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FUNCTIONAL DEPENDENCIES OF THE CHEMICAL COMPOSITION AND STRUCTURES IN THE BALTIC SEA ALGAL COMMUNITIES

Abstract

The composition and structure of galactans from the red algae species *Furcellaria lumbricalis* and *Coccotylus truncatus* (the Baltic Sea, Estonia) were studied. As reference material, the galactans from Far-Eastern species *Ahnfeltia tobuchiensis* and *Eucheuma cottonii* were investigated. The complex polysaccharides were characterized by carbon nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, chemical analysis, inductively coupled plasma optical emission spectrometry, electrothermal atomic absorption spectrometry and size exclusion chromatography methods in comparison with well known commercial galactan preparations. Special attention has been given to the polysaccharides originating from *F. lumbricalis*, their molecular structure, composition and gelling properties in connection with the extraction and chemical treatment conditions. For *C. truncatus*, also the thermal stability characteristics of its native polysaccharides were studied. The possibilities for the determination of micro- and ultramicro elements in the Baltic seawater and in seaweeds were investigated by various spectroscopic methods. The microstructure of galactan gels was studied using a cryofixation method in combination with freeze-drying and scanning electron microscopy (SEM) techniques. To achieve reliability of SEM investigations and to study the gel-forming processes of red algal galactans, a novel structure-preserving preparation technique was developed.

The main components of galactan from *F. lumbricalis* of the Baltic Sea are β-D-galactopyranose (15 ± 1%), β-D-galactopyranose-4-sulfate (36 ± 1.5%), 3,6-anhydro-α-D-galactopyranose (29 ± 1%), α-D-galactopyranose-6-sulfate (6 ± 0.5%), β-D-galactopyranose-6-sulfate and 6-*O*-methyl-β-D-galactopyranose residues. Polysaccharides from *C. truncatus* were found to contain β-D-galactopyranose-4-sulfate (44 ± 2%), 3,6-anhydro-α-D-galactopyranose-2-sulfate (30 ± 1.5%), α-D-galactopyranose-2,6-disulfate (12 ± 2%) and 4',6'-pyruvated β-D-galactopyranose (1.4%) units. The galactans from Far-Eastern species *A. tobuchiensis* are characterized by the main constituents of β-D-galactopyranose (58%), 3,6-anhydro-α-L-galactopyranose (30 ± 3.5%), 2-*O*-methyl-3,6-anhydro-α-L-galactopyranose (8 ± 1%) and β-D-galactopyranose-6-sulfate residues.

For *F. lumbricalis* galactans, the maximum gel strength value of 970 g/cm^2 (for 1.5% gels) is attainable in the case of optimal extraction conditions in the alkaline medium containing Rb^+-ions. The thermally labile galactans from *C. truncatus* are characterized by a weak gelling ability (gel strength of 30–40 g/cm^2 for 2% gels) that does not depend notably on the conditions of extraction.

Extraction of furcellaran in the presence of various alkali metal hydroxides yields products with similar amounts of organic matter; the quota of inorganics varies con-

siderably and is primarily connected with the atomic weight of the introduced alkali metal. The microelement composition of seaweed samples may be elucidated equally well by atomic absorption spectrometry and inductively coupled plasma optical emission spectrometry methods.

Depending on the configuration of 3,6-anhydrogalactose (D- or L-isomer) in the macromolecular chain, the gelation process of algal galactans follows different pathways. The presence of gel promoting cations (K^+, Rb^+, Cs^+) in κ-type carrageenan sols induces the formation of specific subtle tentacle-like structure units responsible for the tightening of the final gel structure.

KEEMILISE KOOSTISE JA STRUKTUURIDE FUNKTSIONAALSÕLTUVUSED LÄÄNEMERE VETIKAKOOSLUSTES

Resümee

Töös uuriti Läänemere punavetikatest *Furcellaria lumbricalis* ja *Coccotylus truncatus* pärinevate sulfaaditud polüsahhariidide keemilist koostist ja struktuuri. Võrdlusainetena kasutati troopilisest vetikaliigist *Eucheuma cottonii* ja Kaug-Ida punavetikast *Ahnfeltia tobuchiensis* eraldatud galaktaane ning karraginaanide ja agarite kommertspreparaate. Polüsahhariide iseloomustati ^{13}C-tuumamagnetresonantsspektroskoopia, Fourier' infrapunaspektroskoopia, spektrofotomeetria, induktiivsidestunud plasma optilise emissioonspektromeetria, elektrotermilise aatomabsorptsioonspektromeetria ja eksklusioonikromatograafia meetoditega. *F. lumbricalis* kinnitumata vormi polüsahhariidide keemilise koostise, molekulaarstruktuuri ning geelistumisvõime vahelisi seoseid uuriti sõltuvalt ekstraktsiooni ja keemilise töötluse tingimustest. Potentsiaalse tööndusliku väärtusega vetikaliigi *C. truncatus* galaktaanide puhul hinnati ka nende polüsahhariidide termostabiilsust. Võrreldi erinevate spektroskoopiliste meetodite rakendusvõimalusi tüüpiliste saasteelementide sisalduste määramiseks Läänemere riimvees ja vetikates. Polüsahhariidgeelide mikrostruktuuri uuriti skaneeriva elektronmikroskoopia vahendusel preparaatide krüofikseerimise ja lüofiliseerimise kaasabil. Töötati välja uudne struktuure säilitav prepareerimistehnika punavetikagalaktaanide geelistumisprotsesside mikroskoopiliseks uurimiseks.

F. lumbricalis galaktaanide põhikomponendid on β-D-galaktoos (15 ± 1%), β-D-galaktoos-4-sulfaat (36 ± 1,5%), 3,6-anhüdro-α-D-galaktoos (29 ± 1%), α-D-galaktoos-6-sulfaat (6 ± 0,5%), β-D-galaktoos-6-sulfaat ja 6-*O*-metüül-β-D-galaktoos. *C. truncatus* polüsahhariidide peamised komponendid on β-D-galaktoos-4-sulfaat (44 ± 2%), 3,6-anhüdro-α-D-galaktoos-2-sulfaat (30 ± 1,5%), α-D-galaktoos-2,6-disulfaat (12 ± 2%) ning 4',6'-püruvaaditud karrabioos-2-sulfaat (1,4%). Kaug-Ida vetikaliigi *A. tobuchiensis* galaktaanide koostisse kuuluvad β-D-galaktoos (58%), 3,6-anhüdro-α-L-galaktoos (30 ± 3,5%), 2-*O*-metüül-3,6-anhüdro-α-L-galaktoos (8 ± 1%) ja β-D-galaktoos-6-sulfaat.

F. lumbricalis galaktaanide maksimaalne geelitugevus 970 g/cm^2 (1,5% geelide puhul) on saavutatav optimaalsete ekstraktsioonitingimuste juures Rb^+-ioone sisaldavas leeliselises keskkonnas. *C. truncatus* polüsahhariidide geelimoodustumisvõime on väike (geelitugevus 30–40 g/cm^2 2% geelide korral) ning ei sõltu oluliselt ekstraktsioonitingimustest.

Furtsellaraani ekstraktsioonil erinevates leelismetallhüdroksiidide vesilahustes saadavad produktid sisaldavad lähedase koguse orgaanikat, mineraalse komponendi osakaal aga varieerub oluliselt, sõltudes peamiselt preparaadi koostisse viidud leelismetalli aatommassist. Mikroelementide määramiseks vetikaproovides on võrd-

selt hästi rakendatavad induktiivsidestunud plasma optilise emissioonspektromeetria ja aatomabsorptsioonspektromeetria meetodid.

Sõltuvalt galaktaani makromolekulaarse ahela koostises esineva 3,6-anhüdrogalaktoosi ruumilisest konfiguratsioonist (D- või L-isomeer) toimub vetikapolüsahhariidide geelistumine põhimõtteliselt erinevalt. Geelistumist soodustavate katioonide (K^+, Rb^+, Cs^+) esinemine κ-tüüpi karraginaanide kuumades vesilahustes indutseerib niitjate, geeli mikrostruktuuri tihendavate lisaelementide moodustumise geelistumisprotsessi käigus.

CONTENTS

LIST OF PUBLICATIONS

This thesis is based on the following papers which are referred by the Roman numerals:

I. **R. Tuvikene**, K. Truus, M. Robal, O. Volobujeva, E. Mellikov, T. Pehk, A. Kollist, T. Kailas, M. Vaher 2009. The extraction, structure, and gelling properties of hybrid galactan from the red alga *Furcellaria lumbricalis* (Baltic Sea, Estonia). – *Journal of Applied Phycology*. (doi: 10.1007/s10811-009-9425-x).

II. **R. Tuvikene**, K. Truus, M. Robal, T. Pehk, T. Kailas, M. Vaher, T. Paalme 2009. Structure and thermal stability of pyruvated carrageenans from the red alga *Coccotylus truncatus*. – *Carbohydrate Research*, 344, 788–794.

III. **R. Tuvikene**, K. Truus, A. Kollist, O. Volobujeva, E. Mellikov, T. Pehk 2008. Gel-forming structures and stages of red algal galactans of different sulfation levels. – *Journal of Applied Phycology*, 20, 527–535.

IV. K. Truus, A. Viitak, M. Vaher, I. Muinasmaa, K. Paasrand, **R. Tuvikene**, T. Levandi 2007. Comparative determination of microelements in Baltic seawater and brown algae samples by atomic absorption spectrometric and inductively coupled plasma methods. – *Proceedings of the Estonian Academy of Sciences, Chemistry*, 56, 122–133.

V. K. Truus, **R. Tuvikene**, M. Vaher, T. Kailas, P. Toomik, T. Pehk 2006. Structural and compositional characteristics of gelling galactan from the red alga *Ahnfeltia tobuchiensis* (Ahnfeltiales, the Sea of Japan). – *Carbohydrate Polymers*, 63, 130–135.

VI. **R. Tuvikene**, K. Truus, M. Vaher, T. Kailas, G. Martin, P. Kersen 2006. Extraction and quantification of hybrid carrageenans from the biomass of the red algae *Furcellaria lumbricalis* and *Coccotylus truncatus*. – *Proceedings of the Estonian Academy of Sciences, Chemistry*, 55, 40–53.

AUTHOR'S CONTRIBUTION

Paper I: The author performed the extractions, rheological and colorimetric measurements, chromatographical analyses, prepared the electron microscopy samples and interpreted the FTIR and ^{13}C-NMR spectra. He interpreted the data, wrote the manuscript and presented the results at the *14th European Carbohydrate Symposium* (2–7 September 2007 Lübeck, Germany) and at the *42nd IUPAC World Polymer Congress, MACRO 2008* (29 June – 4 July 2008 Taipei, Taiwan).

