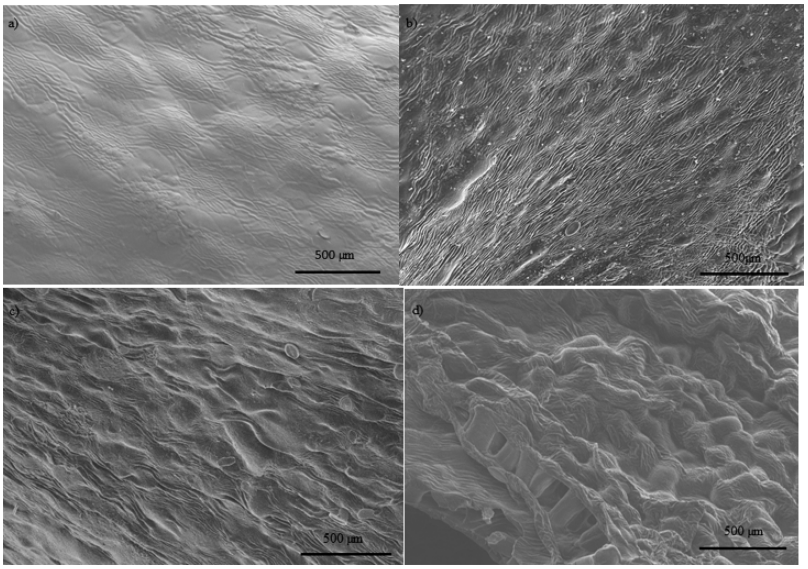

Figure 3. SEM images at 500× magnification of algal leaves of *Porphyra umbilicalis* at different stages of fractionation: (a) before grinding and fractionation, (b) after treatment in 1.0 M Na_2CO_3 for 24 h at room temperature (step (2)), (c) after treatment in Na_2CO_3 and the extraction of carrageenan (step (2b)), and (d) after the extraction of pectin (step (3)).

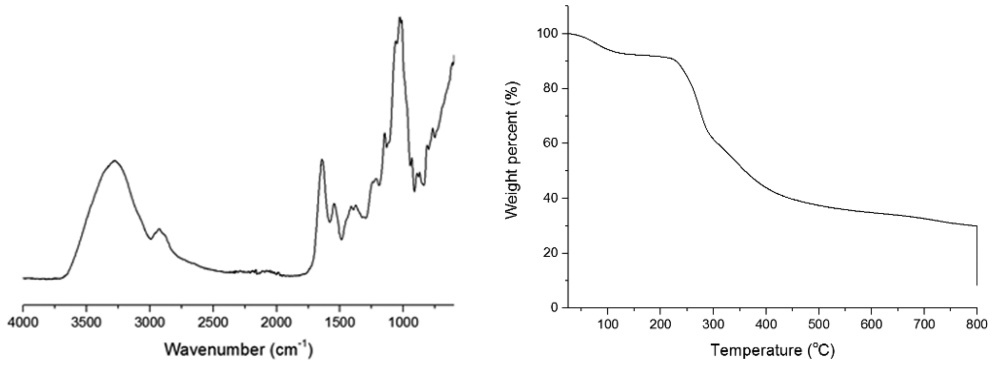

Figure 4. FTIR spectrum (left) and TGA curve (right) of *Porphyra umbilicalis* before fractionation.

Table 2. Char and Ash Contents in % (w/w) in Freeze-Dried *Porphyra umbilicalis* before Fractionation and in Recovered Samples after the Fractionation Steps[a]

	Porphyra umbilicalis	protein-rich fraction	carrageenan-rich fraction	pectin-rich fraction	cellulose-rich fraction
char (% dry weight)	31.9 ± 0.3	34.3 ± 4.2	29.2 ± 0.8	40.6 ± 0.8	13.6 ± 1.9
ash (% dry weight)	9.1 ± 0.3	10.7 ± 2.1	12.1 ± 2.2	7.7 ± 1.6	7.1 ± 2.6

[a]The values were calculated as described in Section 2.4.6. The ash and char data are expressed as percentages (on a dry weight basis). The data are given as mean values of two replicates.

biomass. Thus, as indicated by the FTIR results, polysaccharides are the main components in *Porphyra umbilicalis*. The sharp peak at 1544 cm^{-1} stems from N−H bending in the amines, which indicates the presence of proteins.[16]

The moisture, char, and ash content in freeze-dried *Porphyra umbilicalis* were calculated from the TGA thermogram (see Table 2 and Figure 4, right). The differential weight loss data versus temperature, as measured with TGA, is available in Figure S2 in the Supporting Information. The ash content (8.5%) is lower than other values previously reported for red seaweed.[23,24]

