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The extraction, structure, and gelling properties of hybrid galactan from the red alga *Furcellaria lumbricalis* (Baltic Sea, Estonia)

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Abstract The structure and composition of galactan from *Furcellaria lumbricalis* (furcellaran) were investigated in connection with rheological specificities, gel structure, and extraction conditions. The polysaccharide was characterized by ^{13}C nuclear magnetic resonance (^{13}C-NMR) and Fourier transform infrared spectroscopy, inductively coupled plasma-optical emission spectrometry, electrothermal atomization atomic absorption spectrometry, and gel permeation chromatography methods. The microstructure of polymer gels was studied using a cryofixation method in combination with freeze-drying and scanning electron microscopy (SEM) techniques. The undersulfated furcellaran backbone consists mainly of 3,6-anhydro-D-galactose (28.5–30.1%) and galactose residues, the latter being partly sulfated in positions 4 and 6, which give rise to some specific properties of the gel. Also, residues of 6-*O*-methyl-D-galactose as a minor component are found to be present. The water-extracted furcellaran with the average molecular weight about 290 kDa is rich in nitrogen, calcium, magnesium, and potassium, while the sodium content is rather low. The low sulfur content (5.3%) and ^{13}C-NMR spectra refer to an undersulfated nature of this galactan. The extraction of seaweeds in low concentration alkaline solutions (instead of water) leads to a significant increase of the minimum size of the galactan particles and the value of gel strength (more than 12 times for Rb-containing gels). The properties of the gel are dependent on specific tentacle-like structure units present in furcellaran gels established by a high-resolution SEM.

Keywords Furcellaran · Carrageenan · *Furcellaria lumbricalis* · Gel permeation chromatography · FTIR spectroscopy · NMR spectroscopy

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Introduction

Historically, the galactan mixture from *Furcellaria lumbricalis* (Hudson) J.V. Lamouroux ("furcellaran") was one of the first hydrocolloids to have been industrially produced from red algae. The first attempts to manufacture furcellaran were made in Denmark as early as 1917 (Christiansen 1959). For many decades, the product with specific properties was effectively employed by European food manufacturers (Bird et al. 1991; Briand 1991).

The red algal species *F. lumbricalis* (formerly named *F. fastigiata*) is of wide occurrence in both the eastern and western North Atlantic, from the Barents Sea to the Bay of Biscay, and particularly in the brackish waters of the Baltic Sea. In spite of that, the usable quantities of this seaweed species are usually too dispersed for industrial exploitation. The most abundant community (as a dense voluminous stratum) of *F. lumbricalis* can be found in Estonian waters

in the central Baltic Sea region which is known as the Kassari algal stratum (Martin et al. 1996; Truus et al. 1997). The shape and total biomass (about 140,000 tons in 2005) of the stratum vary slightly from year to year (Martin et al. 2006). Both dominant seaweed species of Kassari algal stratum (*F. lumbricalis* and *Coccotylus truncatus*) are in a loose-lying form due to the sandy bottom of the sea and the lack of hard substrate.

There are some specific aspects in the chemical composition of *F. lumbricalis*. Due to the high concentration of proteinic pigments, this seaweed species is relatively rich in nitrogen (usually varying from 1.1% to 4.8% of dry mass) among the Rhodophyta (Bird et al. 1991). Also, its iodine content has been reported to be moderately high. *F. lumbricalis* is one of the few marine algal species producing histamine (Barwell 1989). By the composition of polysaccharides, *F. lumbricalis* belongs to the family of commercially important carrageenophytes, the red algae producing carrageenans.

Carrageenans are sulfated polysaccharides having usually a linear backbone built up of alternating 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose residues. A substantial part (or all) the α-galactopyranose residues may exist in the form of a 3,6-anhydro derivative. Various hydroxy groups may be substituted with ester sulfate, methyl groups, pyruvic acid acetal, and sometimes, additional monosaccharide residues (Painter 1983; Usov 1998).

By the general primary structure of its major component, furcellaran belongs to the κ family of carrageenans (Fig. 1). The composition of furcellaran has been thoroughly studied using ^{13}C nuclear magnetic resonance (^{13}C-NMR) and ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectroscopy (Knutsen et al. 1990; Knutsen 1992; Van de Velde et al. 2002). The first investigations about the polysaccharide structures from the Kassari algal stratum were performed in 1976–1981 (Yarotsky et al. 1978; Usov and Arkhipova 1981)

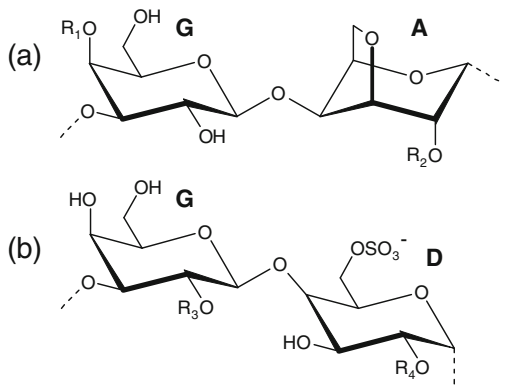

Fig. 1 Disaccharide repeating units of some carrageenans. *a* κ-Carrageenan (R_1=SO_3^-, R_2=H), β-carrageenan (R_1=R_2=H), ι-carrageenan (R_1=R_2=SO_3^-); *b* λ-carrageenan (R_3=R_4=SO_3^-), γ-carrageenan (R_3=R_4=H)

and later corrected (Truus et al. 1997). The approximate content and localization of methyl groups in the polysaccharides from *F. lumbricalis* have also been determined. Among methyl derivatives, 6-*O*-methylgalactose is the most abundant (2.0–2.7%), while 2-*O*-methyl-3,6-anhydrogalactose can be found in lower amounts (Penman and Rees 1973; Truus et al. 1997). Furcellaran has been characterized as a hybrid κ/β-carrageenan complex containing a small amount of ω-carrageenan (Craigie 1990). Also, α- and ι-carrageenans have been tentatively reported to be found in preparations of different origin (Yarotsky et al. 1978; Usov and Arkhipova 1981; Bird et al. 1991). It is generally believed that furcellaran contains different carrageenan segments along the same hybrid macromolecular chain with κ-carrageenan predomination (Knutsen and Grasdalen 1987).

The hybrid nature at the molecular level is responsible for changes in both rheological and conformational properties of carrageenans compared with those of their homopolymeric counterparts. This becomes evident from X-ray diffraction studies of the hybrid polysaccharides. The secondary structure of furcellaran is similar to that of stoichiometrically sulfated κ-carrageenans (Fig. 1 a), a double helix with a period of 2.5±0.02 nm, but undersulfation in the furcellaran molecule causes an axial shift of 0.83 nm from the exact half-stagger position (Cairns et al. 1991).

The degree of polymerization of algal galactans varies significantly and is highly dependent on the conditions of extraction. The average molecular weight of furcellaran has been reported to be around 500 kDa (Yu et al. 2007), which is typical of a wide range of gelling carrageenans. According to literature, the corresponding figure for κ-carrageenan varies from 300 to 1,900 kDa (Abada et al. 2004; Spichtig and Austin 2008), but values as high as 8,000 kDa have also been reported (Montolalu et al. 2008).

An important characteristic of carrageenans discussed here is their gel-forming ability, i.e., the ability to form well-ordered spatial structures during the cooling of their hot polymeric solution. Various aspects of gel structure and gelling mechanism have been studied from the early, classical works of Rees (1969, 1972), Morris et al. (1980), and others. Gel formation in an aqueous solution is a complex process that depends not only on polysaccharide structure, polymer concentration, and temperature, but also on the presence of co- and counter-ions (Morris et al. 1980; Meunier et al. 2001). Certain cations (typically K^+ for κ-carrageenan and furcellaran, and Ca^{2+} for ι-carrageenan) have been found to induce conformational changes in the polymer structure with an initial coil-to-helix transition, which may be followed by the aggregation of these helices to form a gel (Rochas and Rinaudo 1984; Paoletti et al. 1985). The dependence of the gelling properties upon an ionic factor has widely been investigated, discussed, and

reviewed (Borgström 1997; Rinaudo 2005; Piculell 2006). Also, various other aspects of gel properties have been studied during the last decade (Michel et al. 1997; Mangione et al. 2005). Recently, sol–gel transition processes of furcellaran were studied using a cryofixation method in combination with freeze-drying and scanning electron microscopy (SEM) techniques (Tuvikene et al. 2008). Also, the thermal stability of furcellaran (Friedenthal et al. 2000), its inorganic part composition by using neutron activation analysis (Vaher et al. 1998), and extraction dynamics (Tuvikene et al. 2006) as well as other aspects related to this polysaccharide (Mikulich et al. 1988; Tanner et al. 1990; Yu et al. 2007) have been investigated.