Paper II: The author performed the extractions, rheological and colorimetric measurements, chromatographical analyses and interpreted the FTIR and ^{13}C-NMR spectra. He interpreted the data, wrote the manuscript and presented the results at the *9th International Hydrocolloids Conference* (15–19 June 2008 Singapore) and at the *4th International Symposium on the Separation and Characterization of Natural and Synthetic Macromolecules, SCM-4* (28–30 January 2009 Amsterdam, The Netherlands).

Paper III: The author performed the rheological measurements, spectrophotometric analyses, prepared the electron microscopy samples and interpreted the ^{13}C-NMR spectra. He interpreted the data, wrote the manuscript and presented the results at the *World Polymer Congress, 41st International Symposium on Macromolecules, MACRO 2006* (16–21 July

2006 Rio de Janeiro, Brazil) and at the *19th International Seaweed Symposium* (26–31 March 2007 Kobe, Japan).

Papers IV and V: The author performed some of the experimental work, interpreted the results and participated in preparation of the manuscript.

Paper VI: The author performed the extractions, rheological measurements, chemical analyses and interpreted the FTIR spectra. He interpreted the data, wrote the manuscript and presented the results at the *Sustainability of the Agri-Food Chain 2006 EFFoST Annual Meeting* (7–9 November 2006 The Hague, The Netherlands).

In addition some unpublished materials are used in this thesis.

LIST OF ABBREVIATIONS

AAS	atomic absorption spectrometry
AG	3,6-anhydrogalactose
^{13}C-NMR	carbon nuclear magnetic resonance spectroscopy
DSS	2,2-dimethyl-2-silapentane-3,3,4,4,5,5-d_6-5-sulfonate sodium salt
EEO	electroendoosmosis
ETAAS	electrothermal atomic absorption spectrometry
FAAS	flame atomic absorption spectrometry
FTIR	Fourier transform infrared spectroscopy
HPLC	high performance liquid chromatography
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
M_w	average molecular weight
SEC	size exclusion chromatography
SEM	scanning electron microscopy
psu	practical salinity unit

D2S,6S	4-linked α-D-galactopyranose-2,6-disulfate
D6S	4-linked α-D-galactopyranose-6-sulfate
DA	4-linked 3,6-anhydro-α-D-galactopyranose
DA2S	4-linked 3,6-anhydro-α-D-galactopyranose-2-sulfate
G	3-linked β-D-galactopyranose
G4S	3-linked β-D-galactopyranose-4-sulfate
G6S	3-linked β-D-galactopyranose-6-sulfate
G6M	3-linked 6-*O*-methyl-β-D-galactopyranose
G6M,4S	3-linked 6-*O*-methyl-β-D-galactopyranose-4-sulfate
GP	3-linked 4',6'-pyruvated β-D-galactopyranose
L	4-linked α-L-galactopyranose
LA	4-linked 3,6-anhydro-α-L-galactopyranose
LA2M	4-linked 2-*O*-methyl-3,6-anhydro-α-L-galactopyranose

PREFACE

The brackish Baltic Sea is a unique ecosystem, where low salinity forces its marine inhabitants to live under strong environmental stress conditions. For red algae this appears in their morphology, life-history cycle or polysaccharide composition. The gelling ability of such polysaccharides gives the basis for the vital functions of the red algae, as well as for their use in the food industry and microbiology. Gels as finely reticulated structures act as specific molecular sieves and give algae the needed flexibility to withstand marine conditions. Thus the structure-property relations of such polysaccharides can be of interest in both the scientific and industrial field.

Being the main natural object of this thesis, the red algal community of Kassari Bay is a typical example to characterize the ecological conditions in the Baltic Sea environment. Occurring as a dense voluminous stratum, this unique benthic habitat holds the Baltics' largest localized mass of red algae with rather well specified species composition and is the only algal community of industrial use in this region.

In this work the structure and composition of sulfated polysaccharides from the Baltic red algae species *Furcellaria lumbricalis* (**I**) and *Coccotylus truncatus* (**II**) are studied. As reference material, the galactans from Far-Eastern species *Ahnfeltia tobuchiensis* (**V**) and *Eucheuma cottonii* (**I**) were investigated. The polysaccharides were characterized by ^{13}C-NMR and FTIR spectroscopy, chemical analysis, ICP-OES, ETAAS and SEC methods in comparison with well known commercial galactan preparations. Special attention has been given to the polysaccharides originating from *F. lumbricalis*, their molecular structure, composition and gelling properties in connection with the extraction and chemical treatment conditions (**I**, **VI**). For *C. truncatus* as species of potential economic importance, also the thermal stability characteristics of its native polysaccharides were studied (**II**).

The possibilities for the determination of micro- and ultramicro elements in the Baltic seawater and in seaweeds were investigated by ETAAS, FAAS, ICP-MS, ICP-OES methods (I**V**).

The microstructure of galactan gels was studied using a cryofixation method in combination with freeze-drying and SEM techniques (**I**, **III**). To achieve reliability of SEM investigations and to study the gel-forming processes of red algal galactans, a novel structure-preserving preparation technique was developed (**III**).

The main objective of this study was to determine the relationships between the polysaccharide structures from the seaweeds of the Baltic Sea origin, their chemical composition, rheological properties and gel structures.

1. INTRODUCTION

1.1. Red algae of the Baltic Sea

The Baltic Sea is characterized by a salinity gradient ranging from real marine conditions near the Danish straits to almost fresh water environments in the innermost regions. As all marine algae, red seaweeds exhibit strong hypo-osmotic stress at low water salinity resulting in inefficient cellular metabolism. Such conditions can often induce changes in morphology or lead to impairment of the reproductive system of the seaweed (Kirst 1990). While 318 macroalgal species of marine algae can be found in the northern parts of the Kattegat, only 42 occur in the northern Gulf of Bothnia (Nielsen et al. 1995). Regular formation of ice during the winter months with its negative effects due to the reduced light may also influence the distribution of algal species at the northern Baltic. The amount of light in the water column is also affected by the eutrophication process that together with increased sedimentation rate can have a notable effect on the distribution of macroalgal species and their life history generations in the Baltic Sea (Eriksson, Johansson 2005).

Due to the prevailing physical conditions that are unfavourable for the growth of commercial algal species, the Baltic Sea plays a minor role in terms of exploitation of natural seaweed resources. Amongst about 160 red algae species found in the Baltic (Nielsen et al. 1995), *F. lumbricalis* is the only seaweed that has been utilized on a large scale in this region (Schramm 1998). Nevertheless, several other Rhodophyta species are considered of potential economic value in the Baltic Sea, e.g. *Ahnfeltia plicata*, *Ceramium nodulosum*, *C. truncatus*, *Phycodrys rubens*, *Polysiphonia fucoides*, *Rhodomela confervoides*, however, none of these usually occur in populations which are readily exploitable on a commercial scale (Briand 1991).

Furcellaria lumbricalis

The red algal species *F. lumbricalis* (Hudson) J. V. Lamouroux (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. Till the 1960s the world's most remarkable resources of *F. lumbricalis* were located in Kattegat, Denmark (Schramm 1998). In the western Atlantic, *F. lumbricalis* is distributed in the lower Gulf of St. Lawrence and on the Atlantic coast of northern Nova Scotia. In North America, predominantly its unattached (loose-lying) form occurs while in Europe (e.g. the Baltic Sea) both attached and unattached forms of the seaweed species can be found (Chapman, Chapman 1980; Holmsgaard et al. 1981).

Although common in the North Atlantic, the usable quantities of this seaweed species are usually too dispersed for industrial exploitation. Large amounts have been found in the Baltic region where *F. lumbricalis* is the only alga species of industrial use. Historically, the galactan mixture from *F. lumbricalis* was one of the first

hydrocolloids to have been industrially produced from red algae. The first attempts to manufacture furcellaran (so-called Danish agar) 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).

Extensive commercial utilization of this seaweed (on the basis of Kattegat resources) began in the 1940s, but stopped soon as World War II broke out. The manufacture of furcellaran was launched again in the 1950s and began to expand rapidly. The increasing exploitation of *F. lumbricalis* resources in Central-Kattegat (from 2,000 tons wet weight in 1946 to 20,000–30,000 tons in the mid 1960s) led to overharvesting and finally to the depletion of the usable biomass at the end of the 1960s. For this reason, the raw-material originating from Canadian waters was later used for the production of furcellaran in Denmark (Briand 1991).

Nowadays, *F. lumbricalis* is common in the coastal waters of Poland, Lithuania, Latvia, Finland, Sweden, Norway, etc (Schramm 1998). In the 1950s this seaweed species was present in the Puck Lagoon of the Gulf of Gdansk (the Baltic Sea, Poland) in appreciable amount (an estimated value of 11,500 tons wet weight). By 1987, due to pollution and eutrophication, *F. lumbricalis* had practically disappeared from this area (Kruk-Dowgiałło 1991; Ciszewski et al. 1992). Thus, the resources of this valuable seaweed species are decreasing or have already been totally depleted.

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 (Chapman, Chapman 1980; Martin et al. 1996; Truus et al. 1997). The shape and total biomass (about 140 thousand tons of fresh weight in 2005, see Figure 1) of the stratum vary slightly from year to year (Martin et al. 2006). Notably, all the dominant seaweed species of Kassari algal stratum (*F. lumbricalis* with another red alga, *C. truncatus*) are in a loose-lying form due to the sandy bottom of the sea and the lack of hard substrate. Also, smaller communities including these seaweed species can be found in the Turku area along the Finnish coast (Mäkinen et al. 1988). In Eastern Canada maritime regions near Prince Edward Island, *F. lumbricalis* together with *Chondrus crispus* is also found in appreciable amounts (Chopin 1998).

In the northern part of the extremely hyposaline (3.6 psu) Baltic Sea *F. lumbricalis* appears to have lost its diplohaploid life cycle (Kostamo, Mäkinen 2006). Under such conditions vegetative thallus fragmentation and reattachment are the principal means of population regeneration (Kostamo, Mäkinen 2006; Kostamo 2008). As seawater salinity and transparency increases and light period and seawater temperature decreases, it has been reported to be more likely to find *F. lumbricalis* individuals with tetrasporangia (Kostamo, Mäkinen 2006).

Coccotylus truncatus

The red alga *C. truncatus* (Pallas) M. J. Wynne & J. N. Heine (former names *Phyllophora truncata* and *Phyllophora brodiaei*) is common in the North Atlantic, North Pacific, Arctic Sea and in both the White and the Black Sea (Lüning 1990). It is one of the few marine species that can inhabit the brackish water of the Baltic Sea, although as a deep-growing dwarf form. *C. truncatus* is the second dominant species in Kassari algal stratum accounting usually for 30–35% of the fresh biomass of the community (Martin et al. 2006).