The total protein content, calculated as the total nitrogen in crude *Porphyra umbilicalis*, was 30.6% ± 0.7% (dry weight), which is in the same range as previously reported values for the protein contents in *Porphyra* species (22.7%−42.3%).[25,26]

The composition of the freeze-dried *Porphyra umbilicalis* biomass was further assessed using SEM-EDS to map the elemental composition (Table 3) and by HPAEC-PAD to reveal the carbohydrate composition (Table 4). As expected, the most common elements in *Porphyra* are oxygen and carbon, which is consistent with its high content of organic molecules, such as polysaccharides, proteins, and fatty acids. Sulfur (S) is indicative of the sulfate groups in carrageenans and possibly also sulfur-containing amino acids (such as cysteine and/or methionine) in the proteins; however, those amino acids together only represent 1.7% of the total amino acids in crude *Porphyra*.[14] It is important to note that the instrument we used is not equipped to analyze light elements (atom number <10) and the weight percentage values given for the typical elements in organic matter, such as oxygen, nitrogen, and carbon, are

DOI: 10.1021/acs.iecr.7b03768
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Table 3. Elemental Composition (in % as determined by SEM-EDS) of *Porphyra umbilicalis* before Fractionation, and in Recovered Samples after Different Fractionation Steps[a]

element	*Porphyra umbilicalis* before fractionation	carrageenan-rich fraction	pectin-rich fraction	cellulose-rich fraction
C	34.3 ± 10	56.3 ± 1.4	59.3 ± 0.4	57.7 ± 7.7
O	36.6 ± 1.1	23.2 ± 7.3	25.0 ± 1.6	34.4 ± 4.2
Na	17.4 ± 8.4	3.6 ± 1.3	3.1 ± 0.1	*b*
Mg	1.1 ± 1.6	0.5 ± 0.7	1.5 ± 2.1	*b*
Al	0.4 ± 0.1	0.7 ± 0.5	*b*	*b*
S	2.5 ± 2.0	5.7 ± 3.4	*b*	*b*
Cl	7.6 ± 0.3	10.1 ± 7.0	11.1 ± 0.2	1.1 ± 0.1
Ca	0.2 ± 0.3	0.2 ± 0.2	*b*	6.5 ± 3.0

[a]The data are given as the mean values of two replicates. [b]Value is below the detection limit.

highly approximate. Hydrogen cannot be detected at all with this technique and no nitrogen was detected, although *Porphyra umbilicalis* biomass clearly contains proteins. The results are more informative for heavier elements. Approximately 4.5 wt % sodium (Na) was found, and this result was likely due to sodium acting as the counterion to carrageenan. Al, Mg, Cl, and Ca were also found, and their presence may be due to the composition of the seawater in which the algae were collected. These trace elements also contributed to an ash content of ~8.5% (see Table 2). Importantly, no heavy metals or other toxic elements, such as Pb, Hg, Pb, and As, were detected.

Galactose appears to be the most common monosaccharide in *Porphyra umbilicalis*, which indicates the presence of carrageenan and possibly other galactans. The other monosaccharides indicate the presence of other polysaccharides such as cellulose and pectin. No glucuronic acid or galacturonic acid were detected in the *Porphyra* sample. The main reason for this behavior is that conventional harsh acid hydrolysis conditions were used in the pretreatment before HPAEC-PAD analysis, which resulted in total degradation of the uronic acids. HPAEC-PAD analysis allows only for quantification of the monosaccharide building blocks and does not reveal from which polysaccharide the monosaccharides originate. For this reason, the precise amount of the various polysaccharides cannot be determined individually and only a gross carbohydrate amount is known. Therefore, a detailed mass balance is difficult to make. An overall mass balance, based on the total carbohydrate content, is shown in Table S1 in the Supporting Information.

3.3. Specific Analysis of the Protein-Rich Fraction. The protein-rich fraction, which was the first fraction separated in the sequential processing, contained 57.2% ± 3.8% protein on a dry weight basis when the total nitrogen content was analyzed. This was significantly higher than the protein content of the crude seaweed (30.6% ± 0.7%), which indicates that the pH-shift process is efficient for concentrating proteins. Wong et al.[27] reported that seaweed protein extracts from *H. charoides* and *H. japonica* extracted using a method based on alkaline solubilization in combination with 2-mercaptoethanol, followed by precipitation with ammonium sulfate contained 83.1% and 85.0% protein, respectively. As mentioned previously, unlike the food-grade pH-shift process, the use of 2-mercaptoethanol prevents the protein extracts from being food-grade. The pH-shift process has earlier been shown to also concentrate the essential amino acids, compared to the crude seaweed (with the essential amino acids composing 38.5% and 42.4% of the total amino acids in the crude seaweed and in the protein-rich fraction, respectively).[14] The pH-shift process has been classified as Generally Recognized as Safe (GRAS) and, with use of food-grade acids and bases, there is no hindrance from using the protein extract in food. The process is already commercialized at a few places globally for application on muscle food byproducts.