Hence, the natural furcellaran matrix has been characterized from various aspects in the literature. It is evident that some contradictions and shortages exist in results, particularly concerning the presence of the hybrid structural fragments and molecular weight values. The aim of this work is to determine major parameters such as structural and compositional characteristics, molecular weight distribution, and crucial gel properties for furcellaran of the Baltic Sea origin.

Materials and methods

The algae (unattached *Furcellaria lumbricalis*) were collected from Kassari Bay (58°41.60′ N; 22°52.00′ E, Baltic Sea, Estonia) at the end July 2005, using SCUBA down to a depth of 8 m. The attached form of *F. lumbricalis* was obtained from the coastal waters of Tallinn (59°27.72′ N; 24°34.44′ E, Baltic Sea, Estonia) in July 2006. If not specified otherwise, the furcellaran originated from the unattached form of *F. lumbricalis*. The *Eucheuma cottonii* samples were harvested near the southern coast of Bali (Indian Ocean, Indonesia) in June 2006. The samples were thoroughly washed with tap water, then with distilled water and dried at room temperature.

Commercial κ-carrageenan preparations were purchased from Sigma and Fluka. LiCl, NaOH, and KOH were ACS reagents from Fluka; RbCl, CsCl, RbOH, and CsOH originated from Sigma-Aldrich. Dextran markers for gel permeation chromatography analyses were from Fluka.

Extraction, modification, and precipitation

High temperature extraction/modification

The air-dry algal sample was refluxed in a 33-fold mass of the extracting medium (distilled water, KOH, or NaOH solutions of various concentrations); the time of extraction was counted in the boiling state. The hot extract was filtered through a porous glass filter (porosity no. 2) into cold (7°C) isopropanol (99.9% *v*/*v*, 3-fold volume per extract) leading to the precipitation of polysaccharides. The galactans precipitated were separated from the alcohol–water mixture by filtration through a porous glass filter (porosity no. 3) and washed with cold (7°C) isopropanol. The isolated polysaccharide mixture was dried to a constant weight in a drying oven (60°C, 2 days) and then milled.

If needed, additional purification and homogenization were carried out by precipitation of furcellaran in isopropanol (3-fold volume per 2% polymer solution), after that the preparation was dried again at 60°C for 2 days and then milled.

Long-term room temperature modification

The air-dry algal mixture was stored in a 33-fold mass of an alkali solution (0.4 or 0.6 M KOH) for 7 days at room temperature. After that, the algal mass was separated from the solution, washed thoroughly with tap water, then with distilled water, and dried at room temperature. The treated algal mixture was used for the extraction of carrageenans (in a 33-fold mass of boiling water).

Gel testing

For gel strength assessments, a suitable gel tester equipped with a hemispherically tipped plunger (an effective cross-section area of 1 cm^2) was constructed. The gel strength measurements were made in triplicate for 1–2% gels (*w*/*w*) formed by dissolving the dry galactan in hot water (or in a salt solution) after gelling in an air thermostat at 20°C for 4 h. The cylindrical samples were 35 mm in diameter and 35 mm in height. The force needed to rupture the gel by the plunger was expressed in grams per square centimeter; the constant increase of the stress to the gel surface by addition of mass 350 g min^{-1} was achieved.

The melting temperature of the 1.5% gel aged at 20°C for 4 h was determined as the temperature at which a 4-mm tin bead (weight 0.22 g) fell down to the bottom of a capped test tube (9×100 mm) during slow (1.0°C min^{-1}) heating on a water bath. The tube was then cooled (1.0°C min^{-1}) and turned horizontally every minute without removing it from the water bath. The gel setting temperature was determined as the temperature at which the gel would no longer flow. All determinations of melting and gelling points were performed in duplicate.

Syneresis was measured by a centrifugation test by using a Hettich ROTINA 38R centrifuge. The centrifuge tubes were filled with a hot 1.5% (*w*/*w*) galactan solution, allowed to set for 2 h, then closed and stored at 25°C for 7 days. After storage, they were centrifuged at 4,500 rpm for 15 min. Then, the free water was separated, weighed, and expressed as a percentage of the total water content.

Chemical analysis

3,6-Anhydrogalactose content was determined colorimetrically using a resorcinol–acetal method (Yaphe and Arsenault 1965) and fructose as a standard sugar. The total carbohydrate content was estimated using the phenol–sulfuric acid method (Dubois et al. 1956) without previous hydrolysis of the polysaccharide. The nitrogen content was measured by the Kjeldahl procedure. The other elements were determined using the inductively coupled plasma-optical emission spectrometry (ICP-OES) method, except cadmium and arsenic, which were measured by electrothermal atomization atomic absorption spectrometry.

Gel permeation chromatography

Gel permeation chromatography (GPC) of carrageenans was performed on a chromatograph equipped with a PerkinElmer Series 200 pump, a Knauer Smartline 2300 refractive index detector, a Knauer Smartline column thermostat, and two Shodex OHpak SB-806MHQ columns in series. Elution was carried out using a 0.1 M $NaNO_3$ solution as the mobile phase at a flow rate of 0.8 mL min^{-1}. The temperature of the columns was maintained at 60.0°C. A calibration curve was constructed using ten dextran standards (668, 410, 273, 148, 80.9, 48.6, 23.8, 11.6, 5.2, and 1.3 kDa), and the elution volume was corrected to the internal marker of ethylene glycol (0.01% in sample) at 22.89 mL. The equation was log M_w=0.02 x^2 − 1.3503 x+22.361 (M_w, average molecular weight; x, elution volume; R^2=0.9987). The carrageenan concentration used was 0.07% and the sampling volume was 100 μL.

To obtain more reliable results, the galactan samples were dissolved in the same solvent used as an eluent in the GPC system. For better solubilization, the sols were kept overnight under constant shaking at 35°C. The final solubilization was assured by heating the polymeric solutions in a boiling water bath under vigorous stirring for at least 10 min. Then, the hot (60°C) sol was filtered through a 0.45-μm membrane (Spartan 30/0.45RC), allowed to cool, and injected into the HPLC system.

Infrared spectroscopy

The Fourier transform infrared (FTIR) spectra of carrageenan samples were scanned using a PerkinElmer FTIR System Spectrum BX spectrometer (12 scans per spectrum; nominal resolution 4 cm^{-1}) from thin (0.015 mm) films obtained by a slow evaporation of 1% solutions on the surface of the Petri dish. The spectra were recorded in the 4,000–370 cm^{-1} region.

Nuclear magnetic resonance spectroscopy

Proton-decoupled ^{13}C-NMR spectroscopic analyses were carried out using a Bruker AVANCE III spectrometer operating at 800 MHz. The spectra from a 2% carrageenan solution in D_2O (*w*/*w*) were obtained at 50°C, and maximum 100,000 transients were collected before the Fourier transform. The chemical shifts were converted to a DSS scale on the basis of the C-6 signal from the galactose subunit having a constant value of 63.49 ppm for these carrageenans (Van de Velde et al. 2004).

Scanning electron microscopy investigations

Scanning electron microscopy was carried out using a high-resolution LEO Supra 35 electron microscope equipped with a Röntec EDX XFlash 3001 detector and a Thermo Noran Maxray ER Parallel Beam spectrometer. The samples were prepared by inserting preheated (≈90°C) stainless steel or copper capillary tubes (inner diameter 2.0 mm and 1.2 mm, respectively, length 60 mm) into a hot (≈90°C) 1.5% or 2.0% galactan solution (*w*/*w*) and allowing them to fill up. After gelling at 20°C for 4 h, the filled tubes were rapidly frozen in liquid nitrogen to produce small (5 mm in length) open-ended tubes filled with the frozen gel and freeze-dried under vacuum at −60°C. The formed cryogenic gel surfaces were sputter-coated with platinum of about 1 nm thickness using a Polaron High Resolution Sputter Coater SC7640 and examined under an acceleration voltage up to 2.47 kV.

