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Characterization of polysaccharides extracted from brown seaweeds

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Abstract

The structural characteristics of polysaccharides extracted from Quebec's seaweed have not been fully established to date. *Ascophyllum nodosum*, *Fucus vesiculosus* and *Saccharina longicruris* were studied in this research. The seaweeds were treated with selective solvents to extract laminaran and fucoidan in mixture (fraction A), fucoidan (fraction B), and alginate (fraction C). Analyses were performed to determine their content in sulphates, total sugars and uronic acids. H NMR was additionally performed on alginate extract. Results showed that the laminaran extracted from *S. longicruris* contained 99.1% of the total sugars while the extracts from *A. nodosum* and *F. vesiculosus* contained 89.6% and 84.1%, respectively. The fucoidan found in fractions A and B was shown to have different structures. *F. vesiculosus* and *S. longicruris* showed important variations in terms of total sugars, uronic acids and molecular weight of polysaccharide. *A. nodosum*, on the other hand, had a more stable composition, with the exception of the polysaccharide molecular weight. Alginate from *S. longicruris* had the lowest molecular weight (106.6 kDa) with a F_{MM} blocks proportion of 0.07 and F_{GG} blocks of 0.25. Their characterization will lead to a better understanding of their functional characteristics and promote the exploitation of this natural resource.

Keywords: Laminaran; Fucoidan; Sodium alginate; *Ascophyllum nodosum*; *Fucus vesiculosus*; *Saccharina longicruris*; Polysaccharides; Seaweeds

1. Introduction

Many brown seaweeds species are available in Quebec's Saint-Lawrence River: *Ascophyllum nodosum*, *Fucus vesiculosus* and *Saccharina longicruris* formerly named *Laminaria longicruris* (Lane, Mayes, Druehl, & Saunders, 2006). All three contain polysaccharides like laminaran, fucoidan and alginate. The first two seaweed species were studied in other countries but since many factors like geographic location and harvest season influences the polysaccharides content; this work is oriented to study their differences. On the other hand, *S. longicruris* is particularly interesting because few studies were conducted on its polysaccharides. The purpose of this study is to document the polysaccharides composition and characteristics from brown seaweeds found in eastern Canada.

Chemical structures of polysaccharides from seaweeds have been investigated extensively in the past (Chizhov et al., 1998; Duarte, Cardoso, Noseda, & Cerezo, 2001; Fleury & Lahaye, 1991; Haug, 1964; Patankar, Oehninger, Barnett, Williams, & Clark, 1993; Percival & McDowell, 1967). Their structures vary according to the season, age of population, species and geographic location (Graham & Wilcox, 2000). Laminaran and fucoidan are mostly interesting for their potential biological activities where alginate is mostly uses as food ingredients.

Laminaran structure and composition vary according to algae species (Chizhov et al., 1998). Laminaran is composed of (1,3)-β-D-glucan (Zvyagintseva et al., 1999) with β(1,6) branching (Nelson & Lewis, 1973). There are two types of laminaran chains (M or G), which differ in their reducing end. M chains end with a mannitol residue whereas G chains end with a glucose residue. Laminaran's molecular weight is approximately 5000 Da depending on the degree of

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polymerisation (usually 25). The solubility is influenced by the degree of branching. Highly branched laminaran is soluble in cold water whereas lower levels of ramification induce solubility only in warm water (Rupérez, Ahrazem, & Leal, 2002).

Fucoidan is a sulphated fucan; the exact structural characteristics have not yet been elucidated, and only little regularity in the structure is known at the present time. Fucoidan is composed of fucose, uronic acids, galactose, xylose and sulphated fucose. Variations are observed between species which have an impact on the determination of the polysaccharide structure. Fucoidan extracted from *A. nodosum* is mainly composed of fucose linked in α(1,3) and α(1,4) (Chevolot et al., 1999; Chevolot, Mulloy, Ratiskol, Foucault, & Colliec-Jouault, 2001; Daniel et al., 2001; Daniel, Berteau, Jozefonvicz, & Goasdoue, 1999; Marais & Joseleau, 2001). Lateral chains are composed of single or several fucosyl units with branching in position 4. For *F. vesiculosus*, Percival and McDowell (1967) have proposed two possible structures. The first one consists of fucose linked in α(1,2) with sulphate in position 4. The second one is similar to the first structure proposed, but in this case, the fucose units are linked in α(1,3). Patankar et al. (1993) have studied fucoidan from a commercial source, extracted from *F. vesiculosus* and found α(1,3)-linkages between fucose. The ending fucose units were also found to hold branching with α(1,2)- or α(1,4)-linkages. Various molecular weights have been reported in the literature for fucoidan. Some authors have established fucoidan's molecular weight at approximately 100 kDa (Patankar et al., 1993), while others have measured a fraction of 1600 kDa and another of 43 kDa in the same fucoidan sample (Rupérez et al., 2002). Fucoidan is soluble in water and in acid solution (Rupérez et al., 2002).