Based on the TGA analysis, the ash content of the protein-rich fraction is ~10% (Figure 5). This could be compared to the analysis results from classic combustion: the ash content of the pH-shift protein fraction for *Porphyra umbilicalis* was then 4.7% on a dry weight basis.[14] The differential weight loss data versus temperature, as measured with TGA, is available in Figure S3 in the Supporting Information.

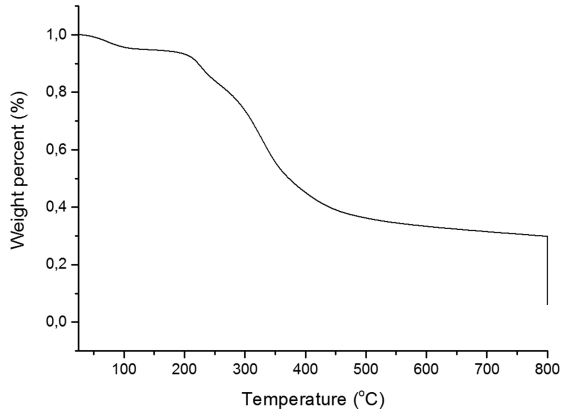

Figure 5. TGA thermogram of the protein-rich fraction.

A SEM micrograph used to analyze the morphology of the protein-rich fraction is shown in the Supporting Information (Figure S1a).

Table 4. Carbohydrate and Uronic Acid Composition, Given as the Relative Percentage of Organic Material, of the Freeze-Dried *Porphyra umbilicalis* before Fractionation, and in Recovered Samples after Different Fractionation Steps[a]

	Composition (%)							
	Ara	Gal	Glu	Xyl	Rha	Man	GlcA	GalA
Porphyra umbilicalis before fractionation	1.6	84.0	1.5	2.4	1.7	8.8	*b*	*b*
carrageenan -rich fraction	*b*	100	*b*	*b*	*b*	*b*	*b*	*b*
pectin-rich fraction	0.9	87.1	5.0	2.2	0.7	1.6	1.8	0.7
cellulose-rich fraction	*b*	11.0	89.0	*b*	*b*	*b*	*b*	*b*

[a]The abbreviations for the carbohydrates are as follows: Ara ≡ arabinose, Gal ≡ galactose, Glu ≡ glucose, Xyl ≡ xylose, Rha ≡ rhamnose, Man ≡ mannose, GlcA ≡ glucuronic acid, and GalA ≡ galacturonic acid. The data are given as the mean values of two replicates. [b]Value is below the detection limit.

DOI: 10.1021/acs.iecr.7b03768
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3.4. Specific Analysis of the Carrageenan-Rich Fraction. A carrageenan-rich fraction was extracted (Figure 2, step (2)), following protein recovery. The FTIR spectrum confirms the presence of the characteristic peaks of carrageenan in the recovered fraction (Figure 6, top) and they have a strong resemblance to the commercial carrageenan sample used as a reference. A list of the identified peaks and peak designations is available in Table S3 in the Supporting Information. The peak at 1257 cm^{-1} corresponds to the sulfate ester groups in carrageenan. The sharp peak at 930 cm^{-1} is characteristic of ι-carrageenan and κ-carrageenan.[28] The peak at 831 cm^{-1} is characteristic of the C−O−SO_3 vibration in λ-carrageenan. In addition, a small peak at 806 cm^{-1} is observed, which indicates the presence of ι-carrageenan.[18] When comparing the FTIR spectrum of the carrageenan fraction (Figure 6, top) to the FTIR spectrum of the freeze-dried *Porphyra umbilicalis* before fractionation (Figure 4, left), it is clear that the former lacks a peak corresponding to N−H bending at ∼1550 cm^{-1}, which indicates that no protein is left in the carrageenan fraction (meaning the carrageenan has successfully been separated from the proteins). In addition, no peak at ∼1700 cm^{-1} was observed in the FTIR spectrum for carrageenan, which indicates that there are no C═O groups in the carrageenan. This means that no uronic acid—and, hence, no pectin—is present in this fraction. Based on FTIR analysis, the extracted carrageenan is a mixture of κ-, ι-, and λ-carrageenan.