Results and discussion

Yields and extraction dynamics

The yield of furcellaran depends greatly on the conditions of extraction and the raw material used. There is a considerable difference in extracting yield between the attached and unattached form of *F. lumbricalis*. While the unattached form of this seaweed species yields only 19% of furcellaran during a 4-h extraction in pure water, the attached one affords 32% under the same conditions. According to literature, high polysaccharide yields from the attached form of *F. lumbricalis* are not rare and can be around 50% (Knutsen and Grasdalen 1987; Yu et al. 2007). The lower yields in the case of the unattached form are apparently caused by the thallus morphology, i.e., by the higher amount of the cortex due to thinner thallus filaments resulting in a lower total content of galactan.

The alkali present in the extracting medium affects notably the course of extraction of carrageenan from the algal material. This was observed already at very low

concentrations of alkali. The yield of carrageenan in the presence of different alkali metal hydroxides in the extracting medium (0.02 mol L^{-1}) increased in the row Na^+, K^+, Rb^+, and Cs^+ with values of 19%, 23%, 25%, and 28%, respectively (during a 4-h extraction). During a 3-h extraction, already 90% of the total yield (compared to a 9-h extraction) was achieved if the concentration of alkali in the extracting medium was low (0.02 mol L^{-1}).

Chemical composition

The composition of furcellaran depends greatly on the conditions of extraction. The slight increase of the 3,6-anhydrogalactose content (from 28.5% to 30.1%) during the alkaline treatment of furcellaran is indicative of the presence of small amounts of alkali-labile precursor structures. This was also confirmed by ^{13}C-NMR. The total carbohydrate (with sulfate) content of furcellaran was estimated at 90±3%. In addition to various saccharic components, furcellaran has a diverse inorganic part in its composition (Table 1).

The sulfur content of furcellaran is relatively low (on an average 5.3% of S or 15.9% of SO_4^{2-}; there is approximately one sulfate group per every three monomer residues) for κ-type carrageenan. This can be attributed to the presence of unsubstituted galactose residues (β-carrageenan fragments). The sulfur content of the native water-extracted galactan from *E. cottonii* is almost theoretical (8.3%) for κ-carrageenan.

The mineral composition of carrageenans depends greatly on the ionic binding between certain metallic cations and on the negative charge of ester sulfate groups. Considering the sulfur content of furcellaran, the extracting media containing KOH at a concentration of as low as 0.02 mol L^{-1} already saturate the galactan matrix with K^+ ions up to 80% during the hot alkali treatment. Furcellaran is characterized by a notably low affinity for Na^+ ions. Considering the element content in seaweed and low extraction yield, no enrichment of the galactan matrix with Na^+ ions occurs as is common for Mg^{2+}, Ca^{2+}, and especially for K^+ ions (Table 1). Compared to galactan from *E. cottonii* and commercial κ-carrageenan preparation (Sigma), the cadmium level in furcellaran was 4.2 to 7.6 times lower. On the other hand, the galactan matrix of furcellaran showed a high affinity for manganese with more than 60 times higher content than in the commercial preparation. The high iron content of furcellaran (5.4 times that of the commercial preparation of Sigma) was attributable to the high concentration of this element in *F. lumbricalis*, that was nearly 80 times that observed in *E. cottonii*.

The hot alkali extraction notably reduced the chemical heterogeneity of furcellaran. Although the chemical modification of the preparation in the presence of K^+ ions did not affect notably its sodium content, the amount of bound Mg^{2+} and Ca^{2+} ions decreased more than twice in comparison with that observed during the treatment of the native water-extracted furcellaran. The long-term room temperature modification of furcellaran by alkali and saturation with K^+ ions increased significantly the mobility of Ca^{2+} ions, resulting in a nearly 2-fold enrichment of the galactan matrix with calcium, compared to the algal raw material. Surprisingly, no substantial rise in potassium concentration was observed. At that, more than 2 times higher gel strength values for treated furcellaran were evidently caused by the bound Ca^{2+} ions.

The nitrogen content of the galactan from *F. lumbricalis* is relatively high, being indicative of the high glycoprotein content that remained in the preparation (Table 1). These

Table 1 Content of some elements in algae and carrageenan samples

Preparation	Element content, %							Element content, mg.kg^{-1}					
	Na	K	Mg	Ca	N	P	S	Mn	Fe	Cu	Mo	Cd	As
F. lumbricalis[a]	0.050	0.32	0.76	1.73	4.18	0.23	4.09	x	2465	20	x	x	6.25
Furcellaran[b]	0.045	0.62	0.87	1.82	0.70	0.07	5.22	x	903	10	x	x	0.71
Furcellaran[c]	0.049	4.98	0.24	0.83	0.66	0.03	5.37	x	481	10	x	x	0.30
Furcellaran[d]	0.040	0.72	0.55	3.32	0.76	0.02	5.26	592	509	10	<0.1	0.015	0.69
E. cottonii[a]	1.04	3.40	0.66	0.89	1.32	0.08	5.95	4.22	31.8	0.6	0.22	0.902	11.8
E. cottonii galactan[b]	1.10	4.11	0.75	0.94	0.32	0.02	7.50	7.27	50.2	3.1	0.12	0.063	0.20
κ-Carrageenan, Sigma	0.59	6.15	0.16	2.71	0.10	0.01	7.19	9.48	166	10	0.20	0.114	0.68

x not measured

[a] Algae washed thoroughly with tap water and distilled water

[b] Extracted 4 h in water, additionally purified by alcohol precipitation

[c] Extracted 4 h in 0.02 M KOH, additionally purified by alcohol precipitation

[d] Algae treated with 0.4 M KOH solution for 7 days, extracted 4 h in water

proteinic pigments cannot be separated from the polymer matrix by ordinary techniques (re-precipitation, gel filtration, electrophoresis) as has been reported earlier (Krasil'nikova and Medvedeva 1975). It has been established that the amount of the galactan-bound nitrogen does not depend on extraction conditions. Compared to furcellaran, the nitrogen content of the galactan from *E. cottonii* was more than twice lower, being closer to that of the commercial κ-carrageenan preparation of Sigma.

Structure

The FTIR spectra of the native and alkali-extracted polysaccharide preparations are shown in Fig. 2. All the galactans under study exhibited absorption bands at 1,375 and 1,248 cm^{-1}, which is indicative of the sulfate ester substitution (Prado-Fernández et al. 2003). The intense signal at 930 cm^{-1} is attributable to 3,6-anhydrogalactose (Pereira et al. 2009). As expected, for κ-carrageenan, the sharp absorption band at 847 cm^{-1} is indicative of an axial sulfate ester at the *O*-4 of a 3-linked galactose. In the spectrum of furcellaran, the weak absorption band at 892 cm^{-1} implies the presence of unsubstituted galactose residues, which is characteristic of both α- and β-carrageenans. The absence of an absorption band of the sulfate group at 804 cm^{-1} at the *O*-2 of 3,6-anhydrogalactose from α-carrageenan suggests the presence of β-carrageenan. Although both the galactan from *E. cottonii* and the commercial κ-carrageenan preparation exhibited a weak absorption band at 804 cm^{-1}, this is quite common for the commercial κ-carrageenan preparation, usually indicating the presence of a low amount of 3,6-anhydrogalactose-2-sulfate from ι-carrageenan.

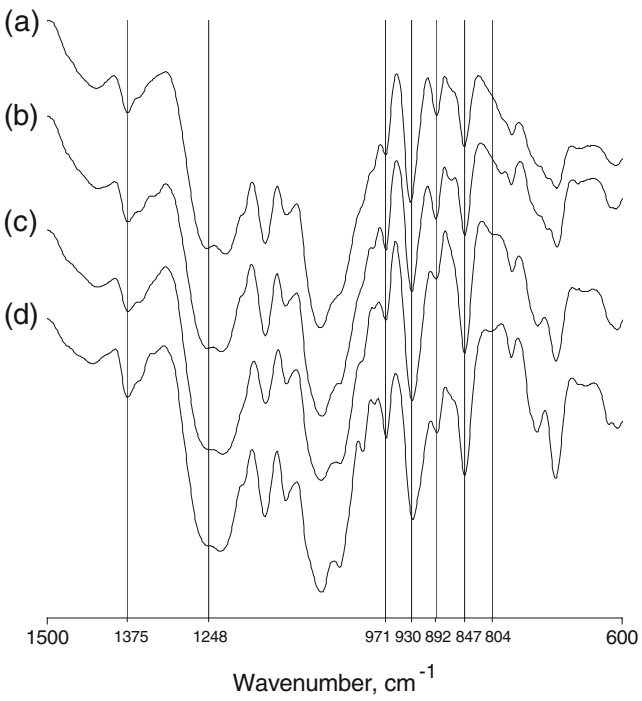

Fig. 2 FTIR spectra of *a* furcellaran extracted 4 h in H_2O, *b* furcellaran extracted 4 h in 0.02 M KOH, *c* galactan from *E. cottonii*, extracted 4 h in H_2O, *d* κ-carrageenan (Sigma)