Alginate is composed of mannuronic (M) and guluronic (G) acid with β(1,4)-linkages and the structure varies according to the monomer position on the chain, forming either homopolymeric (MM or GG) or heteropolymeric (MG or GM) segments. The molecular weight of alginate ranges generally between 500 and 1000 kDa. Its solubility is influenced by factors such as pH, concentration, ions in solution, the presence of divalent ions (Moe, Draget, Skjåk-Braek, & Smidsrød, 1995) and ionic force. Alginate is gelling in presence of divalent ions like calcium (Morris & Norton, 1983) and consequently it is widely used as a food ingredient.

Marine algae remain largely unexploited in the province of Quebec. *S. longicruris*, *A. nodosum* and *F. vesiculosus* are present in sufficient amount for commercial exploitation. In order to promote the emergence of this industry, it is important to establish the structural characteristics of polysaccharides with commercial interest. Furthermore, the structural differences between seaweed species need to be characterized.

2. Materials and methods

2.1. Algal materials

Alginate, fucoidan, laminaran were extracted from *S. longicruris*, *A. nodosum* and *F. vesiculosus*. *A. nodosum* and *F. vesiculosus* were harvested in September of 2002 at Île-Verte (Québec, Canada). *S. longicruris* was harvested at Gaspé (Québec, Canada) in May of 2001. The seaweeds were milled in a Cormitrol Mill fitted with perforated plates of 24.5 and 1 mm, freeze-dried and then kept at 4 °C until use.

2.2. Extraction procedures

All polysaccharides were extracted as described by Mian and Percival (1973) and adapted from Souchet (2004). The extraction procedures are summarized in Fig. 1. Polysaccharides were extracted from the milled seaweeds (30 g) using selective solvents with a constant mechanical stirring between 455 ± 5 rpm. Temperature was controlled using a water bath. First, ethanol 85% (v/v) at 23 °C (2 × 12 h) and 70 °C (2 × 5 h) was used to extract pigments and proteins. The solvent was separated from residual seaweeds by vacuum filtration using Whatman #4 filters. Residual seaweeds were treated with $CaCl_2$ 2% (w/v) at 70 °C (3 × 3 h) in order to precipitate alginates as well as to extract laminaran and fucoidan from the mixture (fraction A) and then, centrifuged. Fucoidan (fraction B) was extracted from the residual seaweeds with HCl 0.01 M, pH 2, at 70 °C (3 × 3 h) and then, centrifuged. Alginate (fraction C) was finally extracted with Na_2CO_3 3% (w/v) at 70 °C (3 × 3 h) and then, centrifuged. All polysaccharides were dialyzed (cut off 1000 Da) during 48 h. Fraction C was precipitated under alkaline conditions with acetone to achieve purification and resuspended in water. All samples were freeze-dried and kept at 4 °C until use.