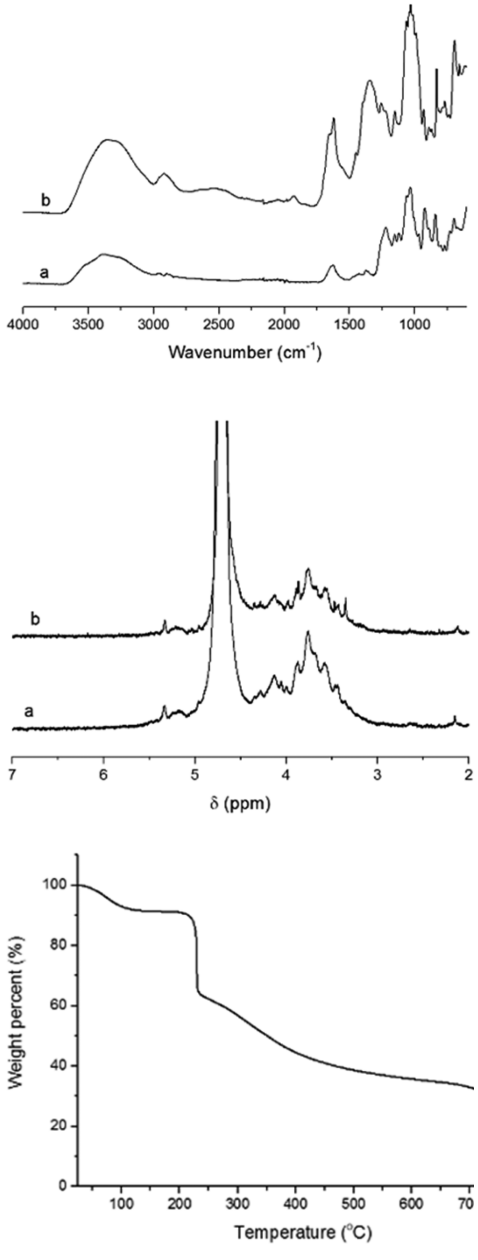

Figure 6. (Top panel) FTIR spectrum of (a) the reference carrageenan and (b) the carrageenan-rich fraction extracted from *Porphyra umbilicalis*. (Middle panel) ^{1}H NMR spectrum of (a) the reference carrageenan and (b) the carrageenan-rich fraction extracted from *Porphyra umbilicalis*. (Bottom panel) TGA thermogram for the carrageenan-enriched fraction extracted from *Porphyra umbilicalis*.

The signals between 4.5 and 3.0 ppm in the NMR spectrum (Figure 6, middle) are characteristic of the ring hydrogens of polysaccharides. The weak signals at 5.17 ppm and 5.33 ppm are characteristic of κ-carrageenan and ι-carrageenan, respectively.[29] No peak for λ-carrageenan was observed probably due to the low resolution caused by the high viscosity of the sample.

The SEC measurements confirmed that the extracted carrageenan fraction is indeed polymeric. A number-average molecular weight (M_n) of 715 000 g/mol, a weight-average molecular weight (M_w) of 1 380 000 g/mol, and a dispersity ($Đ$) of 1.9 were recorded. The molecular weight was quite high and, most likely, an overestimation. SEC estimates the molecular weight by measuring the hydrodynamic volume of the sample relative to a standard of known molecular weight. However, carrageenan molecules (especially κ-carrageenan) have a tendency to aggregate in solution at lower temperatures in the presence of metal ions.[18] Since the SEC analysis was carried out at a rather low temperature (40 °C) and because Na^+ was present in the eluent, aggregate formation was expected under these conditions. Therefore, the estimated hydrodynamic volume was probably the hydrodynamic volume of a cluster of carrageenan molecules rather than the individual chain coils. In addition, the estimated molecular weight was higher than the molecular weight of the pullulan reference sample with the highest molecular weight (708 000 g/mol) which made the calibration less reliable. Finally, the reference sample is a neutral polymer while carrageenan is an anionic polymer. Therefore, the samples were likely to have different affinities to the eluent, so their hydrodynamic volumes were not fully comparable. Still, the recovered carrageenan fraction was undoubtedly polymeric. Previous studies on carrageenan extracted from red seaweed reported a high molecular weight (400 000−2 200 000 g/mol) and dispersities between 2 and 4, so the results are reasonable.[5]

The ash content of the carrageenan-rich fraction was quantified from TGA analysis (see Table 2 and Figure 6, bottom), and the differential weight loss data versus temperature, as measured with TGA, is available in Figure S4 in the Supporting Information. The ash content is somewhat higher than that of the crude algae before fractionation. This is probably because Na^+ acts as a counterion in carrageenan and will remain in the solid phase, rather than being solubilized and enriched in the liquid supernatant in step (2). In addition, sodium was added to the biomass, in the form of Na_2CO_3, which acted as a mediator in the extraction step, allowing more sodium to bind to the carrageenan phase. The effect of this is observed in the results of the elemental composition analysis by SEM-EDS (Table 3), which revealed that the carrageenan-rich fraction contains a significantly higher amount of sodium than