In the 1960s *C. truncatus* was abundant species in the Black Sea occurring in Karkinitsky Bay and together with *Phyllophora crispa* and *Phyllophora pseudoceranoides* in the North-Western part of the sea as a voluminous stratum named as "Zernov's *Phyllophora* field" or "*Phyllophora* meadows". Being once the largest mass of red algae (5 million tons fresh weight) in the world (Lüning 1990), this unique benthic habitat had almost disappeared due to eutrophication by the 1980s but has recovered to some extent at the present time (Minicheva 2007).

As many close relatives of this species are industrially important carrageenophytes, *C. truncatus* has been considered as a seaweed with potential economic value (Bonotto 1979; Mathieson et al. 1984; Usov, Shashkov 1985).

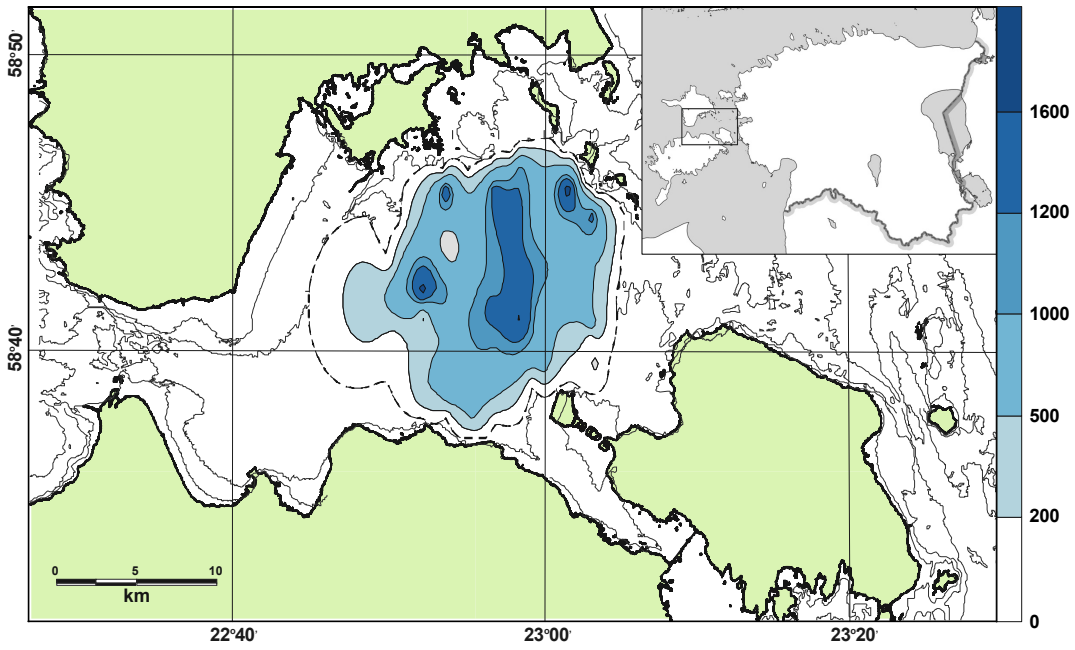
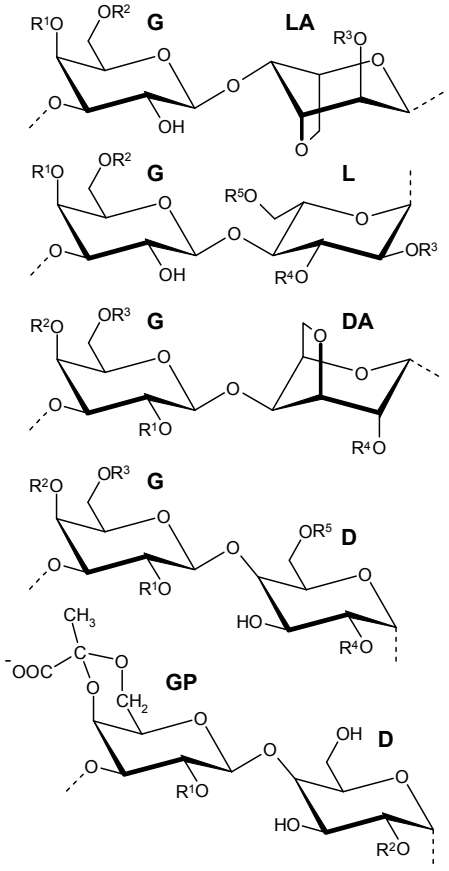


Figure 1. Algal biomass distribution (scale on the right, g/m^2) and the location of the Kassari stratum in the Baltic Sea (summer 2004).

1.2. Algal Galactans

1.2.1. Molecular structure

Red algal galactans are a family of high molecular weight sulfated polysaccharides having usually a linear backbone built up of alternating 3-linked β-D-galactopyranose and 4-linked α-galactopyranose residues. The β-galactose residues always belong to D-series, whereas the α-galactose residues are D in carrageenans and L in agars. A substantial part or all the α-galactopyranose residues may exist in the form of a 3,6-anhydro derivative (Figure 2). Various hydroxyl groups may be substituted by ester sulfate, methyl groups, and sometimes by additional monosaccharide residues (Painter 1983; Usov 1998). Pyruvic acid is an occasional component of agars and many complex carrageenans. It forms a cyclic acetal at *O*-4 and *O*-6 of the 3-linked galactose residues and is related to the idealized structure type of π-carrageenan (DiNinno et al. 1979). Additionally, pyruvic acid acetal may be found in repeating units related to α- and θ-carrageenans (van de Velde et al. 2002a).



agarose	[G-LA]	R^1 = H or SO_3^- R^2 = H or CH_3 or SO_3^- R^3 = H or CH_3
agaran	[G-L]	R^1 = H or SO_3^- R^2 = H or CH_3 or SO_3^- R^3 = H or CH_3 R^4 = H or SO_3^- R^5 = H or SO_3^-
porphyran	(G-L6S)	R^1, R^2, R^3, R^4 = H; R^5 = SO_3^-
α-carrageenan	(G-DA2S)	R^1, R^2, R^3 = H; R^4 = SO_3^-
β-carrageenan	(G-DA)	R^1, R^2, R^3, R^4 = H
θ-carrageenan	(G2S-DA2S)	R^1, R^4 = SO_3^-; R^2, R^3 = H
ι-carrageenan	(G4S-DA2S)	R^1, R^3 = H; R^2, R^4 = SO_3^-
κ-carrageenan	(G4S-DA)	R^1, R^3, R^4 = H; R^2 = SO_3^-
τ-carrageenan	(G4S,6S-DA2S)	R^1 = H; R^2, R^3, R^4 = SO_3^-
ω-carrageenan	(G6S-DA)	R^1, R^2, R^4 = H; R^3 = SO_3^-
γ-carrageenan	(G-D6S)	R^1, R^2, R^3, R^4 = H; R^5 = SO_3^-
δ-carrageenan	(G-D2S,6S)	R^1, R^2, R^3 = H; R^4, R^5 = SO_3^-
λ-carrageenan	(G2S-D2S,6S)	R^1, R^4, R^5 = SO_3^-; R^2, R^3 = H
μ-carrageenan	(G4S-D6S)	R^1, R^3, R^4 = H; R^2, R^5 = SO_3^-
ν-carrageenan	(G4S-D2S,6S)	R^1, R^3 = H; R^2, R^4, R^5 = SO_3^-
ξ-carrageenan	(G2S-D2S)	R^1, R^4 = SO_3^-; R^2, R^3, R^5 = H
ο-carrageenan	(G4S-D2S)	R^1, R^3, R^5 = H; R^2, R^4 = SO_3^-
ψ-carrageenan	(G6S-D6S)	R^1, R^2, R^4 = H; R^3, R^5 = SO_3^-
π-carrageenan	(GP,2S-D2S)	R^1, R^2 = SO_3^-

Figure 2. Idealized disaccharide repeating units of agars and carrageenans. The letter codes given in brackets refer to the alternative nomenclature proposed by Knutsen et al. (1994).

From about 15 principal structure types of carrageenans (Knutsen et al. 1994), κ-, ι-, and λ-carrageenan are the most widely distributed in algae, being used in the food industry as stabilizers, thickeners and gelling agents (Stanley 1990). The structure and rheological properties of agars and carrageenans depend mainly on the species, environment and life history stage of the alga, but also on the isolation and chemical modification procedures (Therkelsen 1993).

Algal polysaccharides often occur as diverse natural mixtures of different types of sulfated galactans along the same hybrid heteropolymeric chain and thus are far from the idealized structure type. Various hybrid polysaccharides have been described, including D/L-galactan copolymers (hybrids of carrageenans and agars), κ/ι-, κ/β-, κ/μ-, ι/ν-, ξ/θ-carrageenan hybrids and more complex structures containing methylated or pyruvated repeating units (Estevez et al. 2001; Viana et al. 2004; Villanueva et al. 2004; van de Velde 2008). The actual distribution of different structures in such hybrid macromolecular chains often remains unknown. For κ/ι-carrageenans, both κ- and ι-structures can occur on the same chain with ι-structures either distributed singly or in short blocks in a random fashion or as a long sequence in a preponderantly κ-carrageenan chain (Guibet et al. 2006; Wichmann et al. 2006).

The hybrid nature of κ/μ- or ι/ν-carrageenans has never been debated as μ- and ν-carrageenans are the biological precursors of κ- and ι-carrageenans, respectively (Anderson et al. 1968; Stancioff, Stanley 1969), and are thus part of the polymeric chains. However, the biosynthetic pathway leading to κ/ι-carrageenan hybrids is still unclear (van de Velde 2008). Therefore, the molecular structure of these hybrids has been a matter of debate.

Furcellaria lumbricalis **galactans (furcellaran).** Furcellaran has been characterized as a hybrid κ/β-carrageenan complex (Painter 1966; Truus et al. 1997) 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, Arkhipova 1981; Bird et al. 1991). The carrageenan chemistry of *F. lumbricalis* does not vary with life-history generations (Bird et al. 1991), unlike that of the Gigartinaceae in which case the shift from the κ- to λ-carrageenan occurs with the change from gametophyte to sporophyte. It is generally believed that furcellaran contains different carrageenan segments along the same hybrid macromolecular chain with κ-carrageenan predomination (Knutsen, Grasdalen 1987). This becomes evident from x-ray diffraction studies of the polysaccharides from *F. lumbricalis*. The secondary structure of furcellaran is similar to that of stoichiometrically sulfated κ-carrageenans, 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).