Separation of laminaran and fucoidan from fraction A was realized with a DEAE sepharose CL-6B anion exchanger (Sigma, USA) equilibrated with HPLC grade

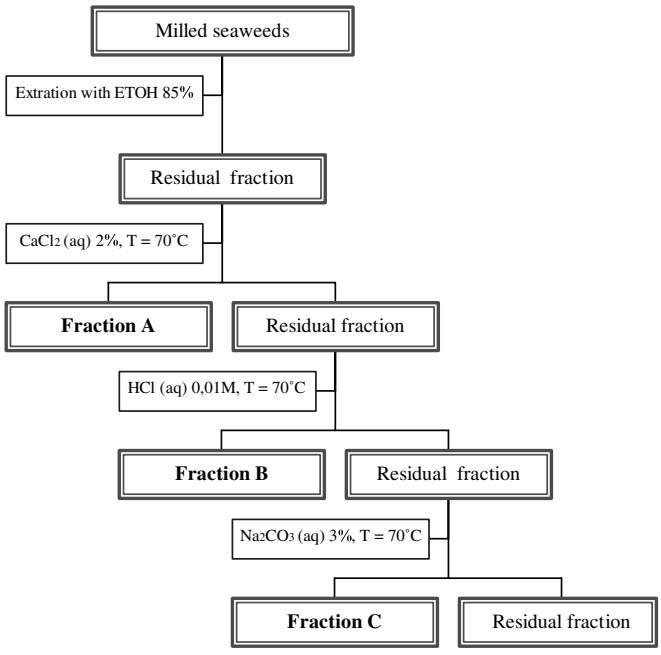

Fig. 1. Extraction of polysaccharides with selective solvents.

water. Fraction A samples were adjusted to pH 7 and precipitated in imidazole (Sigma, USA) 50 mM, to remove resulting proteins, then dialysed for 72 h. Degassed samples were injected in column and eluted with two columns volume of HPLC grade water to elute laminaran. Fucoidan was eluted with two columns volume of 2 M NaCl. All fractions were evaporated and dialysed for 72 h, freeze-dried and stored at 4 °C until use.

2.3. Chemical composition

The crude seaweed analysis provided moisture, protein, ash and lipid contents, and was carried out following AOAC official methods (1990). Total sugars and uronic acids were quantified for each polysaccharide using phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) which uses glucose as the standard, and the Blumenkrantz method (Blumenkrantz & Asboe-Hansen, 1973) where glucuronic acid is used as the standard and 3-phenyl-phenol as the reagent. Absorbance measurements were realised in triplicate. A microplate reader (Molecular Devices, Ottawa, Canada) was used for total sugars analysis. An HP-8453 spectrophotometer (Hewlett Packard, Mississauga, Canada), equipped with UV–visible ChemStation software, was used to measure uronic acids. Sulphur was quantified by ICP-OES (inductively coupled plasma-optical emission spectroscopy) using the model Optima 4300DV from Perkin-Elmer (Boston, USA) equipped with Winlab32 software. The sulphate content was deduced from the amount of sulphur determined by ICP using the following equation: % sulphate group $= 3.22 \times S$ (Roger, Kervarec, Colliec-Jouault, Ratiskol, & Chevolot, 2004).

2.4. Molecular weight determination

Polysaccharides weight average, M_w, was determined by HPSEC–MALLS (high performance size exclusion chromatography–multiangle laser light scattering (HPSEC–MALLS)). The HPSEC system consisted of a Waters 515 HPLC pump (Waters Chromatography S.A., Milford, USA), a 500 µl manual injecting loop and a Wyatt 903 refractometer (Wyatt Technology, Santa Barbara, USA). The MALLS apparatus consisted of a Wyatt Dawn-DSP laser photometer (Wyatt Technology, Santa Barbara, USA) equipped with a K5 flow cell and a He–Ne laser operating at $\lambda = 632.8$ nm. The detection of scattering light was possible at 18 angles.

Three columns were used in line: TSK-guard column PWXL (6 mm × 40 mm), TSK-G5000 PWXL (7.5 mm × 300 mm) and a TSK-G3000 PWXL (7.5 mm × 300 mm) (Tosoh Bioscience, Montgomeryville, USA). Molecular weight separation range for polysaccharides was established from 1 to 1000 kDa by the supplier. The mobile phase consisted of 0.1 M NaCl solution realised with HPLC grade water filtrated on 0.22 µm filters. The flow rate was 0.5 ml/min and analyses were performed at room temperature. Samples were dissolved in 0.1 M NaCl solution and filtrated on 0.45 µm filters to eliminate dust particles. The MALLS instrument was placed directly after the HPSEC columns and before the refractive index detector (DRI).