The alkaline treatment only slightly modified the FTIR spectrum of furcellaran. The spectrum of the native furcellaran exhibited a shoulder near 867 cm^{-1} that can be attributed to a sulfate group at the *O*-6 of the 4-linked galactose. In this region, the small difference in spectra between the native and alkali-extracted furcellaran, implying the presence of alkali-labile μ- or γ-carrageenans, was evident. The alkaline treatment also favored absorption at 971 cm^{-1}, which has previously been observed for the pyruvated galactans from some *Erythroclonium* and *Claviclonium* species (Chiovitti et al. 1997, 2004). The absorbance of furcellaran at 847 and 1,248 cm^{-1} was substantially weaker than that of the commercial κ-carrageenan. This implies the lower sulfur content as has been proven by ICP-OES studies and is in accordance with the presence of characteristic resonance signals in the ^{13}C-NMR spectra corresponding to β-carrageenan residues.

The ^{13}C-NMR spectra of galactans investigated are shown in Fig. 3. The main components of the polysaccharide from *F. lumbricalis* were β-D-galactose-4-sulfate, unsubstituted β-D-galactose, and 3,6-anhydro-α-D-galactose, indicating the κ/β-carrageenan backbone. Similar hybrid κ/β-carrageenan blends (undersulfated κ-carrageenans) have been isolated from the Far-Eastern seaweeds *Tichocarpus crinitus* (Yermak et al. 1999; Barabanova et al. 2005) and *Eucheuma gelatinae* (Greer and Yaphe 1984). The values of chemical shifts of basic carbon signals of the galactans investigated are presented in Table 2. These are in accordance with literature data (Pereira et al. 2003; Van de Velde et al. 2004).

As a minor component, the alkali-labile α-D-galactose-6-sulfate from γ-carrageenan, a biological precursor to β-carrageenan (Greer and Yaphe 1984), is also present in furcellaran. This was confirmed by detectable signals for G-1 at 106.9 ppm and for D-1 at 98.2 ppm although resonances for D-6 around 70.4 ppm and for G-4 around 67.9 ppm were poorly resolved due to the overlapping noise in this region of the spectra. The precursor structure appeared to be present in galactans from the both studied *F. lumbricalis* forms in almost equal amounts. Much higher γ-carrageenan contents have been found in the polysaccharides from the attached *F. lumbricalis* growing in more saline environments (Knutsen and Grasdalen 1987).

In the case of furcellaran from the attached form of *F. lumbricalis*, the presence of β-D-galactose-6-sulfate from ω-carrageenan was confirmed by characteristic signals for G-4, G-5, and G-6 at 68.10, 75.10, and 69.40 ppm, respectively. Traces of ω-carrageenan segments are probably present also in the galactans from unattached form of

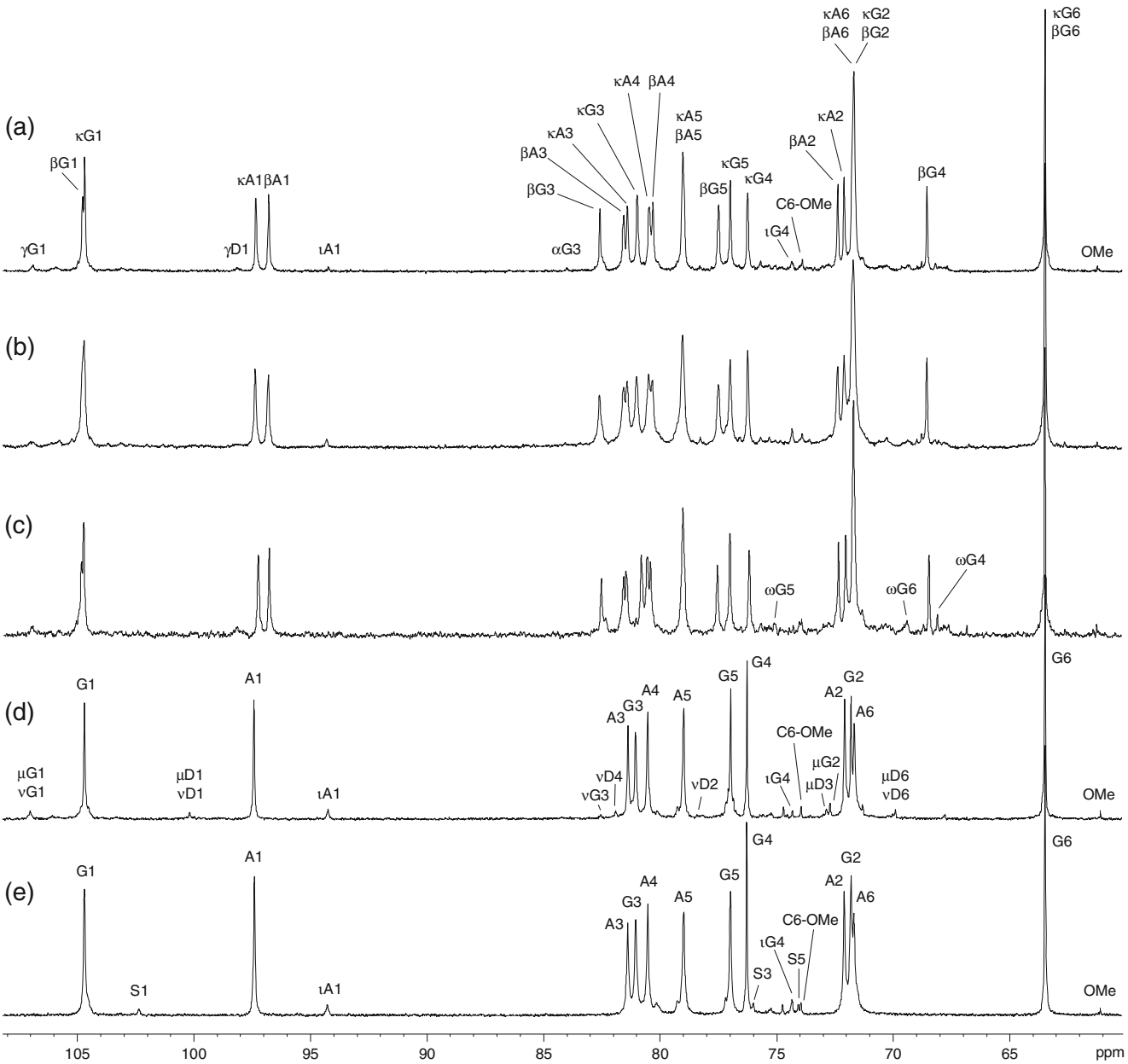

Fig. 3 ^{13}C-NMR spectra of carrageenan preparations recorded at 50° C. *a* Water-extracted furcellaran, 100,000 transients; *b* alkali-extracted furcellaran, 100,000 transients; *c* water-extracted furcellaran from the attached form of *F. lumbricalis*, 23,000 transients; *d* water-extracted galactan from *E. cottonii*, 61,000 transients; *e* κ-carrageenan (Sigma), 56,000 transients collected

the seaweed. A very weak signal at 84.0 ppm in the furcellaran sample may be attributed to the C-3 of an unsubstituted galactose from α-carrageenan. Also, traces of 3,6-anhydro-α-D-galactose-2-sulfate from ι-carrageenan were found, originating presumably from the minute amounts of *C. truncatus* (Usov and Shashkov 1985), another dominant seaweed species present in the Kassari algal stratum that forms hardly separable mixtures with *F. lumbricalis*.