Coccotylus truncatus **galactans.** Very little research has been conducted on the polysaccharides from *C. truncatus*. By the general primary structure of its major component, polysaccharides from this species belong to the ι-carrageenan pattern (Usov, Shashkov 1985; Truus et al. 1997). As is common for Phyllophoraceae, the carrageenan chemistry of *C. truncatus* varies considerably with life history gene-

rations. Based on immunoprecipitation and FTIR studies it has been found that the carrageenans from the gametophytic phase of this seaweed species belong to ι-family, while the sporophytic galactans contain both ι- and λ-structures (McCandless et al. 1981; McCandless et al. 1982). The detailed compositional and structural peculiarities of *C. truncatus* polysaccharides have still remained unresolved.

***Ahnfeltia tobuchiensis* galactans.** Depending on climatic conditions, some species of Ahnfeltia can produce agar as well as carrageenan type galactans (Levring et al. 1969). Although galactans from *A. tobuchiensis* have often been referred to as agar or agarose and utilized as gelling agents, the fine chemical nature and structure of this polysaccharide have never been established.

***Eucheuma cottonii* galactans.** Tropical seaweed *E. cottonii* (also named as *Kappaphycus alvarezii*) is extensively utilized for the industrial production of κ-carrageenan (Glicksman 1983). Thus, the composition of this seaweed galactan has been widely investigated. The hybrid polysaccharides from this species consist mainly of κ-carrageenan with small amounts of ι-structure (Aguilan et al. 2003). Also, minor quantities of μ-carrageenan, ν-carrageenan and **G**, **L** and **G6M,4S** residues have been reported to be components of this polysaccharide (Estevez et al. 2000; Estevez et al. 2004).

1.2.2. Inorganic and minor components

Industrially important seaweeds usually contain galactans 30–60% of dry-weight with some examples with phycocolloid content up to 80% (Therkelsen 1993). The exact chemical nature, inorganic part composition and purity of the separated polysaccharides are strongly dependent on the isolation procedures.

Carrageenans represent a group of highly sulfated polysaccharides, usually having sulfur content more than 5%. For agars, this value normally remains below 1.5% (Matsuhashi 1990). The sulfur content in a less charged fraction (defined as agarose) usually does not exceed 0.05% whereas sulfate rich fractions (agaropectin, defined as the fraction with sulfur content over 0.2%) can contain sulfur up to 2.5% (Armisén, Galatas 2000). Nevertheless, exceptionally high sulfur contents (over 6%) have been reported for *Gelidium vittatum* agars, with the respective values for the non-gelling fraction around 15.5% (Furneaux, Miller 1986). Somewhat lower sulfur contents (6.8%) have been observed for the highly charged fraction of some commercial grade agars (Izumi 1971).

The sulfur content of the three main carrageenan types usually remains in the range of 7–10% for κ-carrageenan, 9.5–11.5% for ι-carrageenan and 11–13% for λ-carrageenan. For furcellaran as a κ/β-carrageenan hybrid galactan, this value is between 5.5–6.5% (Armisén, Galatas 2000), whereas pure β-carrageenan does not contain sulfur in its molecule. Based on the idealized structure types, the theoretical (calculated) sulfur content values of the highly sulfated carrageenans appear to be somewhat higher: 8.3% for κ-carrageenan, 13.8% for ι-carrageenan and 17.1% for λ-carrageenan.

Ash content in agar varies from about 1.3% in highly purified samples to 3–4% in commercially prepared agar (Humm 1947; Matsuhashi 1990) and is below 6.5% in most cases. For carrageenans the ash content normally remains between 15–40%. The mineral composition of red algal galactans depends greatly on the ionic binding between certain metallic cations and on the negative charge of ester sulphate groups. The most abundant cations associated with the polysaccharide are Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and NH_4^{+}. Samples of different origin can also contain significant amounts of iron, aluminium and silicon (Matsuhashi 1990). Due to the strong tendency for carrageenans to sequester metal ions, the limits apply to the content of such heavy metals as Pb, As, Cd and Hg in food grade carrageenans (limits 5, 3, 2 and 1 mg/kg, respectively). Also, Cu and Zn can be found in relatively high amounts. Boron is a minor but a noteworthy constituent of algal galactans, in agars it rarely exceeds a level of 100 mg/kg (Matsuhashi 1990). This element occurs in the composition of red algae as free borate or is found in complexes with saccharides (Dembitsky et al. 2002).

Nitrogen has been reported to remain in crude polysaccharide preparations in appreciable amounts and could be removed by purification procedures to only a limited extent. It has been proposed that most of the remaining nitrogen is proteinaceous in nature and is strongly absorbed or covalently linked to the carrageenan matrix (Matulewicz, Cerezo 1980; King, Lauterbach 1987). For furcellaran, it has been found that the proteinic substances cannot be separated from the polysaccharide by re-precipitation, gel filtration or electrophoresis (Krasil'nikova, Medvedeva 1975).

The principal storage glucan of red algae, floridean starch (Yu et al. 2002) has often been reported to remain in the crude galactan preparations, sometimes in relatively high amounts (Knutsen, Grasdalen 1987; Estevez et al. 2008). Also, xylose has been reported to be contained in many galactan samples. This sugar can originate from a separate xylan (Turvey, Williams 1970; Myslabodski 1990) or occur as a chemically bound substituent on a galactan molecule (Furneaux et al. 1990; Duarte et al. 2004). Furthermore, commercial food grade carrageenans may contain varying amounts of standardizing additives, for example, sucrose, dextrose, KCl and buffer salts (Therkelsen 1993).

1.2.3. Molecular weight distribution and structural stability

Furcellaran and other carrageenans exist naturally in highly polymerized states. Also, the degree of polydispersity is high. Size exclusion chromatography (SEC) coupled with the light scattering or/and refractive index detection has been extensively used to determine the molecular weight distribution of red algal galactans (Lecacheux et al. 1985; Slootmaekers et al. 1991). The investigations often involve calibration with dextran, pullulan or poly(ethylene oxide) standards of low polydispersity (Villanueva et al. 2004), and sometimes with carrageenan markers (Spichtig, Austin 2008). Also, capillary gel electrophoresis and flow field-flow fractionation techniques have been demonstrated to be advantageous for such kind

of analyses (Roberts et al. 1998; Viebke, Williams 2000). The estimation of the molecular weight of κ-type carrageenans is often complicated due to the high aggregation behaviour of these polysaccharides, especially in the presence of co- and counter-ions. For this reason, in order to keep polysaccharic chains in a complete random coil form with the lowest hydrodynamic radii, high temperatures and low galactan concentrations are commonly used. 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 1900 kDa (Abad et al. 2004; Spichtig, Austin 2008), but values as high as 8000 kDa have also been reported (Montolalu et al. 2008).

An important characteristic of food grade algal galactans is their structural stability derived from the configuration of AG (D- or L-isomer) and the substitution pattern of hydroxyl groups. The destructive effect of acidic medium on agars and carrageenans has been widely investigated (Karlsson, Singh 1999), several reports have been published on the influence of ultrasound and radiation (Lii et al. 1999; Relleve et al. 2005). The thermal degradation of carrageenans has been elucidated for both the dry preparations and the water solutions (Bradley, Mitchell 1988; Friedenthal et al. 2000; Lai et al. 2000). For furcellaran, the intense destruction of polymeric chains has been reported to begin at temperatures above 115 °C (Friedenthal et al. 2000).

The susceptibility to degradation by high temperatures, ultrasound and acidic media usually follows the order of agarose > κ-carrageenan > ι-carrageenan, and has been related to the chain rigidity and sulfation degree (Lii et al. 1999). For carrageenans, a different response to radiation has been observed, in which case the respective order is λ-carrageenan > ι-carrageenan > κ-carrageenan, attributable to the conformational state of these galactans (Relleve et al. 2005). The stability optimum for carrageenans is around pH 9; severe degradation takes place at pH values below 3–4. Acid catalyzed hydrolysis of algal galactans occurs mainly at the α-1,3-glycosidic linkages; the process is enhanced by the presence of strained AG residues whereas sulfate groups at *O*-2 of α-galactopyranose units will reduce hydrolysis (Therkelsen 1993).

Estimation of the degradation degree of algal galactans is of particular importance for carrageenan type polysaccharides. As degraded carrageenans with M_w below 50 kDa (named as poligeenans) have been associated with negative health effects (Spichtig, Austin 2008), controlling their presence in food-grade carrageenans and in various food products is required.

1.2.4. Gelation process and rheology

An important characteristic of many algal galactans is their gel-forming ability, i.e. the ability to form well-ordered spatial structures during the cooling of their hot

polymeric solution. The gelling ability of such polysaccharides gives the basis for the vital functions of the red algae, as well as for their use in the food industry and microbiology. From more than 5000 red algae (Rhodophyta) species known at present (Graham, Wilcox 2000), only about 70–80 species are used for the industrial production of gelling galactans.

Gel formation in an aqueous solution is a complex process that depends 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, Ca^{2+} for ι-carrageenan) are found to induce conformational changes in the polymer with the initial coil-to-helix transition, which may be followed by subsequent aggregation of these helices to form a gel (Rochas, Rinaudo 1984; Paoletti et al. 1985). The final gel structure is often determined by mutual interactions between conformational transition, molecular cross-linking and phase-separation processes.

It is generally accepted that gelation mechanisms for agars and carrageenans follow different pathways. The association processes of polymer chains have been extensively investigated beginning with well-known research by Rees (Rees 1969; Rees 1972). From this time on, the studies on gelation of carrageenans and agars have mainly been concerned with the structure details of the junction zones of the gel-forming elements (Morris 1986; Piculell 1995), and some different models of gel formation have been proposed (Figure 3). Anderson et al. (1969) suggested that the junctions are formed by intertwined double helices, whereas Morris et al. (1980) and Robinson et al. (1980) independently proposed that they are caused by cation-mediated aggregates of double helices. According to the model of Smidsrød and Grasdalen (1982), the junctions in the carrageenan networks are formed by cation-specific salt bridges between ordered chain segments. The sequence of steps leading to gelation and the type of conformational transition (coil-helix or coil-double helix) are still a matter of study and discussion.

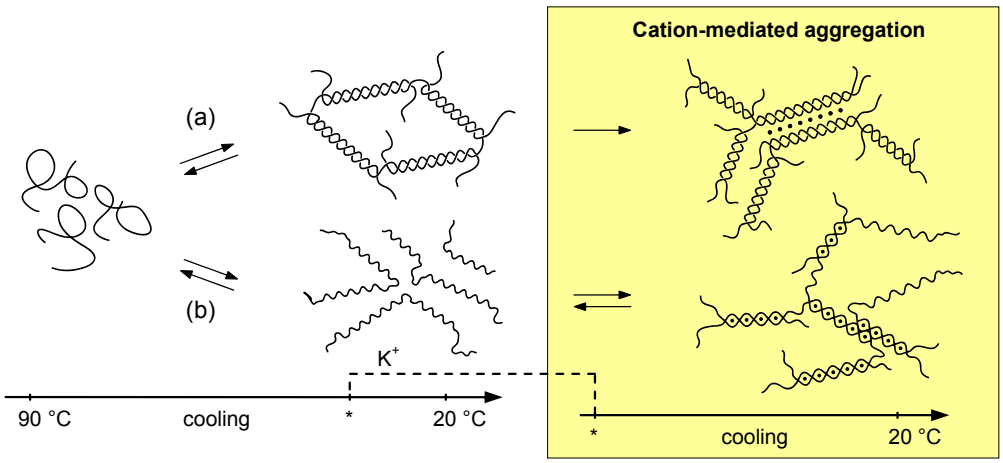


Figure 3. Models of gel formation of red algal galactans: (a) domain model proposed by Morris et al. (1980) and Robinson et al. (1980), and (b) nested, single-helix model proposed by Smidsrød and Grasdalen (1982). Dots indicate gel-promoting cations.