DOI: 10.1021/acs.iecr.7b03768
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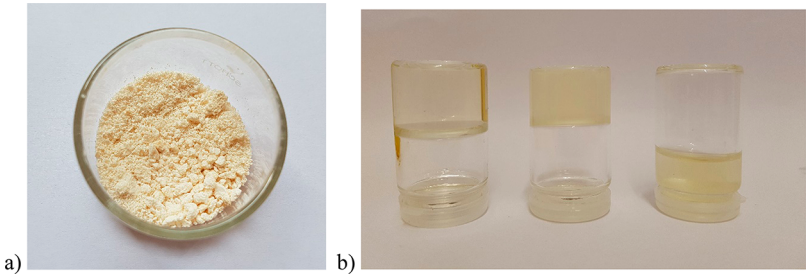

Figure 7. (a) Carrageenan-rich fraction extracted from *Porphyra umbilicalis*. (b) Samples from the gelling experiments: (left) commercial κ-type carrageenan mixed with K^+, (middle) the carrageenan-rich fraction extracted from *Porphyra umbilicalis* mixed with K^+, and (right) the carrageenan-rich fraction extracted from *Porphyra umbilicalis* mixed with Ca^{2+}.

the crude algal biomass. The carrageenan-rich fraction also contain sulfur that is indicative of a sulfated polysaccharide.

A SEM micrograph used to analyze the morphology of the carrageenan-rich fraction is shown in the Supporting Information (Figure S1b).

As discussed above, the FTIR analysis indicated that the extracted carrageenan-rich fraction is a mixture of κ-, ι-, and λ-carrageenans. The different hybrids of carrageenan have different gelling behaviors; the κ-type forms gels in the presence of K^+, ι-carrageenan forms gels in the presence of Ca^{2+}, and λ-carrageenan tends to not form gels at all under these conditions.[9] To test the gelling ability of the carrageenan-rich fraction extracted from *Porphyra umbilicalis*, aqueous solutions of the isolated and dried carrageenan-rich fraction (Figure 7a) were mixed with either K^+ or Ca^{2+} ions, heated, and then cooled to room temperature. A commercial κ-type carrageenan was used as a reference sample and treated in the same way as the extracted carrageenan-rich fraction from *Porphyra umbilicalis*. The commercial κ-type carrageenan readily formed a gel with K^+ (Figure 7b, left), as expected. Similarly, the extracted carrageenan-rich fraction formed a gel with K^+ (Figure 7b, middle), and no gel was formed when the extracted carrageenan-rich fraction was mixed with Ca^{2+} (Figure 7b, right). In the latter case, an opaque solution formed but it did not gel, even after an extended period of time. This indicates that the extracted carrageenan-rich fraction contains a fair amount of the κ-type carrageenan hybrid.

3.5. Specific Analysis of the Pectin-Rich Fraction. The FTIR spectra of the freeze-dried *Porphyra umbilicalis* biomass before fractionation and the carrageenan and pectin fractions also show peaks characteristic of polysaccharides (Figure 8, top). A list of the identified peaks and peak designations is available in Table S4 in the Supporting Information. The bands from O−H, C−H, and C−O−C stretching are observed along with peaks for bounded water and β-glycoside bonds. However, there are some differences between the FTIR spectra of the carrageenan fraction and the pectin fraction. No characteristic peaks for the sulfated moieties abundant in carrageenan can be observed in the FTIR spectrum of the pectin fraction, which means that there is little or no carrageenan in this fraction. In addition, a small peak is observed at ∼1700 cm^{-1} that overlaps with the peak for absorbed water at 1642 cm^{-1}. This peak may originate from the C═O stretching of the carboxyl group in galacturonic acid or glucuronic acid, and it is indicative of a pectic substance.

The NMR spectrum of the pectin fraction (Figure 8, middle) shows a cluster of overlapping peaks at 4.5−3.0 ppm that are characteristic of polysaccharide ring hydrogens. Studies have shown that the characteristic peaks for pectin protons appear at