The principal storage glucan of red algae, floridean starch, has been reported to remain in the crude furcellaran preparation in relatively high quantities (Knutsen and Grasdalen 1987) and could be readily detected by ^{13}C-NMR. Nevertheless, no characteristic bands were detected in the spectra of either the native or alkali-extracted furcellaran or the galactan from *E. cottonii*. The chemical shifts of glucose units of C-1, C-3, and C-5 at 102.36, 76.00, and 74.08 ppm, respectively, were clearly seen to be present in the commercial κ-carrageenan preparation (Sigma). The commercial κ-carrageenan preparations often contain low quantities of ι-carrageenan as impurity detectable in a characteristic band for A-1 at 94.3 ppm. The presence of ι-fragments was observed in both the commer-

Table 2 ^{13}C-NMR chemical shifts (ppm) for basic signals of carrageenan structures

Galactan	Unit	^{13}Carbon chemical shift					
		C-1	C-2	C-3	C-4	C-5	C-6
κ-Carrageenan	G	104.70	71.82	81.04	76.28	76.99	63.49
	A	97.41	72.11	81.40	80.53	78.99	71.69
E. cottonii galactan	G	104.69	71.81	81.05	76.28	76.98	63.49
	A	97.42	72.09	81.39	80.54	79.00	71.68
Water-extracted furcellaran	κG[a]	104.68	71.70	80.98	76.23	76.99	63.49
	κA[a]	97.34	72.11	81.42	80.46	79.01	71.70
	βG[b]	104.76	71.70	82.58	68.55	77.48	63.49
	βA[b]	96.79	72.39	81.55	80.32	79.01	71.70

[a] Corresponding units from κ-carrageenan

[b] Corresponding units from β-carrageenan

cial κ-carrageenan (Sigma) preparation and galactan from *E. cottonii*. Being common for κ-carrageenan, a small amount of 6-*O*-methyl-β-D-galactose residues was found in all galactan samples under study. In ^{13}C-NMR spectra, these residues give specific signals for OMe at 61.1, for the substituted C-6 at 74.0, and for the neighboring C-5 at 75.5 ppm. In the spectrum of *E. cottonii* galactan, weak resonance signals, which are indicative of an alkali-labile α-D-galactose-6-sulfate from μ-carrageenan, a biological precursor to κ-carrageenan (Anderson et al. 1968), were detected at 107.02, 100.18, 72.87, and 72.71 ppm attributable to G-1, D-1, D-3, and G-2, respectively. Also, a small amount of α-D-galactose-2,6-disulfate from ν-carrageenan was present as revealed by a characteristic signals for G-3 and D-4 at 82.57 and 81.91 ppm, respectively. The lack of a signal around 100.2 ppm in the furcellaran preparation implies the absence of μ- and ν-carrageenans, revealing that γ-carrageenan residues are the only precursor structures in this hybrid galactan.

The ^{13}C-NMR spectra of native and alkali-extracted furcellarans somewhat differ. In the case of the latter, the lower-resolution stems from the higher viscosity of the galactan. The spectrum of the native furcellaran demonstrates that the content of β-carrageenan is higher as seen from more intense bands at 96.79, 80.32, and 82.58 ppm, which correspond to A-1, A-4, and G-3, respectively. This is in accordance with the lower sulfur content of the native furcellaran measured by ICP-OES. The lower β-carrageenan content of the alkali-extracted preparation is not due to the simple conversion of other galactan residues to β-carrageenan segments or degradation of the κ-carrageenan part, but due to complex processes taking place during extraction and hydrolysis, and chemical changes occurring at both polysaccharide and seaweed levels in hot alkaline media. The same tendency can be seen in ^{13}C-NMR spectra of an earlier investigation concerned on furcellaran, where similar hot alkaline extraction was performed (Truus et al. 1997). Usually, the treatment of the native furcellaran with alkali slightly increases its β-carrageenan content due to the presence of alkali-labile α-D-galactose-6-sulfate residues from γ-carrageenan in the galactan matrix (Knutsen 1992).

Molecular weight distribution

Figure 4 shows the molecular weight distribution of the carrageenans under study. Using the sample solvent of the same composition as that of the GPC eluent (0.1 M $NaNO_3$) enabled interferences (broad negative peaks) to be reduced in the region of an internal ethylene glycol standard at 22.89 mL.

An average molecular weight (M_w) of the Baltic furcellaran (after a 4-h water extraction at 100°C) is about 290 kDa. According to literature data, this is the lowest value for furcellarans, indicating that extensive hydrolysis takes place during the 4-h water extraction of the polysaccharide probably because of the slightly acidic extracting medium. The treatment of the native preparation with weak alkali (1 h in a 0.02-M KOH solution at 100°C as a 0.1% sol) also induces the cleavage of polysaccharide chains, resulting in the final products with M_w values as low as 182 kDa. Compared to furcellaran, galactan from *E. cottonii* showed somewhat higher resistance to hydrolytic degradation during hot water extraction.

The M_w assessment of the alkali-extracted carrageenan is complicated due to its abundant inorganic part which induces the aggregating behavior of those galactans. The very low galactan concentration (0.07%) and high temperature (60°C) used in the analyses are believed to prevent conglomeration of "minimum size" molecular particles as much as possible. In the case of alkaline extraction of *F. lumbricalis* with 0.02 M KOH solution, the measured M_w exceeded the highest standard value used to construct the calibration curve and was estimated at over one million. This is comparable to the M_w values of the galactan from *E. cottonii* extracted under the same conditions. A similar degree of polymerization was also observed in the case of the commercial carrageenan preparations. The highest M_w

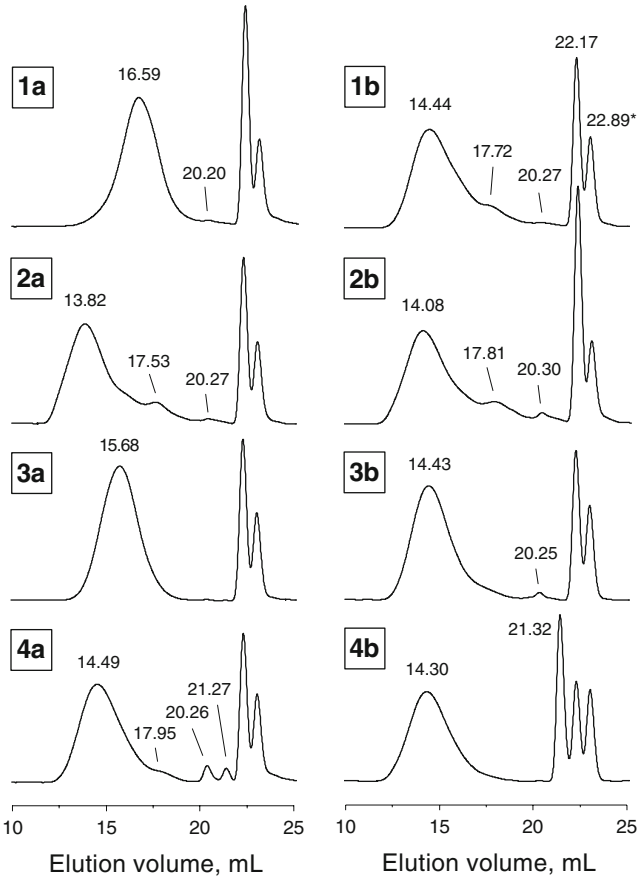

Fig. 4 Gel permeation chromatography elution profiles for furcellaran extracted 4 h (*1a*) in water, *1b* in 0.02 M KOH solution; furcellaran extracted in 0.02 M NaOH solution (*2a*) for 1 h, *2b* for 9 h; galactan from *E. cottonii* extracted 4 h (*3a*) in water, *3b* in 0.02 M KOH solution; commercial κ-carrageenan preparation from *4a* Sigma and from *4b* Fluka. *Peak at 22.89 mL corresponds to the internal marker of ethylene glycol

values characterize the preparations obtained by a short-term extraction (1 h) in 0.02-M NaOH solution; at that, a slight degradation of polymeric chains took place during the first 9 h of extraction.

Indicative of polymeric chains cleavage and the release of sulfate from the carrageenan, minor peaks at 20.25±0.05 mL were present in some GPC elution profiles. Typically, the alkaline extraction caused a partial hydrolysis of the galactan matrix and the formation of a low molecular weight component. As seen from chromatograms, the commercial carrageenans of Sigma and Fluka also contain low molecular constituents as hydrolysis products and/or standardization additives. The presence of a disaccharide component in the Fluka carrageenan is evidenced by a high peak around 21.32 mL and rheological behavior (a poor linear correlation between gel concentration and strength, see Table 3). As a result of the alkaline treatment of furcellaran, the separation of the polysaccharic fraction with M_w of about 50 kDa noted as a shoulder around 17.7 mL is clearly seen in GPC profiles. In all chromatograms, a characteristic peak at 22.17 ±0.01 mL was observed. This is due to the small difference in concentration between the HPLC eluent and the solvent used to prepare the galactan solution.