Of all natural polymer matrices, agarose gives the strongest thermoreversible hydrogels. High quality (low-sulfated) agars can form workable gels already at concentrations as low as 0.15% whereas for κ-carrageenan the polymer content for measurable gel strengths usually must exceed 0.5% in water systems and 0.1% in some bicolloidal systems (Therkelsen 1993). The gelation of agar-type polysaccharides (as 1% sols) usually occurs at 35–50 °C, whereas the melting of the gel normally takes place at temperatures above 80 °C. The gelling temperature is affected by the polysaccharide concentration as well as by the methoxylation degree of agar – a greater methoxylation at C-6 will correspond to a higher gelling temperature, the methoxylation of the rest of the carbons usually reduces both the gelling temperature and gel strength (Guiseley 1970; Armisén, Galatas 2000).

The melting and gelling temperature of carrageenans are strongly influenced by the presence of certain cations, at that, gel promoting cations have the effect of increasing these characteristics. Depending on the inorganic part, the gelation of κ-carrageenan usually occurs between 30–60 °C whereas the gel melting temperature remains 5–20 °C higher. For ι-carrageenan the hysteresis behaviour (gelling/melting temperature interval) is considerably lower, being about 5 °C. A positive correlation exists between gelling temperature and carrageenan molecular weight.

The role of counter-ions to promote carrageenan gelation is very specific. Based on theoretical and experimental evidence, it has been concluded that some cations (e.g. K^+, Rb^+) bind specifically to carrageenan helices, decreasing the net charge of the chains and favouring gelation (Nilsson, Piculell 1991). For κ-type carrageenans the ability to form gels follows the sequence Li^+, Na^+, NR_4^+ << Ca^{2+}, Cu^{2+}, NH_4^+ < K^+, Cs^+ < Rb^+ (Ciancia et al. 1997b; Michel et al. 1997). Also, the effect of anionic substances on gel formation has been studied (Takemasa, Nishinari 2004). Anions differ in their capacity to stabilize the κ-type carrageenan helix in the sequence Cl^- < NO_3^- < Br^- < SCN^- < I^-; the iodide and thiocyanate anions impede aggregation and gel formation (Zhang et al. 1991).

The presence of AG in the galactan molecule leads to increased flexibility of the polymer chain allowing a larger contraction of the random coil structure (Therkelsen 1993). It also allows a helical secondary structure which is essential for the formation of a gel network. Thus, increasing the AG content results in a higher gelling capacity for both agar and carrageenan type polysaccharides. For that reason the natural galactan mixtures are often submitted to alkaline treatment procedures, where α-galactose residues containing sulfate group at position *O*-6 and hydroxyl at *O*-3 (or *vice versa*) are converted into their respective anhydro forms (Ciancia et al. 1993; Ciancia et al. 1997a; Viana et al. 2004).

Gelation of agar occurs only by its agarose content (Armisén, Galatas 2000), resulting in rigid, brittle gels with high syneresis. Among carrageenans, κ-carrageenan gives the strongest gels, especially in the presence of both K^+- and Ca^{2+}-ions. However, κ-carrageenan in the pure K^+-form shows weaker gelling ability, affording gels with more elastic properties (Stanley 1990; Chen et al. 2002). The gel formation of ι-carrageenan is most favoured in the presence of Ca^{2+}-ions, resulting in elastic tixotropic gels. λ-Carrageenan does not form a gel as helix formation of its

molecule is sterically hindered by the presence of sulfate group at the *O*-2 of β-galactose residue.

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. The presence of precursor structures (e.g. μ- and ν-units) can result in a rapid decrease in gel strength values even at considerably low concentrations. It has been shown that above 20 mol% ν-units ι-carrageenan completely loses its ability to form a gel (van de Velde et al. 2002b).

κ-Carrageenan being its major component, furcellaran is generally similar to this polymer in both gelling properties and applications. However, due to the interaction with milk proteins, the texture of its milk gels is smoother and less brittle (Laos 2005; Laos et al. 2007). This generates a specific mouthfeel sensation of diary products containing furcellaran, making it a valuable raw material for the food industry. On the other hand, furcellaran does not combine synergistically with locust bean gum, as does κ-carrageenan, to produce firmer gels with less syneresis (Glicksman 1983; Chen et al. 2001). The lack of this phenomenon deteriorates slightly possibilities of using furcellaran in certain food applications. Finally, furcellaran can be precipitated by much lower KCl concentrations because its molecule contains fewer charged groups. Also, less alkali metal ions are needed to saturate the galactan matrix of furcellaran. Thus, lower concentrations of salt or hydroxide are required to achieve optimal gel strength (Bird et al. 1991).

2. OBJECTIVES

The general aim of this study was to investigate the structure-property relations of the galactans from the red algae *F. lumbricalis* and *C. truncatus*. The main objectives of this thesis can be formulated as follows.

- To characterize the composition and molecular structure of the gelling galactans from the Baltic red algae species *F. lumbricalis* (both attached and loose-lying forms) and *C. truncatus* (loose-lying form) comparatively with well-known commercial galactan preparations and with polysaccharide structures obtained from the seaweeds *A. tobuchiensis* and *E. cottonii*.
- To investigate the influence of extraction conditions on the molecular structure, inorganic part composition, molecular weight distribution and rheological characteristics of the polysaccharides from the biomass of Kassari red algae community and to study thermal stability properties of these galactans.
- To elucidate principal stages in the gel formation process of red algal galactans (carrageenans and agars) at the macroscopic level and to investigate the influence of counter-ions on the gel structure of furcellaran.
- To investigate the feasibility of some spectroscopic methods for the analysis of typical toxic elements in the brackish Baltic seawater and in seaweeds.

3. MATERIALS AND METHODS

3.1. Galactan and algae samples

Galactans form *F. lumbricalis*, *C. truncatus* and *E. cottonii* were isolated from the seaweed raw material according to the methodology described in section 3.2. The reference κ-carrageenan preparations were purchased from Sigma (**I**, **VI**) and Fluka (**I**, **III**); commercial ι-carrageenan preparations also originated from Sigma (**VI**) and Fluka (**II**). Agarose preparations for the comparative study were from Serva (standard EEO, Heidelberg, Germany) (**III**), LKB (medium EEO) and Sigma (type I-A, low EEO) (**V**). Agarose from *A. tobuchiensis* (**V**) was obtained from the pilot production plant of the Institute of Chemistry, Estonian Academy of Sciences.

Origination and characteristics of the seaweed species used for polysaccharide extraction in this work are presented in Table 1. Algae samples from Kassari Bay (the Baltic Sea, Estonia) were collected using SCUBA diving, the attached form of *F. lumbricalis* was a storm cast from the Baltic Sea, *E. cottonii* originated from the seaweed farm near the southern coast of Bali (Indian Ocean, Indonesia). The specimens of *C. truncatus* were separated carefully from the algal mixture (accounting for 38% of *C. truncatus* of the total wet biomass) containing *F. lumbricalis* as a dominant species. The algae samples were washed thoroughly with tap water, then with distilled water and dried at room temperature, except the raw material collected in 2004 (**VI**) that was dried at outdoor temperature after washing with tap water. If not specified otherwise, the furcellaran originated from the unattached form of *F. lumbricalis*.

Table 1. Characteristics of the seaweeds used for carrageenan extraction

Seaweed	Form*	Source	Location	Collection time		Depth	Paper
F. lumbricalis	U	Kassari Bay	58°40.40' N; 23°01.56' E	August	2004	6–8 m	**VI**
F. lumbricalis	U	Kassari Bay	58°41.60' N; 22°52.00' E	July	2005	8 m	**I**, **III**
F. lumbricalis	A	Kakumäe	59°27.72' N; 24°34.44' E	July	2006	shore	**I**
C. truncatus	U	Kassari Bay	58°41.08' N; 22°50.16' E	August	2007	8 m	**II**
E. cottonii	A	South of Bali	8°50.12' S; 115°12.98' E	June	2006	1–3 m	**I**

* U – unattached vegetative form, A – attached form

3.2. Extraction, modification and purification

Galactan extraction and precipitation procedures were carried out according to Figure 4. High temperature extraction/modification step (Figure 4a) was employed in publications **I–III** and **VI**. As a rule, the air-dry algal sample was refluxed in a 33-fold mass (**I–III**) of the extracting medium (distilled water, LiOH, NaOH, KOH, RbOH and CsOH solutions of various concentrations) but also algae/extracting agent mass ratios of 1:20 and 1:27 were used (**VI**). Due to the high viscosity of the extract, the mass ratio of 1:100 was used in the case of *E. cottonii*. 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 ºC) ethanol (95% v/v) or isopropanol (3-fold volume per extract) leading to the precipitation of polysaccharides. Bulk of the solvent was removed by passing through a thick nylon cloth (**I–III**). The galactans precipitated were finally separated from the alcohol-water mixture by filtration through a porous glass filter (porosity No. 3) and washed with cold (7 ºC) ethanol (95% v/v) or isopropanol. The isolated polysaccharide mixture was dried to a constant weight in a drying oven (60 ºC, as a rule for 2 days) and then milled.