Prior to measurements, the Dawn apparatus was calibrated using HPLC grade toluene and normalized using 47,300 Da pullulan standard from P-82 kit (Shodex, Japan) in 0.1 M NaCl. The performance of the HPSEC–MALLS system was checked with monodisperse pullulan of various molecular weights. Also, the specific refractive index increment (dn/dc) was determined using an Optilab DSP interferometric refractometer (Wyatt Technologies, Santa Barbara, CA). Fucoidan and alginate were dissolved in 0.1 M NaCl in increasing concentration from 0.1 to 0.8 mg/ml in order to determine the slope of increment. The dn/dc value of 0.155 for alginate and of 0.129 for fucoidan was obtained.

Data were collected from the DRI and MALLS and evaluated with the ASTRA software 4.70.07. Since alginate and fucoidan are polydisperse polysaccharides, only average weight were compared. Results were estimated using second-order Zimm for alginate and fucoidan.

2.5. High performance anion exchange chromatography (HPAEC)

Polysaccharide identification was done by anion exchange chromatography using Waters system (Waters Chromatography S.A., Milford, USA) equipped with auto injector (Wisp 717). The anion exchange column was a Phenomenex Rezex RPM monosaccharide, 100 × 7.8 mm, equipped with a security cartridge (Phenomenex, 4 × 3). The column was eluted with isocratic HPLC grade water at a flow rate of 0.6 ml/min. The eluent was quantified with a SEDEX 75 evaporative light scattering detector (Sedere, Lawrenceville, USA) at a temperature of 40 °C, a pressure of 2 bars and a gain of 10. The Waters system works with a Millennium 3.2 program. The calibration curves were realized following Souchet (2004). Polysaccharides identification was achieved using the appropriate standards of laminaran (Sigma, Oakville, Canada), fucoidan (Sigma, Oakville, Canada) and alginate (Nealanders, Dorval, Canada).

2.6. H NMR spectroscopy

In order to reduce sample viscosity, alginate samples (20 mg) were dissolved in 5 ml of water and heated at 90 °C for 1 h. Then, 1 ml of HCl 0.1 N was added to each tube and heated at 90 °C for a further 2 h. Samples were consecutively cooled, dialysed for 48 h, freeze-dried and washed twice with deuterium oxide 99.96 atom % D (CDN isotopes, Pointe-Claire, Canada) (0,5 ml) to reduce the level of water present in the sample. Then, 10 mg of the hydrolysed alginate was dissolved in 1 ml of deuterium oxide for GG, MM and GM blocks determination. H NMR analyses were performed using a Varian Inova 400 MHz spectrometer

(Varian Inc., Palo Alto, USA). Samples were analysed at 60 °C, with a sweep width of 4400 Hz, a 45° pulse, an acquisition time of 3.7 s and a number of transients of 32 with a relaxation delay of 5 s. The spectra interpretation was realised following Grasdalen, Larsen, and Smidrød (1979).

2.7. Statistical analysis

Composition and structural data was analysed using SAS 8.2 software (SAS Institute Inc., Cary, USA). Comparison between species was analysed with the measure of the least significance difference (LSD) at a significant level of 5%. Means with the same letter are not significantly different (at 0.05).

3. Results and discussion

The chemical composition of all seaweeds is presented in Table 1. *S. longicruris* had the highest percentage of ash, protein and lipids compared to the other species of seaweeds. The high proportion of minerals observed in all species can be explained by the inorganic salt in the water absorbed by the seaweed or by the association of cations with algal polysaccharides (Lahaye, 1991). Results obtained for *A. nodosum* and *F. vesiculosus* are in good agreement with the literature (Bobin Dubigeon et al., 1997). *S. longicruris* results are similar to those obtained by Souchet (2004). Yields of extraction (Table 2) were determined for all fractions; results show few differences for fraction A and B between each seaweed species. Alginate (fraction C) extracted from *A. nodosum* has a higher concentration due to the species' own characteristics. For *S. longicruris*, the amount obtained is in good agreement with Souchet (2004). Results are also similar to those obtained by Mian and Percival (1973), for other seaweed species. Laminaran and fucoidan from fraction A were separated from each other by anion exchange chromatography. The proportion of laminaran was very small with typical yields below 0.004%. Other research had found much more polysaccharides within other species (Black, 1950). Still, since laminaran and fucoidan recovery is strongly linked to the environment and the harvest season (Painter, 1983), it is predictable to have varying yields of extraction. Polysaccharide identification was achieved using HPAEC with the appropriate standard. Structural characteristics of polysaccharides were determined for each fraction, but they were analysed separately in order to highlight the structural differences between species.