Gelling ability

As its major component is κ-carrageenan, furcellaran is generally similar to this polymer in gelling properties. The hot alkaline treatment of the seaweeds with 0.02 M KOH solution increased the stiffness of furcellaran gels more than 11 times compared to the gels of water-extracted furcellaran (Table 3). For this polysaccharide backbone, the maximum gel strength was achieved in the case of the Rb^+ form of the polymer. The formation of a characteristic gel network is not favored in the presence of Na^+ ions, as seen from the notably low gel strength values of the NaOH-treated furcellaran. While in the case of the water-extracted furcellaran both KCl and CsCl showed similar effect to the gel strength, the treatment with a 0.02 M CsOH solution resulted in a noticeable decrease, which was probably caused by a destructive effect of such a strong alkali.

It is known that ionic co-solutes affect the solubility properties of algal galactans with increased phase separation rates during the gelation process. This usually causes notable changes in the physical properties of the gels. At low concentrations, alkali metal chlorides favor the formation of the gel network, having a positive effect on the gel strength of furcellaran (Fig. 5). The concentration of K^+, Rb^+, and Cs^+ chlorides of more than 0.1 mol L^{-1} led to a noticeable drop in gel strength. However, this was not observed when NaCl or LiCl was added to galactan sol, while within the concentration range under study, a positive correlation was observed to exist between gel strength values and salt concentration.

The gel strength values of the commercial κ-carrageenan (Sigma) were higher than those of furcellaran and *E. cottonii* galactan. This was mainly caused by the higher content of 3,6-anhydrogalactose (32.3%) and compositional specificity (more K^+ and Ca^{2+} ions bound to the galactan matrix) of this commercial preparation. Another κ-carrageenan preparation of Fluka had a very weak gelling ability at a 1% galactan concentration, but the gel strength values of more concentrated gels were relatively high. This may be attributed to impurities, probably some low molecular carbohydrates used for standardization purposes, as was confirmed by gel permeation chromatography (Fig. 4).

Due to the high pigment content of *F. lumbricalis*, the color and transparency of the gel obtained from this seaweed galactan depends greatly on the conditions of extraction. The long-term room temperature alkaline modification resulted in furcellaran gels with moderate strength and reduced optical density (more than 20% lower absorbance at 400 nm for

Table 3 Rheological characteristics of galactan gels

Galactan	Gel strength, g/cm^2			Gel melting temperature, °C	Gel setting temperature, °C
	1%	1.5%	2%		
Furcellaran, native[a]	19±2.4	75±2.9	134±2.6	41.0	25
Furcellaran, Na^+ form[b]	10±0.03	16±1.6	34±0.4	35.5	21
Furcellaran, K^+ form[b]	437±28.8	862±30.3	1,152±17.5	59.5	36
Furcellaran, Rb^+ form[b]	521±50.2	970±7.1	1,412±11.9	63.8	40
Furcellaran, Cs^+ form[b]	349±15.6	636±25.7	805±38.7	57.4	33
Furcellaran[c]	147±14.0	417±9.3	653±6.0	51.8	28
Galactan from *E. cottonii*[a]	NG	37±1.6	86±4.9	34.7	19
Galactan from *E. cottonii*[b]	23±2.6	146±0.8	384±7.9	40.6	27
κ-Carrageenan, Sigma	624±5.1	1,349±25.0	2,146±13.7	49.8	29
κ-Carrageenan, Fluka	17±0.9	318±4.0	1,066±53.5	40.3	29

NG not gelling

[a] Extracted for 4 h in water

[b] Extracted for 4 h in 0.02 M alkali metal hydroxide solution

[c] Algae treated with 0.6 M KOH solution for 7 days, extracted for 4 h in water

1.5% gels), compared to preparations of hot alkaline extraction, but the absorbance was still 2.5 times higher than in the case of the commercial κ-carrageenan preparation of Sigma. Although the optical density of the native water-extracted furcellaran was considerably lower than that of the preparation extracted in hot alkaline media, the nitrogen content of both preparations was quite similar (Table 1). Thus, the coloration of the samples can be attributed to hot alkali modification of the proteinic pigments present in both preparations.

All the galactan preparations under study were characterized by low syneresis. In the case of the furcellaran preparation extracted in an alkali metal hydroxide solution, a good positive linear correlation (r=0.997) exists between gel strength and syneresis values. The Na^+ form of furcellaran with a very low gel strength showed no tendency towards syneresis. The syneresis values of K^+, Rb^+, and Cs^+ forms were 1.1%, 1.3%, and 0.9%, respectively. While the water-extracted furcellaran exhibited a very low syneresis (0.1%), the respective values of the commercial κ-carrageenan preparations were significantly higher (1.1% for Sigma and 0.4% for Fluka preparation).

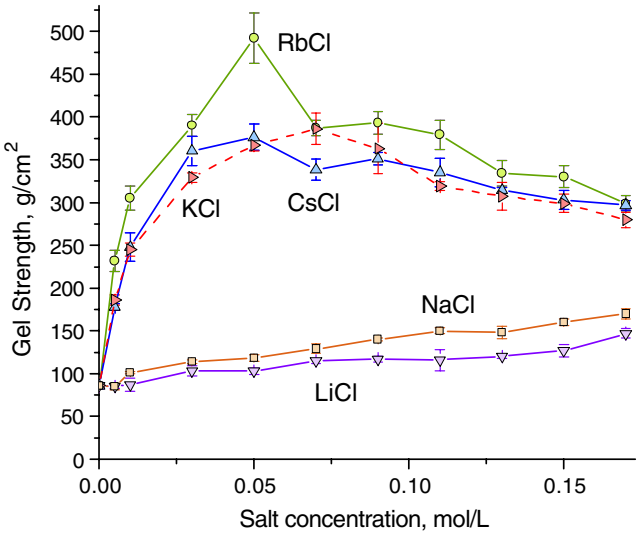

Fig. 5 Effect of cations on gel strength (for 1.5% gels) of water-extracted furcellaran

Scanning electron microscopy

To protect fragile SEM preparations, the gels were inserted into metal capillary tubes before cryofixation. It was observed that the results were not affected by the material of the tubing (stainless steel or copper) used. SEM micrographs showed a characteristic honeycomb pattern to be present in furcellaran gels. Similar supramolecular structures have also been described in the case of mixed gels (Dunstan et al. 2001).

The presence of alkali metal chlorides (KCl, RbCl, CsCl) in the furcellaran sol induced the formation of some subtle (tentacle-like) constituents responsible for the tightening of its final structure (Fig. 6). The increase of the gel strength in the presence of metal chlorides was apparently caused by an aggregative effect of those structure units (Figs. 6b, c and 7). The shape and arrangement of the tentacle-like components were affected by chloride ions. While in the gels co-soluted with salt they were more subtle and arranged less regularly, alkali-extracted furcellaran afforded

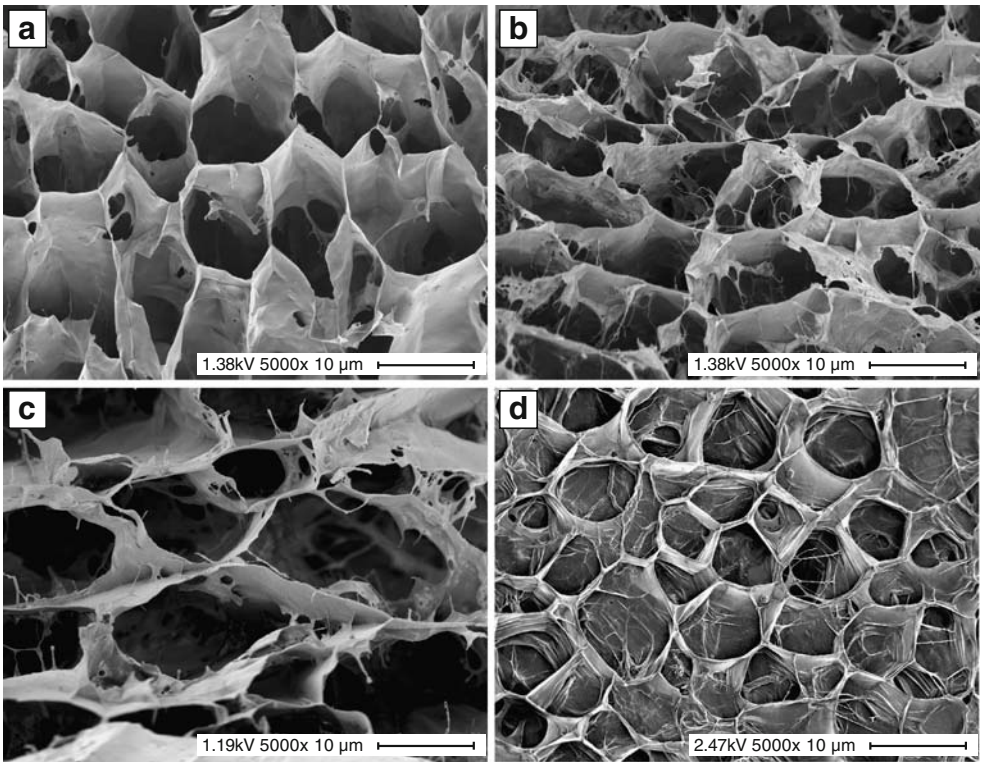

Fig. 6 SEM images of gel network structures of 1.5% water-extracted furcellaran gelled in **a** water, **b** 0.01 M RbCl, **c** 0.05 M KCl, and **d** 2.0% alkali-extracted (0.02 M KOH, 4 h) furcellaran, gelled in water