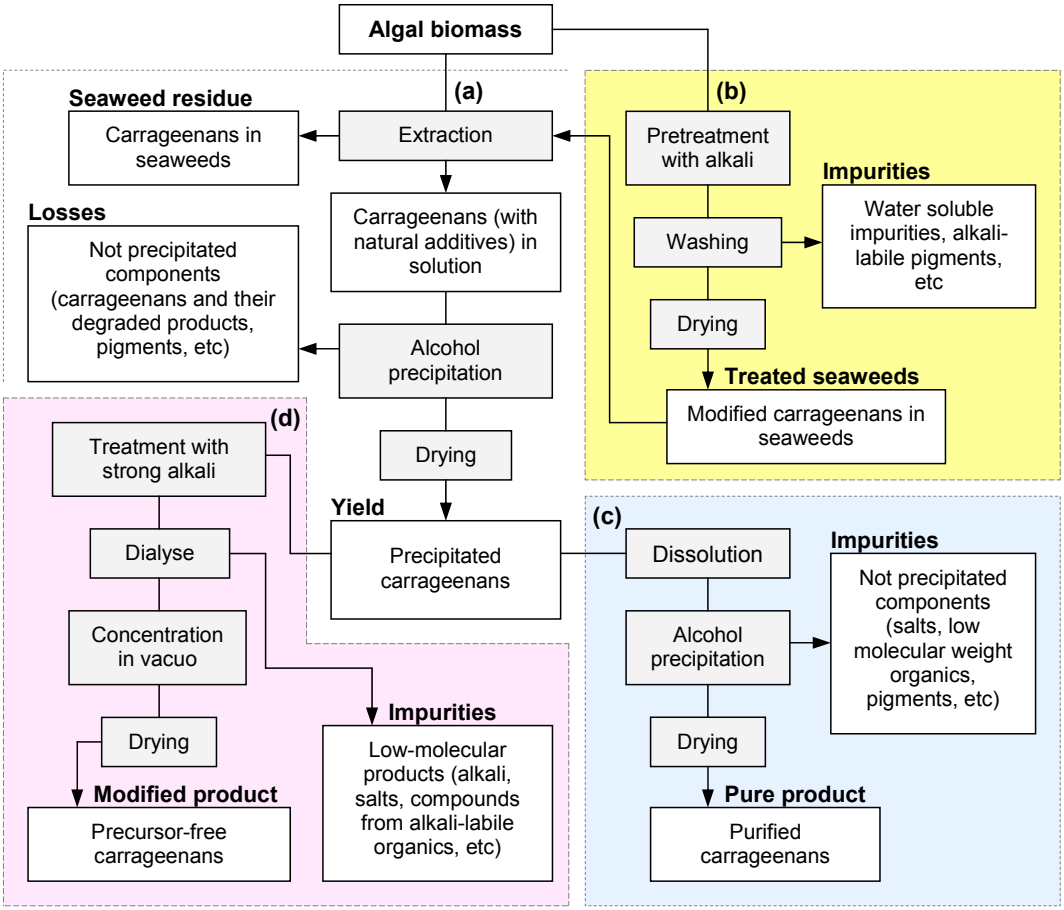


Figure 4. General isolation process of carrageenans from algal biomass: (a) high temperature extraction/modification step, (b) long-term room temperature modification step, (c) additional purification and homogenization step, (d) precursor removal step.

If needed, additional purification and homogenization (Figure 4c) were carried out (**I**) by precipitation of furcellaran in isopropanol (3-fold volume per 2% polymer solution), after that the preparation was dried again at 60 ºC for 2 days and then milled.

In order to decrease the amount of alkali-labile galactan-bound proteinic substances in the final product, long-term room temperature modification step (Figure 4b) was used (**I**, **VI**). The air-dry algal mixture was stored in a 33-fold (or 25-fold) mass of an alkali metal (Na^+ or K^+) hydroxide solution (0.1–0.6 mol/L) 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 boiling water).

In order to determine the percentage of alkali-labile carrageenan precursor units (**D2S,6S** and **D6S** residues) the native galactan preparation was subjected to a chemical modification procedure (**II**, Figure 4d). For this, the 1 M NaOH solution containing 0.5% of $NaBH_4$ and 0.25% of polysaccharide was heated in a water bath at 80 °C for 3 hours. After cooling, the solution was dialyzed (molecular weight cut-off 12 kDa) against bidistilled water, concentrated in vacuo, and freeze-dried.

3.3. Chemical analysis

AG content was determined (**I**, **II**, **V, VI**) colorimetrically using a resorcinol-acetal method and fructose as a standard sugar (Yaphe, Arsenault 1965). Galactose content was estimated (**II**) by anthrone assay (Yaphe 1960). The total carbohydrate content was estimated (**I**, **V**) using the phenol-sulfuric acid method (DuBois et al. 1956) without a previous hydrolysis of the polysaccharide. Pyruvate acetal substitution was detected (**II**) as the 2,4-dinitrophenylhydrazone derivative of pyruvic acid (Sloneker, Orentas 1962), the value corrected according to Duckworth and Yaphe (1970). Methoxy group content was analyzed (**V**) by a classical method (Cheronis, Ma 1964) and a standard microchemical apparatus (Steyermark et al. 1956). The nitrogen content was measured (**I**, **II**) by the Kjeldahl procedure. The other elements were determined (**I, II, IV**) using the ICP-OES, ICP-MS, ETAAS and FAAS methods. Sulfur released from the galactan matrix during the degradation process was estimated (**II**) chromatographically (Karlsson, Singh 1999). Ash content was determined by heating the moisture-free samples in a muffle furnace at 550 °C for 6 hours.

3.4. Spectroscopic methods

The FTIR spectra of carrageenan samples were scanned (**I, II, V, VI**) using a PerkinElmer FTIR System Spectrum BX spectrometer (12 scans per spectrum; nominal resolution: 4 cm^{-1}) from thin (about 0.015 mm) films obtained by a slow

evaporation of 1% solutions in polystyrene Petri dishes at room temperature. The spectra were recorded in the 4000–370 cm^{-1} region.

^{13}C-NMR analyses were carried out using a Bruker spectrometer operating at 500 MHz (**III**, **V**) or 800 MHz (**I**, **II**). The spectra from a 2–4% carrageenan solution in D_2O were obtained at 40–85 °C, and maximum 100 thousand transients were collected before the Fourier transform. The chemical shifts were converted to a tetramethyl silane scale on the basis of the C-6 signal from the galactose subunit having a constant value of 61.3 ppm (**II**, **III**, **V**) for these carrageenans (Usov, Shashkov 1985) or to a DSS scale on the basis of the C-6 signal at 63.49 ppm (**I**) (van de Velde et al. 2004). The spectra presented in this thesis are given on the basis of the DSS scale.

Optical density measurements were made (**III**) on a temperature controlled Shimadzu UV-1601 spectrophotometer at 260 or 400 nm against water as a blank. Hot (≈90 °C) galactan solution was inserted into a 2-mm cuvette, sealed and thermostated in the cuvette holder at 95 °C for 5 min to allow sample equilibration. Thereafter, with continuous absorbance recording, the temperature was decreased slowly (0.5 °C/min) to 20 °C, followed by isothermal hold for 50 min.

3.5. Size exclusion chromatography

SEC of carrageenans was performed (**I**, **II**) 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 protected by a Shodex OHpak SB-G guard column. 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. The temperature of the columns was maintained at 60.0 °C. A calibration curve was constructed using dextran (668, 410, 273, 148, 80.9, 48.6, 23.8, 11.6, 5.2, 1.3 kDa) or pullulan (788, 404, 212, 112, 47.3, 22.8, 11.8, 5.9, 1.32, 0.342 kDa) standards, the elution volume was corrected to the internal marker of ethylene glycol (0.01% in sample) at 22.89 mL. The equations of the curves were as follows: $\log M_w = 0.02\,x^2 - 1.3503\,x + 22.361$ for dextran ($R^2 = 0.9987$) and $M_w = -0.0205\,x^2 + 0.1981\,x + 7.5575$ for pullulan ($R^2 = 0.9995$) standards (M_w, average molecular weight; x, elution volume). The carrageenan concentration used was 0.07% and the sampling volume 100 µl. The M_w values are given relative to pullulan calibration in this thesis.

To obtain more reliable results, the galactan samples were dissolved in the same solvent used as an eluent in the SEC 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 10 minutes. The hot (60 °C) sol was filtered through a 0.45 µm membrane (Spartan 30/0.45RC), allowed to cool down and then injected into the HPLC system.

3.6. 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 (**I**–**III** and **VI**) in triplicate for 1–2% gels formed by dissolving the dry galactan in hot water (or a salt solution) after gelling in an air thermostat at 20 °C for 4 h. The cylindrical samples were 35 mm in diameter. The force needed to rupture the gel by the plunger was expressed in g/cm^2; the constant increase of the stress to the gel surface by addition of mass 350 g/min was achieved. If not stated otherwise, the gel strength values are given for 1.5% gels. All percentages in this thesis are expressed as weight percentages (w/w), unless otherwise indicated.

The melting temperature of the 1.5% gel aged at 20 °C for 4 hours was determined (**I**, **III**) 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) heating on a water bath. The tube was then cooled (1.0 °C/min) and turned horizontally every minute without removing it from the water bath. The gel-setting temperature was determined (**I**, **III**) 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 (**I**) by a centrifugation test by using a Hettich ROTINA 38R centrifuge. The centrifuge tubes were filled with a hot 1.5% galactan solution, allowed to set for 2 hours, then closed and stored at 25 °C for 7 days. After storage they were centrifuged at 4500 RPM for 15 min. Then the free water was separated, weighed and expressed as a percentage of the total water content.

3.7. Scanning electron microscopy

Scanning electron microscopy was carried out (**I**, **III**) on a high resolution LEO Supra 35 electron microscope equipped with Röntec EDX XFlash 3001 detector and Thermo Noran Maxray ER Parallel Beam spectrometer. Samples were prepared by inserting preheated (90–95 °C) stainless steel or copper capillary tubes (inner diameter 2.0 mm and 1.2 mm, respectively, length 60 mm) into hot (90–95 °C) 1.5–2% galactan solution allowing them to fill up. After gelling for 4 hours at 20 °C, the filled tubes were tightly closed. The prepared tubes were then heated on a water bath at 98 °C for 30 min, followed by slow (0.5 °C/min) cooling to 20 °C. The samples removed from the thermostat during the cooling stage (at predetermined temperature) were rapidly frozen in liquid nitrogen at different stages of gel formation (from 90 to 20 °C), cryofractured twice to produce small (5 mm in length) open-ended tubes filled with 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.

4. RESULTS AND DISCUSSION

4.1. Yields and extraction dynamics

The yield and quality of carrageenan depend notably on the algal raw material, life-history stage of the seaweed, isolation procedures, extraction duration, and the medium used. Although pure water is an effective medium to isolate furcellaran from the algal tissue (**VI**), the separation of the galactans from alcohol/water mixture can be problematic due to the subtle nature of the precipitate formed at low cation concentrations.

The total yields (obtained through an 8-hour process) for carrageenan extraction from the biomass of *F. lumbricalis* remained around 30% (**VI**). During the first 2 hours already bulk of the galactans were extracted from the algal raw material (in most cases significantly over 50% of the total yield). Actually, the process could be interrupted after 4 hours to prevent excessive destruction of the polysaccharic chains but still achieving rather high extraction yield.