Table 2
Yields of extraction for each fraction from *A. nodosum*, *F. vesiculosus* and *S. longicruris* given in % of dry weight

Species	EtOH	A	B	C
Ascophyllum nodosum	11.3 ± 2.3	2.2 ± 0.2	1.1 ± nd	24.0 ± 0.3
Fucus vesiculosus	18.3 ± 4.3	2.6 ± 0.2	1.4 ± nd	16.2 ± 3.2
Saccharina longicruris	19.4 ± 15.9	1.3 ± 0.82	1.3 ± 0.8	20.0 ± 1.1

3.1. Laminaran

Laminaran is the principal reserve polysaccharide and is composed of β-glucan (Nagoaka et al., 2000). Total sugar and uronic acid contents were measured on this fraction. Laminaran contains a large amount of neutral sugars with a low proportion of uronic acids (Table 3). *S. longicruris* contained 99.1% of neutral sugars while *A. nodosum* and *F. vesiculosus* showed lower concentrations: 89.6% and 84.1%, respectively. The uronic acid content differed significantly for all species: 9.3%, 5.6% and 7.5% were found in *A. nodosum*, *F. vesiculosus* and *S. longicruris*, respectively. Overall, *S. longicruris* was found to be the most different from the other seaweed species. The molecular weight of laminaran was not measured due to the low yields of extraction and thus the lack of polysaccharide available for testing. Laminaran is known to have a small molecular weight; around 5000 Da (Patier, Yvin, Kloareg, Liénart, & Rochas, 1993).

Harvesting period is probably responsible for these low yields of extraction. Algae generate their biomass reserve after the rapid grow phase in spring in order to survive the winter where hardly any photosynthesis occurs (Painter, 1983). As a result, a larger amount of laminaran is found during the winter season. Since the algae were harvested in May for *S. longicruris* and in September for the other two species, it was not the optimum period for laminaran.

3.2. Fucoidan

Total sugar and uronic acid contents were determined on fraction A and B in order to compare the different

Table 3
Global composition of laminaran

Species	Total sugar (%)	Uronic acids (%)
Ascophyllum nodosum	b 89.6 ± 4.2	a 9.3 ± 0.3
Fucus vesiculosus	b 84.1 ± 2.6	b 5.6 ± 0.6
Saccharina longicruris	a 99.1 ± 5.7	c 7.5 ± 0.6

Column with the same letter are not significantly different at $p < 0.05$.

Table 1
Initial composition of each crude seaweeds given in % of dry weight

Species	Ash (%)	Proteins (%)	Lipids (%)	Sugars[a] (%)
Ascophyllum nodosum	ab 22.5 ± 0.1	b 1.2 ± 0.1	b 1.2 ± 0.1	b 69.6 ± 0.2
Fucus vesiculosus	bc 24.8 ± 0.2	b 1.4 ± 0.1	b 1.4 ± 0.1	b 65.7 ± 0.4
Saccharina longicruris	c 27.7 ± 1.5	a 12.4 ± 1.3	a 2.1 ± 1.2	a 57.8 ± 2.8

[a] Sugars content were obtained by difference, since no official method exists. Column with the same letter are not significantly different at $p < 0.05$.

Table 4
Global composition of fucoidan from *A. nodosum*, *F. vesiculosus* and *S. longicruris*

Species	Sources	Total sugars (%)	Uronic acids (%)	Sulphates (%)	Molecular weight (kDa)
Ascophyllum nodosum	A	ab 45.4 ± 2.2	a 9.9 ± 1.3	22.1 ± 3.5E-5	417
	B	ab 46.5 ± 3.5	a 9.3 ± 0.2	22.3 ± 2.1E-5	1323
Fucus vesiculosus	A	a 42.1 ± 2.9	a 8.6 ± 0.6	22.4 ± 2.2E-5	529
	B	c 25.3 ± 1.1	c 3.0 ± 0.3	19.0 ± 3.3E-5	877
Saccharina longicruris	A	b 50.5 ± 2.1	b 22.0 ± 0.7	12.0 ± 1.3E-5	454
	B	c 24.4 ± 5.7	a 8.2 ± 0.7	14.2 ± 1.8E-5	576