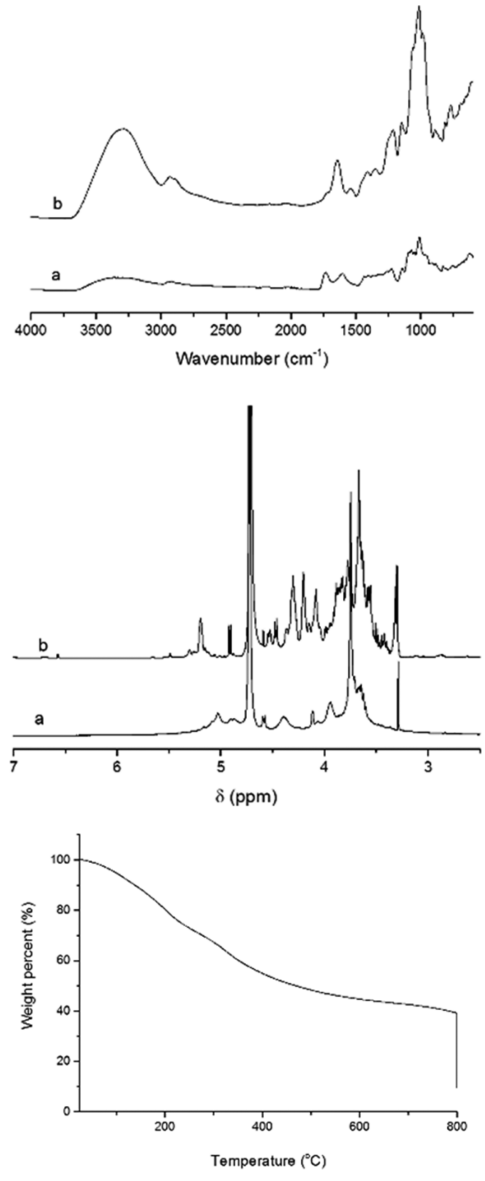

Figure 8. (Top panel) FTIR spectrum of the reference pectin (spectrum a) and pectin extracted from *Porphyra umbilicalis* (spectrum b). (Middle panel) ^1H NMR spectrum of the reference pectin (spectrum a) and pectin extracted from *Porphyra umbilicalis* (spectrum b). (Bottom panel) TGA curve for carrageenan extracted from *Porphyra umbilicalis*.

3.73, 3.80, 3.98, 4.41, 4.90, 4.95, and 5.08 ppm.[19] Here, two characteristic peaks for pectin can be observed at 3.73 and 4.90

DOI: 10.1021/acs.iecr.7b03768
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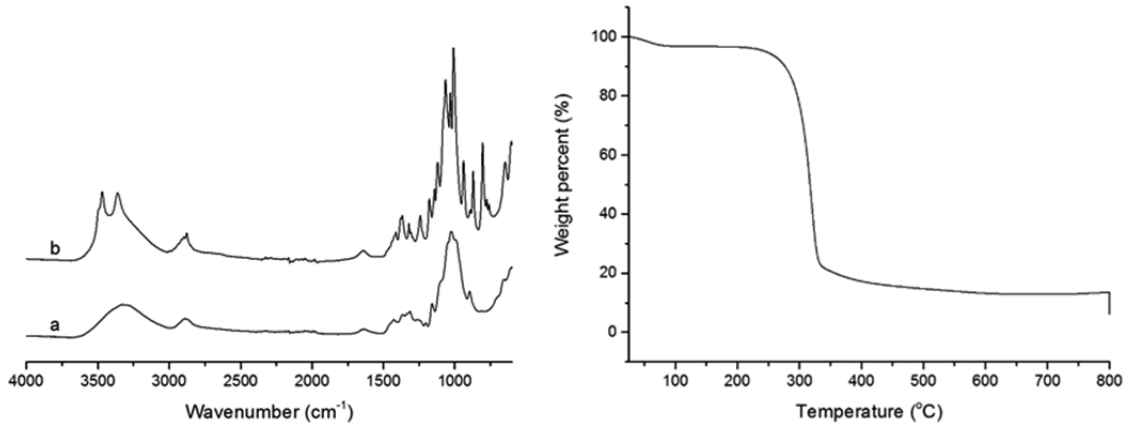

Figure 9. (Left) FTIR spectrum of the α-cellulose reference from spruce wood (spectrum a) and the cellulose-rich fraction extracted from *Porphyra umbilicalis* (spectrum b). (Right) TGA curve for the cellulose-rich fraction.

ppm in the pectin-enriched fraction extracted from *Porphyra umbilicalis*. The pectin composition is known to vary significantly, depending on the source; therefore, the comparison to a commercial lemon pectin in Figure 8 is only valid to some extent.

The lack of sulfate bands in the FTIR spectrum was consistent with the results of the elemental analysis (Table 3); no sulfur (S) was detected in this fraction. These results indicate that the pectin-rich fraction is essentially free from carrageenan. Sodium, which is known to associate strongly with carrageenan, is less abundant in this fraction than in the *Porphyra umbilicalis* biomass before fractionation, and it is somewhat lower than in the carrageenan-rich fraction. The ash content remaining after TGA analysis (Figure 7, bottom) is slightly lower than the ash content of *Porphyra umbilicalis* before fractionation and significantly lower than the ash contents of the protein-rich and the carrageenan-rich fractions, which suggests that ions such as Mg and Na were both extracted before this stage. The differential weight loss data versus temperature, as measured with TGA, is available in Figure S5 in the Supporting Information.