As the extracting media containing 0.02 mol/L alkali were found to give products with the highest gel strength values (**VI**), this concentration was predominantly employed in isolation procedures involving hot alkaline media. The yields of furcellaran in the presence of various alkali metal hydroxides in the extracting medium (0.02 mol/L) increased in the row Li^+, Na^+, K^+, Rb^+, and Cs^+ with values of 23%, 24%, 24%, 26%, and 28%, respectively (during a 4-hour extraction). The notable variance in extraction yields is not only due the better extracting ability of the more active alkali but is the result of the mutual interaction of different processes occurring in the both seaweed and polysaccharide levels. The presence of gel promoting cations in the extraction medium leads to coarser precipitate that can be easily separated from alcoholic solution, thus minimizing the extraction losses (Figure 5).

Another important factor affecting the yield of carrageenans is the proportion of inorganic cations bound to the polysaccharide matrix during the extraction process. For the furcellaran backbone having the overall negative charge to bind 5% of K^+-ions, the extraction yield of 24% for the K^+-form would have the following values in the case of the total ionic exchange: 23.0% (Li^+-form), 23.5% (Na^+-form), 25.4% (Rb^+-form), and 26.9% (Cs^+-form). This is also in accordance with the ash percentage values of these preparations (see section 4.2.2). Within the range of Li^+, Na^+, K^+, and Rb^+, there is a good positive linear correlation (R^2 = 0.9997) between the ash contents and the calculated (theoretical) yields, while the respective values for Cs^+-form of furcellaran considerably lower the correlation (R^2 = 0.9903). This reveals that for the studied furcellaran matrix the capacity to bind Cs^+-ions is slightly lower than the theoretical approximations suggest.

Taking into account the actual yields, it becomes evident that the higher extraction yields in the presence of heavier alkali metal ions are primarily caused by their higher atomic weights. Thus, the extraction of seaweeds in different low concent-

ration alkali solutions leads to products with virtually the same amount of organics (Figure 5). Similarly, the lower extraction losses in the case of stronger alkalis are connected with the absorption of heavier alkali metal ions on the seaweed residue.

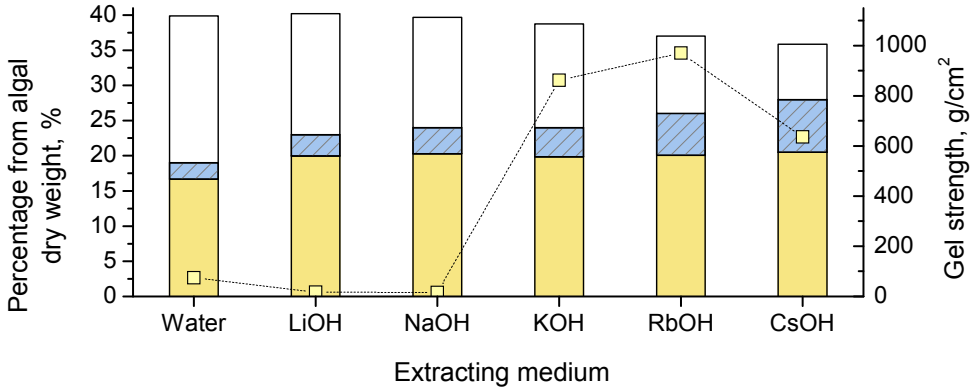


Figure 5. Characteristics of carrageenan extraction from the red alga *F. lumbricalis*. Galactans extracted 4 hours in 0.02 M alkali metal hydroxide solutions or in pure water. (▨) *A*, ash content of the extracted polysaccharide; (▭) *B*, calculated organic matter content (*B* = *Y* - *A*; *Y*, yield); (▭) *C*, extraction losses (*C* = 100 - *R* - *Y*; *R*, seaweed residue percentage after extraction); (- -▭- -) gel strength values for the 1.5% galactan gels (right scale).

The galactan content can be considerably influenced by the life-history stage of the alga. For the Far-Eastern seaweed species, *Tichocarpus crinitus*, which has a close polysaccharide composition to furcellaran, a significant difference in yields have been confirmed for the vegetative (21%) and reproductive (37%) forms (Barabanova et al. 2005). Due to the low salinity, mainly vegetative *F. lumbricalis* forms are found in Estonian waters.

A considerable difference in extracting yields between the attached and unattached form of *F. lumbricalis* was established. While the loose-lying form yields only 19% of the polysaccharides during a 4-hour water extraction, the respective characteristic for the attached *F. lumbricalis* is 32% (**I**). High galactan yields in the case of attached *F. lumbricalis* are not rare, even the values over 50% have been described in the literature (Knutsen, Grasdalen 1987; Yu et al. 2007). Also, no seasonal variation in the yield have been observed for this species (Knutsen, Grasdalen 1987).

Another dominant seaweed species in Kassari algal stratum, *C. truncatus*, is characterized by a relatively low galactan content. Water extraction of this species affords maximum yields (17%), lower recoveries (12%) were obtained by the process involving hot alkaline media (0.02 M KOH, extraction duration 4 hours) (**II**). Evaporation of the alcoholic solution used for the precipitation of water-extracted polysaccharides indicated that a notable amount of the components soluble in hot water (4% of the starting raw-material) were not removed by

filtration. Thus, it can be predicted that the actual galactan percentage of this species can be around 20%.

The galactan content of *C. truncatus* has been reported to depend considerably on seasonality; according to the literature, the extraction yields usually remain around 11–20%, however, values as low as 1–2% have been observed for specimens collected in colder season (Mathieson et al. 1984; Truus et al. 1997). Considerably low extraction yields of *C. truncatus* have previously been attributed to the morphological peculiarities (e.g. thick cortex) of this seaweed species (Truus et al. 1997). Nevertheless, this was not confirmed in the present study, as the further extraction from the seaweed residue afforded notably low yields (0.2–0.3%) (**II**).

A widely utilized commercial carrageenophyte *E. cottonii* affords notably high yields, more than twice greater than those observed in the case of the seaweeds from Kassari algal stratum. Extraction of carrageenans from this species resulted in 61% of polysaccharides during the 4-hour extraction in pure water, slightly lower yields (57%) were obtained if 0.02 M KOH solution was used as the extracting medium. Somewhat lower yields in the case of alkaline extraction media (compared to pure water) have also been reported for other *Eucheuma* species (Freile-Pelegrín et al. 2006).

4.2. Chemical composition and molecular structure

The structure of algal galactans was investigated by ^{13}C-NMR and FTIR spectroscopy. The polysaccharides were characterized by ICP-OES, ETAAS and chemical methods in comparison with well known carrageenan and agarose preparations.

As was confirmed by the FTIR investigations (**I**, **II**, **VI**), the galactans from both of the dominant seaweed species of Kassari algal stratum belong to the family of sulfated galactans. Based on colorimetric analyses, the polysaccharides were found to contain a similar amount of galactose with the respective values of 40 ± 1% for furcellaran and 38 ± 1.5% for *C. truncatus* galactans and more variable percentages of AG (29 ± 1% and 20 ± 1.5%, respectively). Also, a considerable amount of pyruvate (0.5%) was detected in the composition of *C. truncatus* polysaccharide, making it a moderately pyruvated galactan. The total carbohydrate content (with sulfate) of furcellaran was estimated at 90 ± 3%, the same characteristic for *C. truncatus* galactan was somewhat lower (86 ± 3%). In addition to various saccharic components, both of the studied galactan preparations where found to contain a diverse inorganic part in their composition (see section 4.2.2).

A slight increase in the AG content by 1.6% for furcellaran and by 2.1% for *C. truncatus* galactans during the alkaline extraction in 0.02 M KOH solution was observed (compared to the water-extracted preparation), indicating the presence of alkali-labile precursor residues. In order to determine the total amount of cyclizable precursor units, alkaline modification of galactans in the presence of $NaBH_4$ was performed. The analysis of the modified polysaccharides showed an increase in the AG content by 3.6% for furcellaran and by 4.8–6.7% for *C. truncatus* galactans.

It was evidenced that after the chemical modification process, the content of AG in these galactans was similar to the values observed for their respective commercial preparations from Sigma (κ-carrageenan, 32.3% AG; ι-carrageenan, 26.5% AG) and Fluka (ι-carrageenan, 28.3% AG).

Figure 6 shows the molecular weight distribution of the water-extracted galactan preparations under study. Compared to dextran calibration, the M_w values for the studied galactans remain substantially lower when expressed relative to pullulan standards. As this is apparently the effect of the branched pattern of the dextran molecule, the M_w values in this work are given relative to pullulans with more linear chain structures. While the water-extracted galactans from *C. truncatus* and *E. cottonii* show similar M_w values, the polymerization degree of furcellaran remains more than two times lower being closer to the characteristic observed for *A. tobuchiensis* galactans (Figure 6). Unlike noted for *C. truncatus* galactans, the extraction parameters can have a notable effect on the M_w characteristics of furcellaran (see section 4.4.2).

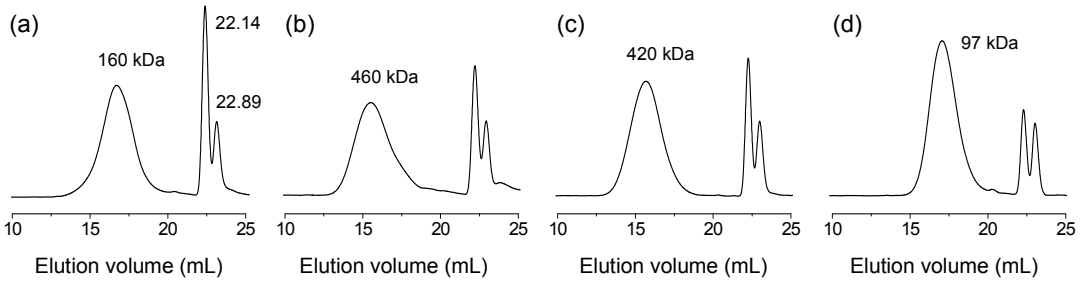


Figure 6. Size exclusion chromatography elution profiles for water-extracted galactans from (a) *F. lumbricalis*, (b) *C. truncatus*, (c) *E. cottonii*, and (d) *A. tobuchiensis*. Peak at 22.89 mL corresponds to the internal marker of ethylene glycol. Peak at 22.14 mL is due to the small difference in $NaNO_3$ concentration between the HPLC eluent and the sample solution.

4.2.1. Structure of the galactans from the Kassari algal community

The ^{13}C-NMR spectra of the galactans from the dominant seaweeds of the Kassari algal community is presented in Figure 7 and the values of chemical shifts of carbon signals are summarized in Figure 8.