Column with the same letter are not significantly different at $p < 0.05$.

sources of fucoidan (Table 4). Since the interaction between the species and the sources (A or B) is significant, both factors were analysed at the same time. *A. nodosum* contained 45.4% and 46.5% of neutral sugars and 9.9% and 9.3% of uronic acids for fraction A and B, respectively. However, *F. vesiculosus* and *S. longicruris* showed a significant reduction of neutral sugars, from 42.1% to 25.3%, and a reduction of uronic acid from 8.6% to 3.0% from fraction A to B, respectively. The polysaccharides from fraction A were purified by ion exchange chromatography to separate laminaran from fucoidan with 2 M NaCl as elution buffer to recover fucoidan. This treatment could have selected a fraction containing higher amount of neutral sugars and uronic acids. Also, the results might be explained by the difference in the solubility of the polysaccharide. Other researches have demonstrated the impact of extraction procedures on polysaccharide structure (Mabeau, Kloareg, & Joseleau, 1990; Ponce, Pujol, Damonte, Flores, & Stortz, 2003). Few differences were observed between seaweed species from the same source. For fraction A, *S. longicruris* is the only one significantly different from the others for its uronic acid content. For fraction B, *A. nodosum* is significantly different in total sugars whereas *F. vesiculosus* is significantly different in uronic acids.

The amount of sulphates was determined for all fractions. There was no significant difference found between fractions A and B. However, *S. longicruris* was found to contain a significantly lower amount of sulphate: 12% and 14.2% for fractions A and B, respectively, while an average of 20% was observed for all other species. The lower amount of sulphates found in *S. longicruris* might be explained by the presence of unsulphated fucoses in the linear part of the polysaccharides which was observed in the case of *Laminaria brasiliensis* (Pereira, Mulloy, & Mourão, 1999). Also, the difference between fraction A and B for *S. longicruris* may be linked to the branching of the polysaccharide; branched zone containing sulphated fucose leading to the increase of the amount of sulphate.

Other researches have been performed on the same seaweed species (Table 5). The fucoidan extracted from *F. vesiculosus* contained around 48% of neutral sugars, 9% of uronic acids and 12% of sulphates (Rupérez et al., 2002). Others measured 37% of neutral sugars, 17% of uronic acids and 39% of sulphates for an acidic extract of fucoidan (Mabeau et al., 1990). Since the analysed material was harvested at different places and season periods, the chemical composition is likely to vary. Therefore, all extractions and analytical protocols are different. For *A. nodosum*, fucoidan contained approximately 31.3% of neutral sugars, 5.7% of uronic acids and 26.1% of sulphates (Nardella et al., 1996) which are all higher than what was observed in this study. Since, the extraction procedure is not fully described, the difference might be attributed to variation in the extraction protocol or the method used to determine the chemical composition. For *S. longicruris*, few researches have been conducted. However, the seasonal variation of fucoidan and other polysaccharides has been studied (Souchet, 2004). Two samples were analysed in May and the average results were compared with those from this study; 12.5% and 3.8% of neutral sugars and uronic acids, respectively. These results are not in good agreement with those obtained from this research. Since the extraction protocol was slightly different, it is possible that we may have modified the fucoidan structure causing variation among the

Table 5
Comparative results[a] for fucoidan from various seaweed species

	Laminaria longicruris[b]	*Fucus vesiculosus*[c]	*Fucus vesiculosus*[d]	*Ascophyllum nodosum*[e]
Total sugars (%)	12.5[f]	48.4[g]	37[h]	31.3[h]
Uronic acids (%)	3.8[i]	8.8[j]	17[k]	5.7[l]
Sulphate (%)	nd	11.5[m]	39[n]	26.1[n]

[a] All results are presented on a dry weight basis; nd, not determined value.
[b] Data from Souchet, 2004. This seaweed species is now named *Saccharina longicruris*.
[c] Data from Rupérez et al., 2002.
[d] Data from Mabeau et al., 1990.
[e] Data from Nardella et al., 1996.
All methods are described in their respective article references: [f]Phenol–sulphuric acid method; [g]Anthrone method; [h]Cysteine–H_2SO_4 method; [i]Blumenkrantz method; [j]Method of Scott; [k]*m*-Hydroxydiphenyl–sulphuric method; [l]Carbazole–H_2SO_4 method; [m]Gelatine–barium chloride turbidimetric method; [n]Elemental analysis method.