gels whose tentacle-like units were responsible for zippering together of the honeycomb walls, thus having a more pronounced constructional function (Fig. 6d). Such structural differences between alkali-extracted and chloride-containing furcellarans affect significantly their gel strength values, which are much lower in the case of the latter (cf. Table 3 and Fig. 5).

The walls of honeycomb structures were too thin (less than 100 nm) for conventional SEM investigations. These double-layered films (Fig. 8) were probably formed by the agglomeration of water-holding compartments during the gelation process.

Conclusion

The major components of galactan from *F. lumbricalis* (furcellaran) of the Baltic Sea are β-D-galactose-4-sulfate, unsubstituted β-D-galactose, and 3,6-anhydro-α-D-galactose, being indicative of the κ/β-carrageenan backbone. Also, the α-D-galactose-6-sulfate from γ-carrageenan is present in low amounts, and traces of ω- and α-carrageenan segments were detected. The 3,6-anhydrogalactose content of furcellaran is relatively stable, varying from 28.5% to 30.1% depending on the chemical treatment procedures. The undersulfated carrageenan with sulfur content of 5.3%

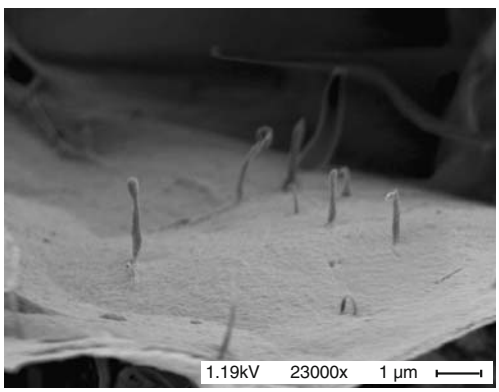

Fig. 7 Micrograph of tentacle-like structures in 1.5% water-extracted furcellaran gel (gelled in 0.05 M KCl solution)

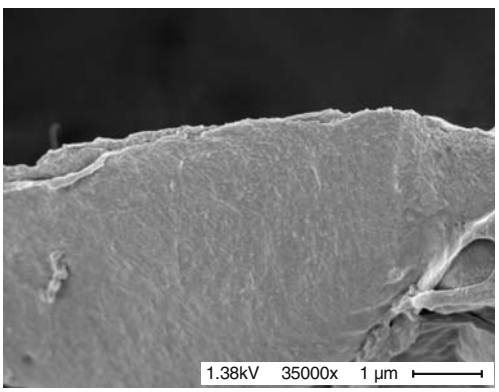

Fig. 8 Microstructure of double-layer honeycomb walls (water-extracted furcellaran, 1.5% gel in 0.05 M RbCl)

and the average molecular weight of about 290 kDa for water-extracted preparation exhibit low affinity towards Na^+ ions and high binding capacity to K^+ ions. The high nitrogen content of furcellaran may be attributable to the high pigment concentration and does not depend on the extraction conditions. Depending on the alkali used in the extracting medium after a 4-h extraction, the total yield of furcellaran varied from 19%, when using NaOH, to 28% in the case of using CsOH (0.02 M solutions). Furcellaran is characterized by low syneresis. For this polysaccharide backbone, the maximum gel strength is achieved in the presence of Rb^+ ions (970 g/cm^2 for 1.5% gel). This is over 12 times higher than that of the water-extracted preparation. In the furcellaran gel, K^+, Rb^+, and Cs^+ chlorides as co-solutes at concentrations below 0.1 mol L^{-1} favor the gelation process most. The characteristic honeycomb pattern observed in furcellaran gels exhibited a tightening tendency in the presence of alkali metal chlorides. At the same time, the formation of subtle tentacle-like gel structure units was observed.

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References

Abada LV, Nasimova IR, Relleve LS, Aranilla CT, De la Rosa AM, Shibayama M (2004) Dynamic light scattering studies of irradiated kappa carrageenan. Int J Biol Macromol 34:81–88

Anderson NS, Dolan TCS, Lawson CJ, Penman A, Rees DA (1968) Carrageenans. Part 5. The masked repeating structures of λ- and μ-carrageenans. Carbohydr Res 7:468–473

Barabanova AO, Yermak IM, Glazunov VP, Isakov VV, Titlyanov EA, Solov'eva TF (2005) Comparative study of carrageenans from reproductive and sterile forms of *Tichocarpus crinitus* (Gmel.) Rupr (Rhodophyta, Tichocarpaceae). Biochem (Moscow) 70:350–356

Barwell CJ (1989) Distribution of histamine in the thallus of *Furcellaria lumbricalis*. J Appl Phycol 1:341–344

Bird CJ, Saunders GW, McLachlan J (1991) Biology of *Furcellaria lumbricalis* (Hudson) Lamouroux (Rhodophyta: Gigartinales), a commercial carrageenophyte. J Appl Phycol 3:61–82

Borgström J (1997) Phase separation, aggregation and gelation of a charged helix forming polysaccharide in aqueous salt solution. Doctoral thesis, Lund University, Sweden

Briand X (1991) Seaweed Harvesting in Europe. In: Guiry MD, Blunden G (eds) Seaweed resources in Europe: uses and potential. Wiley, Chichester, pp 259–308

Cairns P, Atkins EDT, Miles MJ, Morris VJ (1991) Molecular transforms of kappa carrageenan and furcellaran from mixed gel systems. Int J Biol Macromol 13:65–68

Chiovitti A, Bacic A, Craik DJ, Munro SLA, Kraft GT, Liao M-L (1997) Cell-wall polysaccharides from Australian red algae of the family Solieriaceae (Gigartinales, Rhodophyta): novel, highly pyruvated carrageenans from the genus *Callophycus*. Carbohydr Res 299:229–243

Chiovitti A, Bacic A, Craik DJ, Kraft GT, Liao M-L (2004) A nearly idealized 6′-*O*-methylated ι-carrageenan from the Australian red alga *Claviclonium ovatum* (Acrotylaceae, Gigartinales). Carbohydr Res 339:1459–1466

Christiansen E (1959) Danish agar. In: Whistler RL (ed) Industrial gums. Academic, New York, pp 51–54

Craigie JS (1990) Cell walls. In: Cole K, Sheath R (eds) The biology of red algae. Cambridge University Press, Cambridge, pp 221–257

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Dunstan DE, Chen Y, Liao M-L, Salvatore R, Boger DV, Prica M (2001) Structure and rheology of the kappa-carrageenan/locust bean gum gels. Food Hydrocoll 15:475–484

Friedenthal M, Eha K, Viitak A, Lukas A, Siimer E (2000) Effects of drying on the gel strength and cation mobility of furcellaran. Innovat Food Sci Emerg Tech 1:275–279

Greer CW, Yaphe W (1984) Characterization of hybrid (beta-kappa-gamma) carrageenan from *Eucheuma gelatinae* J. Agardh (Rhodophyta, Solieriaceae) using carrageenases, infrared and ^{13}C-nuclear magnetic resonance spectroscopy. Bot Mar 27:473–478

Knutsen SH (1992) Isolation and analysis of red algal galactans. Doctoral thesis, University of Trondheim, Norway

Knutsen SH, Grasdalen H (1987) Characterization of water-extractable polysaccharides from Norwegian *Furcellaria lumbricalis* (Huds.) Lamour. (Gigartinales, Rhodophyceae) by IR and NMR spectroscopy. Bot Mar 30:497–50