An analysis of the carbohydrate composition (Table 4) revealed some differences between the crude algae and the pectin-enriched fraction. No glucuronic acid or galacturonic acid was found in the *Porphyra umbilicalis* sample, but small amounts were found in the pectin-rich fraction. The main reason for this behavior is probably that the pectin fraction was subjected to methanolysis and mild TFA hydrolysis, instead of conventional hydrolysis with sulfuric acid. The milder treatment resulted in a lower degree of degradation of the hydrolytically unstable uronic acids, and the uronic acids could thus be detected in the subsequent carbohydrate analysis with HPAEC-PAD. By adding the masses of the monosaccharides, we obtain ~600 mg per 1 g of sample, which indicates that only ~60% of the total mass is pectin. In addition, the amount of galacturonic acid in the pectin fraction was very low, compared to the monosaccharide content, which is quite different from other pectin sources, such as lemon, in which galacturonic acid is the main component. The contents of the neutral sugars xylose, arabinose, and glucose in the pectin fraction were also consistent with the expected values for a pectic substance. Even though the pectin fraction is polymeric, the molecular weight is quite low, compared to the carrageenan fraction; M_n = 5090 g/mol, M_w = 11 300 g/mol, and $Đ$ = 2.2 were recorded by SEC analysis for the pectin fractions. Given the hydrolytic instability of pectic substances, it is likely that the fraction was somewhat degraded during fractionation and that the native molecular weight is higher than the recorded values.[30]

A SEM micrograph used to analyze the morphology of the pectin-rich fraction is shown in the Supporting Information (Figure S1c).

3.6. Specific Analysis of the Cellulose-Rich Fraction.

Similar to the spectra for the carrageenan-and pectin-rich fractions, the FTIR spectrum of the cellulose-rich fraction (Figure 9, left) shows characteristic polysaccharide peaks, including O−H, C−H, and C−O−C stretching, bounded water and the β-glycoside bonds. A list of the identified peaks and peak designations is available in Table S5 in the Supporting Information. An important difference between the spectrum of the cellulose-rich fraction and the spectrum from the reference sample of cellulose is the presence of two peaks, at 931 cm^{-1} and 806 cm^{-1}, that are characteristic of κ and ι-carrageenan, respectively. Evidently, some residual carrageenan was present, which indicates that the cellulose-rich fraction was not a pure cellulose fraction. However, the elemental analysis (Table 3) does not detect any sulfur in this fraction, and the sodium content is also much lower than that of the freeze-dried *Porphyra umbilicalis* biomass before fractionation; thus, the carrageenan content could not be too high. A quantification of the carbohydrate composition of the cellulose-rich fraction reveal that the glucose content is very high (89%; see Table 4), while galactose accounts for the rest, confirming the above conclusion that some carrageenan remained in the fraction. If purified further, the algae cellulose could present a valuable component in renewable materials. Cellulose extracted from *Cladophora* algae have been shown to work as reinforcing additives in a plastic matrix.[31] Other potential uses of algae cellulose as a material include filter materials and consistency regulators in water dispersions.[32]

Overall, the ash content (Table 2) of this fraction, as measured with TGA, was lower than that of the other fractions, which suggests that inorganic ions were largely co-extracted with the other biopolymers. Only calcium remained to a measurable extent in this fraction. The TGA thermogram (Figure 9, right) and the differential weight loss data versus temperature (Figure S6 in the Supporting Information) further shows that this fraction was the most thermally stable, and it had the highest onset of thermal degradation. A SEM micrograph used to analyze the morphology of the cellulose-

DOI: 10.1021/acs.iecr.7b03768
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rich fraction is shown in the Supporting Information (Figure S1d).