Table 6
Global composition of alginate

Species	Total sugar (%)	Uronic acids (%)	Molecular weight (kDa)
Ascophyllum nodosum	nd ± nd	a 32.3 ± 2.3	a 177.3
Fucus vesiculosus	nd ± nd	b 29.6 ± 2.4	a 154.9
Saccharina longicruris	nd ± nd	c 23.6 ± 2.9	a 106.6

nd. Alginates are only partially hydrolysed; no tests were performed on those fractions. Column with the same letter are not significantly different at $p < 0.05$.

results. The results obtained with fucoidan for fraction B were compared with other researches. Results have shown that the extraction method as well as the chemical method, the harvest period and the location did have an influence on the structural characteristics of fucoidan. Other researches have reported such variations (Painter, 1983; Percival & McDowell, 1967).

Surprisingly, the molecular weights determined for fractions A and B were very different among all species even within the same seaweed sources except for *S. longicruris*. Fractions A and B from *A. nodosum* revealed molecular weights of 417 and 1323 kDa and of 529 and 877 kDa for *F. vesiculosus*, respectively. The molecular weight for fraction A of *S. longicruris* was around 454 kDa and fraction B revealed a molecular weight of 576 kDa. No alginate fragments were present in these fractions which did not induce the higher molecular weight of fraction B (confirmed by HPAEC). In all cases, the extraction of the fraction A was realised in water. Those fractions contained small molecular weight polysaccharide. Kloareg and Quatrano (1988) have reported that fucoidan was located in the cell walls of the seaweed. Fucoidans are either or both free and part of an acid-labile supramolecular complex with ascophyllan and are progressively secreted into the intercellular matrix (Kloareg & Quatrano, 1988). It is logical that in the water extract, small molecular weight polysaccharide like those found in fraction A of *A. nodosum*, *F. vesiculosus* and *S. longicruris* would be liberated easily.

The molecular weight found in fraction B of *S. longicruris* is considerably small compared to the other fraction B from *A. nodosum* and *F. vesiculosus*. This result may be explained by a precipitation problem while dialysing the fucoidan solution from *S. longicruris*. A precipitate was found at the end of the dialysis process. The precipitate was not soluble in aqueous solution neither in acidic or alkaline solution. The proportion of insoluble fucoidan was small and might contained high molecular weight fucoidan because this pellet revealed the presence of neutral sugars when analysed by phenol–sulphuric acid method.

It is obvious that the fucoidan extracted from *S. longicruris* is different from the other seaweeds according to their composition in uronic acids and sulphates. Even if there are similarities in molecular weight for *S. longicruris*, their structures may be different (branching, chain length, sulphate position among the chains) between fraction A and B. Thus, no molecular weight reference for *S. longicruris* polysaccharides is available at the present time and does not allow comparison.

3.3. Sodium alginate

Uronic acids, molecular weight and MM/GG ratio were determined on the sodium alginate samples (Table 6 and 7). Total sugar analyses were not performed because the samples were only partially hydrolyzed. There were important differences observed between species. *A. nodosum* showed a higher proportion of uronic acids and molecular weight than *F. vesiculosus* and *S. longicruris*. This indicates important differences in the structural features of alginate from the different seaweeds species, such as longer chain length. Since they were all extracted following the same protocol, the extraction process could not be responsible for this variation between the results. The harvest region, on the other hand, may be responsible. Waves, sea current, light available for photosynthesis as well as water temperature are just a few of the many factors that can influence polysaccharides and their structure (Percival & McDowell, 1967).