Knutsen SH, Myslabodski DE, Grasdalen H (1990) Characterization of carrageenan fractions from Norwegian *Furcellaria lumbricalis* (Huds.) Lamour. by ^{1}H-n.m.r. spectroscopy. Carbohydr Res 206:367–372

Krasil'nikova SV, Medvedeva EI (1975) Some characteristics of the alkali-soluble protein from the red alga *Furcellaria fastigiata*. Khim Prir Soedini 11:400–404 (in Russian)

Mangione MR, Giacomazza D, Bulone D, Martorana V, Cavallaro G, San Biagio PL (2005) K^+ and Na^+ effects on the gelation properties of κ-carrageenan. Biophys Chem 113:129–135

Martin G, Paalme T, Kukk H (1996) Long-term dynamics of the commercially useable *Furcellaria lumbricalis–Coccotylus truncatus* community in Kassari Bay, West Estonian Archipelago, the Baltic Sea. In Proceedings of Polish–Swedish Symposium on Baltic coastal fisheries. Resources and Management. 2–3 April 1996, Gdynia, Poland (pp. 121–129). Gdynia: Sea Fisheries Institute

Martin G, Paalme T, Torn K (2006) Seasonality pattern of biomass accumulation in a drifting *Furcellaria lumbricalis* community in the waters of the West Estonian Archipelago, Baltic Sea. J Appl Phycol 18:557–563

Meunier V, Nicolai T, Durand D (2001) Structure of aggregating κ-carrageenan fractions studied by light scattering. Int J Biol Macromol 28:157–165

Michel A-S, Mestdagh MM, Axelos MAV (1997) Physico-chemical properties of carrageenan gels in presence of various cations. Int J Biol Macromol 21:195–200

Mikulich DV, Voloshchenko IA, Medvedeva EI (1988) The jelly-forming capacity of polysaccharides from the red algae *Furcellaria lumbricalis* (Huds.) Lamour and *Phyllophora truncata* (Pall.) Newroth et Taylor (the Bight of Kassari of the Baltic Sea). Rast Resur 24:456–460 (in Russian)

Montolalu RI, Tashiro Y, Matsukawa S, Ogawa H (2008) Effects of extraction parameters on gel properties of carrageenan from *Kappaphycus alvarezii* (Rhodophyta). J Appl Phycol 20:521–526

Morris ER, Rees DA, Robinson G (1980) Cation-specific aggregation of carrageenan helices: domain model of polymer gel structure. J Mol Biol 138:349–362

Painter TJ (1983) Algal polysaccharides. In: Aspinall GO (ed) The polysaccharides, vol. 2. Academic, New York, pp 195–285

Paoletti S, Delben F, Cesàro A, Grasdalen H (1985) Conformational transition of κ-carrageenan in aqueous solution. Macromol 18:1834–1841

Penman A, Rees DA (1973) Carrageenans. Part IX. Methylation analysis of galactan sulfates from *Furcellaria fastigata*, *Gigartina canaliculata*, *G. chamissoi*, *G. atropurpurea*, *Ahnfeltia durvillaei*, *Gymnogongrus furcellatus*, *Eucheuam isiforme*, *Eucheuma uncinatum*, *Aghardiella tenera*, *Pachymenia hymantophora*, and *Gloiopeltis cervicornis*. Structure of ξ-carrageenan. J Chem Soc, Perkins I 1:2182–2187

Pereira L, Sousa A, Coelho H, Amado AM, Ribeiro-Claro PJA (2003) Use of FTIR, FT-Raman and ^{13}C-NMR spectroscopy for identification of some seaweed phycocolloids. Biomol Eng 20:223–228

Pereira L, Amado AM, Critchley AT, Van de Velde F, Ribeiro-Claro PJA (2009) Identification of selected seaweed polysaccharides (phycocolloids) by vibrational spectroscopy (FTIR-ATR and FT-Raman). Food Hydrocoll. doi:10.1016/j.foodhyd.2008.11.014

Piculell L (2006) Gelling carrageenans. In: Stephen AM, Phillips GO, Williams PA (eds) Food polysaccharides and their applications. 2nd edn. CRC, Boca Raton, pp 239–287

Prado-Fernández J, Rodríguez-Vázquez JA, Tojo E, Andrade JM (2003) Quantitation of κ-, ι- and λ-carrageenans by mid-infrared spectroscopy and PLS regression. Anal Chim Acta 480:23–37

Rees DA (1969) Structure, conformation, and mechanism in the formation of polysaccharide gels and networks. Adv Carbohydr Chem Biochem 24:267–332

Rees DA (1972) Polysaccharide gels. A molecular view. Chem Ind 19:630–636

Rinaudo M (2005) Advances in characterization of polysaccharides in aqueous solution and gel state. In: Dumitriu S (ed) Polysaccharides. Structural diversity and functional versatility. Marcel Dekker, New York, pp 237–252

Rochas C, Rinaudo M (1984) Mechanism of gel formation in κ-carrageenan. Biopolymers 23:735–745

Spichtig V, Austin S (2008) Determination of the low molecular weight fraction of food-grade carrageenans. J Chromatogr B 861:81–87

Tanner SF, Morris VJ, Belton PS (1990) Multinuclear N.M.R. studies of group I cations in furcellaran gels. Int J Biol Macromol 12:302–304

Truus K, Vaher M, Usov AI, Pehk T, Kollist A (1997) Gelling galactans from the algal community of *Furcellaria lumbricalis* and *Coccotylus truncatus* (the Baltic Sea, Estonia): a structure–property study. Int J Biol Macromol 21:89–96

Tuvikene R, Truus K, Vaher M, Kailas T, Martin G, Kersen P (2006) Extraction and quantification of hybrid carrageenans from the biomass of the red algae *Furcellaria lumbricalis* and *Coccotylus truncatus*. Proc Est Acad Sci Chem 55:40–53

Tuvikene R, Truus K, Kollist A, Volobujeva O, Mellikov E, Pehk T (2008) Gel-forming structures and stages of red algal galactans of different sulfation levels. J Appl Phycol 20:527–535

Usov AI (1998) Structural analysis of red seaweed galactans of agar and carrageenan groups. Food Hydrocoll 12:301–308

Usov AI, Arkhipova VS (1981) Polysaccharides of algae. XXX. Methylation of κ-carrageenan type polysaccharides of the red seaweeds *Tichocarpus crinitus* (Gmel.) Rupr., *Furcellaria fastigiata* (Huds.) Lam. and *Phyllophora nervosa* (De Cand.) Grev. Bioorg Khim 7:385–390 (in Russian)

Usov AI, Shashkov AS (1985) Polysaccharides of algae. XXXIV: detection of iota-carrageenan in *Phyllophora brodiaei* (Turn.) J. Ag. (Rhodophyta) using ^{13}C-NMR spectroscopy. Bot Mar 28:367–373

Vaher M, Truus K, Taure I, Leito I, Lahe L, Kallavus U, Pehk T (1998) Composition and structure of summary polysaccharide from the red alga *Furcellaria lumbricalis*. Proc Est Acad Sci Chem 47:51–59

Van de Velde F, Knutsen SH, Usov AI, Rollema HS, Cerezo AS (2002) ^{1}H and ^{13}C high resolution NMR spectroscopy of carrageenans: application in research and industry. Trends Food Sci Technol 13:73–92

Van de Velde F, Pereira L, Rollema HS (2004) The revised NMR chemical shift data of carrageenans. Carbohydr Res 339:2309–2313

Yaphe W, Arsenault GP (1965) Improved resorcinol reagent for the determination of fructose, and of 3,6-anhydrogalactose in polysaccharides. Anal Biochem 13:143–148

Yarotsky SV, Shashkov AS, Usov AI (1978) Polysaccharides of algae. XXV. Application of ^{13}C-NMR spectroscopy for structural analysis of κ-carrageenan group polysaccharides. Bioorg Khim 4:745–751 (in Russian)

Yermak IM, Kim YH, Titlynov EA, Isakov VV, Solov'eva TF (1999) Chemical structure and gel properties of carrageenans from algae belonging to the Gigartinaceae and Tichocarpaceae, collected from the Russian Pacific coast. J Appl Phycol 11:41–48

Yu G, Yang B, Ren W, Zhao X, Zhang J, Barrow C (2007) A comparative analysis of four kinds of polysaccharides purified from *Furcellaria lumbricalis*. J Ocean Univ China 6:16–20