In summary, the presented sequential fractionation method allows for the recovery of several macromolecule extracts from the same biomass. With this, we hope to contribute to a more resource efficient approach with the potential of adding value to seaweed and other biomasses by utilizing several components. Consistent with the concept of biorefining (sustainable processing of biomass into a spectrum of marketable products, such as chemicals, materials, fuel, and power), a sequential process allows for the production of several valuable component fractions. The additional value and yield from the algal biomass of course require additional processing and energy consumption. Therefore, it is important not only to use green raw materials but also to develop as green and efficient processes as possible and to benchmark new processes from a lifecycle analysis perspective against conventional processes.[33] Still, a process that may not be cost-effective based on one component only can be enabled if other components are also utilized and sold as valuable new materials. While vegetarian proteins are highly interesting food and feed ingredients, the polysaccharides represent new material feedstock with many opportunities for the polymer processing industry to shift from petroleum hydrocarbons to renewable resources. Regarding proteins, total protein levels in most seaweed species are too low to directly use them as protein ingredients in feed.[34] Also, the though polysaccharide-rich cell wall and the abundance of polyphenols reduce the digestibility of the proteins.[35,36] To be able to utilize seaweed as a protein source, it is therefore necessary to extract and concentrate the proteins, as demonstrated in this work. Furthermore, this will increase the value of the seaweed proteins. So far, there has been no use of the residual sediment from the first step in the pH-shift process. In our biorefinery process, the sediment byproduct from this process is the raw material used for the extraction of carrageenan. Converting this and other biopolymers from *Porphyra* to valuable products would mean that useful renewable materials are made available to the market, and it was possible to downgrade them to solid fuel or field fertilization by composting after their service life. Another benefit with macroalgal biorefining is the possibility of recovering biomolecules that are not accessible elsewhere and that mankind cannot (at least not yet) produce synthetically. Carrageenan is a good example of such a unique component. A sequential extraction like this will minimize transportation and make more efficient use of the already-existing infrastructure. In the presented process, the yield of cellulose is small and this fraction may not be viable as a materials feedstock if the produced volume is minor. However, the yields of pectin and carrageenan are substantial. Both of these fractions can be obtained in a reasonable pure state. In addition, the polysaccharide fractions are free from heavy metals such as As, Cd, and Pb that otherwise would limit the safe use of these components in materials design.

4. CONCLUSIONS

Fractions enriched in proteins, carrageenan, pectin, and cellulose (insoluble fraction) were successfully extracted from the red macroalgae *Porphyra umbilicalis* in a sequential fractionation protocol that was purposely developed to enable the recovery of multiple biomacromolecules from seaweed biomass in a biorefinery approach. The yield of protein in the protein-enriched fraction was 15.0% (w/w), while its weight contributed to 7.5% of the initial seaweed mass. Regarding the carrageenan- and pectin-rich fractions, extraction weight yields were close to 20% (w/w) each, and they were recovered in subsequent steps. The protein-enriched fraction contained 57% protein. The carrageenan-rich fraction, which was extracted with an aqueous Na_2CO_3, had a very high molecular weight. ^1H NMR and FTIR analyses indicated that this fraction was a mixture of κ-carrageenan, ι-carrageenan, and λ-carrageenan and was essentially free from proteins and carboxylated pectins. The carrageenan-rich fraction formed gels in aqueous solution upon the addition of K^+ ions. The pectin-rich fraction was isolated after the carrageenan-rich fraction was essentially free from carrageenan. The pectic substance contained galactose and uronic acid building blocks. After the final step, a cellulose-rich insoluble phase remained.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.iecr.7b03768.

SEM micrographs of the proteins and polysaccharide fractions extracted from *Porphyra umbilicalis*; table giving the overall composition of *Porphyra umbilicalis* (Table S1); FTIR peak values of *Porphyra umbilicalis* (Table S2), the carrageenan-rich fraction (Table S3), the pectin-rich fraction (Table S4), and the cellulose-rich fraction (Table S5); TGA curves of *Porphyra umbilicalis* (Figure S2), the protein-rich fraction (Figure S3), the carrageenan-rich fraction (Figure S4), the pectin-rich fraction (Figure S5), and the cellulose-rich fraction (Figure S6) PDF)

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Author Contributions

The manuscript was written through contributions of all authors. Regarding the experimental work, Hanna Harrysson and Ingrid Undeland performed the protein extraction and protein analyses. Niklas Wahlström and Ulrica Edlund performed all subsequent extractions and analyses of the carrageenan-rich, pectin-rich and cellulose-rich fractions, as well as the SEM and SEM-EDS analyses. All authors have approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank The Swedish Foundation for Strategic Research (SSF) for their financial support (Project No. RBP14-0045). We thank Pär Lindén, Jennie Berglund, and Nicola Giummarella at WWSC, KTH Royal Institute of Technology, for their help and support during the SEC-measurements and HPAEC-PAD analysis of the carbohydrate composition of the pectin. Finally, we thank Gunnar Cervin, Friederike Eimer, Henrik Pavia, and Göran Nylund at the Sven Lovén Center of Marine Science at Tjärnö (University of Gothenburg) for cultivating and harvesting *Porphyra umbilicalis*.

DOI: 10.1021/acs.iecr.7b03768
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DOI: 10.1021/acs.iecr.7b03768
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