The molecular weights of alginate extracted from *A. nodosum*, *F. vesiculosus* and *S. longicruris* ranged between 106.6 and 177.3 kDa (Table 6). The molecular weights of laboratory extract alginate obtained from *L. digitata* and *L. hyperborea* were 544 and 940 kDa, respectively (Moe et al., 1995). These values are considerably higher then those obtained with the alginates in this study. However, the results from Turquois and Gloria (2000) are more in line with our results with a molecular weight between 115.0 and 321.7 kDa for commercial alginates. These molecular weights were related to interesting application for these alginates.

Single monomers and blocks were determined by H NMR (Table 7). The results showed important differences between species; *F. vesiculosus* was significantly different than the two others in terms of the proportion of F_M and F_G. Surprisingly, *S. longicruris* had a low proportion of F_{MM}. Since *S. longicruris* had the lowest amount of mannuronic acids and highest amount of guluronic acids with intermediate GG blocks amount, it can be expected that the MM block proportion would be inferior with a more important proportion of MG or GM blocks. The

Table 7
H NMR results for alginate samples

Species	F_M	F_G	F_{MM}	$F_{GM,MG}$	F_{GG}	n
Ascophyllum nodosum	b 0.46	a 0.54	a 0.28	b 0.18	a 0.36	0.72
Fucus vesiculosus	a 0.59	b 0.41	a 0.39	b 0.19	b 0.22	0.80
Saccharina longicruris	b 0.41	a 0.59	b 0.07	a 0.34	b 0.25	1.41

Column with the same letter are not significantly different at $p < 0.05$.

importance of monomers and block proportion is essential to the understanding of gelling process and characteristics of alginate gels. A high proportion of GG blocks lead to a rigid and brittle gel where MM blocks will induce a soft and elastic gel and MG blocks will give flexibility (Draget et al., 2000). The proportion of block obtained differs with the literature. *L. longicruris* (now named *Saccharina longicruris*) showed a low proportion of MM blocks and intermediate results for GG blocks ($F_{MM} = 0.07$; $F_{GG} = 0.25$) where other research have found an F_{MM} of 0.57 and F_{GG} of 0.23 (Moe et al., 1995). Craigie, Morris, Rees, and Thom (1984) had also studied *L. longicruris* (now named *Saccharina longicruris*) block proportion by circular dichroism. They have found various block proportion according to seaweed body part used. MM and GG blocks were found to be approximately 40% and 3% in the algae's blade and 27% and 18% in the stipe, respectively. Different harvest locations were also studied for *L. longicruris* (now called *Saccharina longicruris*) and the results show different block amounts depending on the harvest site (Craigie et al., 1984). The scenario is the same for *A. nodosum* where literature results demonstrate an F_{MM} of 0.84 and F_{GG} of 0.04 in young algae and F_{MM} of 0.44 and F_{GG} of 0.16 in old tissue (Moe et al., 1995) which can be compared to an F_{MM} of 0.28 and F_{GG} of 0.36 from this study. Since the extraction process was probably not the same, such block ratio variation is not surprising. Once again, the harvest period and location will also have an influence on the results, not to mention the part of the seaweed that is used.

4. Conclusion

After analysis, the three seaweed species studied are quite different from each other. Their polysaccharides have similarities, but at the same time demonstrate important differences on the base of their molecular weight and sulphate content. More structural investigation is needed on these polysaccharides. Laminaran is a difficult polysaccharide to extract considering the fact that a separation from fucoidan is required which leads to a great loss of the polysaccharide. Thus, further analysis is needed to determine its molecular weight and confirm the presence of glucose as the only monosaccharide. Fucoidan is known to have various biological activities. More investigations are necessary to determine the general chain length and the branching of fucoidan. Since low molecular weight fucans are usually reported to have biological activities, fraction A and B from *S. longicruris* might be the most interesting seaweed in Quebec. Additional research is necessary to improve the structural comprehension of this polysaccharide because it has not yet been documented. Further analysis has to be performed on alginate. Its chain length has to be determined to understand the results published in this paper (molecular weight and uronic acids). All of the differences reported on each polysaccharide from the different sources will likely have varying influences on flow behaviour. This functionality is dependent on the structure, the seaweed species, the extraction protocol and the harvest period. The study of these characteristics for fucoidan and alginate is particularly interesting because few researches have been able to identify the link between the structure and the rheological behaviour of these polysaccharides.

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