The major components of the galactan from *F. lumbricalis* of the Baltic Sea were **G** (15 ± 1%), **G4S** (36 ± 1.5%) and **DA** (29 ± 1%) residues, being indicative of the κ/β-carrageenan (**G4S-DA**/**G-DA**) backbone. Also, the **D6S** (6 ± 0.5%) units from γ-carrageenan (**G-D6S**) were present in low amounts, and traces of **G6S** residues from ω-carrageenan (**G6S-DA**) and **G6M** units were detected. Based on ^{13}C-NMR investigations, possibly minute amounts of α-carrageenan diads (**G-DA2S**) were

also present. The spectrum also indicated the presence of ι-carrageenan as a minor component, originating presumably from the minute amounts of *C. truncatus*, another dominant seaweed species present in the Kassari algal stratum that forms hardly separable mixtures with *F. lumbricalis*. This presumption is further confirmed by the fact that no ι-carrageenan segments were detected in the furcellaran preparation originating from the attached form of *F. lumbricalis*.

The precursor **D6S** structures appeared to be present in galactans from both of the studied *F. lumbricalis* forms in almost equal amounts. According the literature, much higher γ-carrageenan contents have been found in the polysaccharides from the attached *F. lumbricalis* growing in more saline environments (Knutsen, Grasdalen 1987). The only notable structural difference between the studied furcellaran preparations of different origin was the significantly higher **G6S** content in the case of the polysaccharides from the attached form of the seaweed (**I**).

The main components of the polysaccharide from *C. truncatus* were **G4S** (44 ± 2%) and **DA2S** (30 ± 1.5%) residues, indicating the ι-carrageenan (**G4S-DA2S**) backbone. As the minor components, alkali labile **D2S,6S** (12 ± 2%) units from ν-carrageenan (**G4S-D2S,6S**) and **GP** (1.4%) residues from pyruvated α-carrageenan (**GP-DA2S**) were also present in the galactan. The presence of **GP** residues is commonly associated with agar-type polysaccharides, although many complex carrageenans can contain those structures in low amounts (Stevenson, Furneaux 1991). The ^{13}C-NMR spectrum of the galactan revealed also the presence of κ- and β-carrageenans (**G4S-DA**; **G-DA**) both of which were considered as contaminants from *F. lumbricalis*.

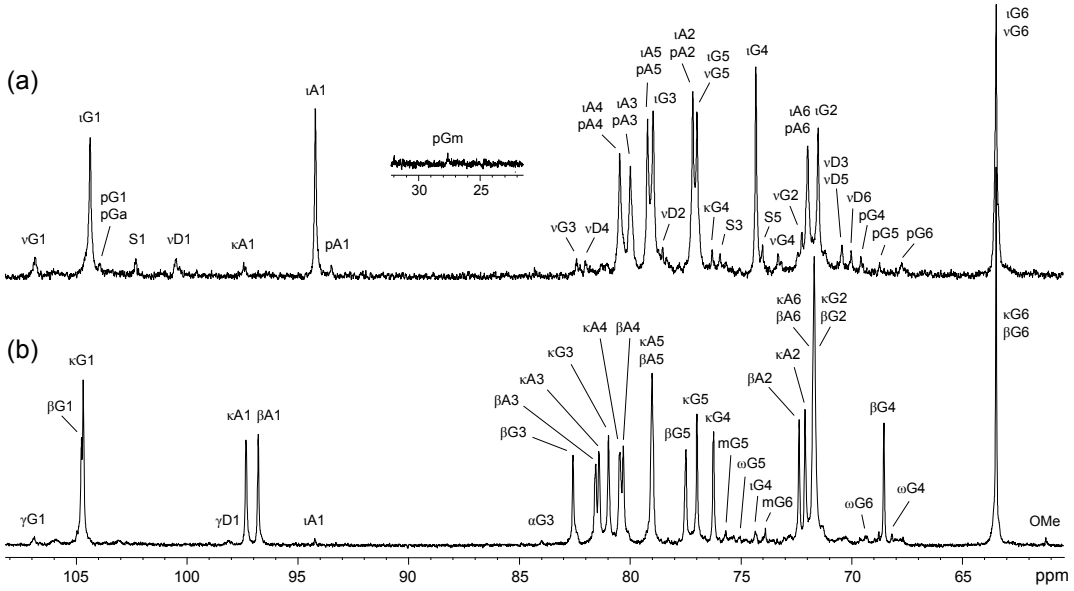


Figure 7. ^{13}C-NMR spectra of water-extracted galactans from the Kassari algal community. (a) Galactans from *C. truncatus*, 38,000 transients, recorded at 40 °C; (b) galactans from *F. lumbricalis*, 100,000 transients, recorded at 50 °C. S – glucose units in floridean starch. For other signal marks and chemical shift values, see Figure 8.

For reference, the structure of some other galactan preparations was also investigated (**I**, **III**, **V**). The ^{13}C-NMR spectrum of the polysaccharide from *E. cottonii* confirmed the presence of κ-carrageenan (**G4S-DA**) as a major component, but also indicated the presence of minor amounts of **DA2S**, **D6S**, **D2S,6S** and **G6M** residues (**I**). The commercial κ-carrageenan preparation from Sigma was free of precursor structures, but contained also traces of **DA2S** and **G6M** units (**I**). The main components of *A. tobuchiensis* galactan were **G** (58%), **LA** (30 ± 3.5%), **LA2M** (8 ± 1%) and **G6S** residues, indicating agarose backbone (**V**). For the other studied agarose preparations, the methoxy groups were located mainly at the C-6 position of the galactose subunit (**G6M** residues) (**III**, **V**).

The sulfur content of the galactans from *F. lumbricalis* inhabiting the Kassari algal stratum is relatively high (on an average 5.3% of S) for furcellaran, indicating that there is approximately one sulfate group per every three monomer residues (**I**). According to the literature, sulfur level for the galactans from this seaweed species usually remains in the range of 2.7–6.3% (Bird et al. 1991). Previously, considerably low sulfur levels (2.7–3.3%) have been reported (Truus et al. 1997) for the furcellaran from the Kassari stratum, but the values as high as 6.1% have also been found (Laos, Ring 2005) for galactans from this raw material.

The galactans from *C. truncatus* are characterized by the sulfur content (on an average 8.6% S) considerably lower than is usual for ι-type carrageenans (**II**). Previously, low levels of this element (6.3–7.4%) have also been reported for the Baltic *C. truncatus* galactans (Truus et al. 1997; Mikulich, Kopytov 2002), as well as for the gametophytic polysaccharides (about 6% S) originating from this seaweed species (McCandless et al. 1982). On the other hand, carrageenans from the sporophyte form of this alga have been characterized as more highly sulfated galactans with sulfur content over 10% (McCandless et al. 1982).

For the galactans from both of the dominant species of the Kassari algal stratum, the sulfur content varies to a slight degree by the extraction medium. Surprisingly, higher values were observed for the alkali-extracted preparations. The phenomenon is an object of discussion in section 4.2.2.

The sulfur content of the native water-extracted galactan from *E. cottonii* is almost theoretical for κ-carrageenan being considerably higher than was found in the commercial κ-carrageenan preparation from Sigma. Lower sulfur percentage in the latter is attributable to the more abundant inorganic part of the commercial preparation. Compared to carrageenans, the sulfur content of 0.2% measured for the galactan from *A. tobuchiensis* is fairly low, but is still the highest conventional limit for an agar-type polysaccharide (**V**).

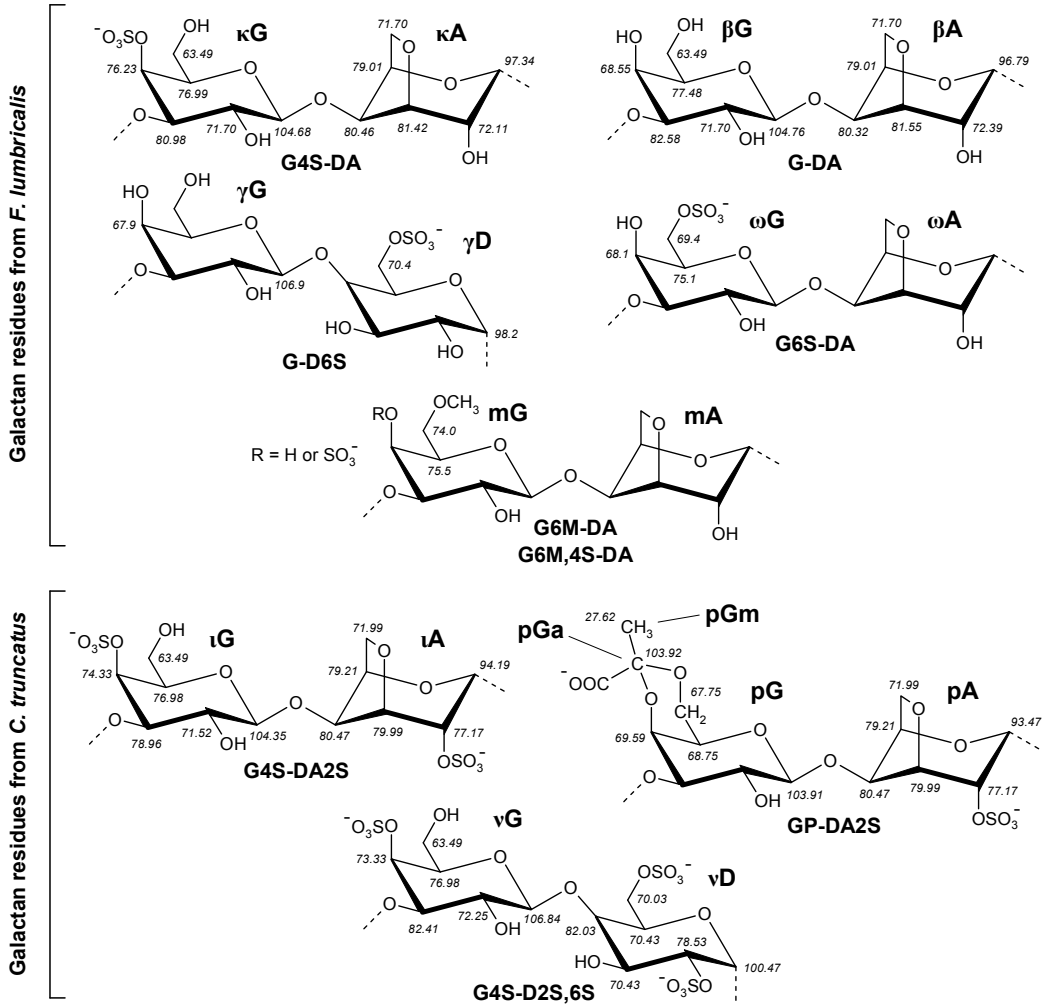


Figure 8. Galactan structures from the dominant seaweeds of Kassari algal stratum. The numbers in italic indicate ^{13}C-NMR chemical shift values.

The nitrogen content in the galactans from both of the dominant species of the Kassari algal stratum is relatively high, indicating a notable amount of proteinic substances remaining in the preparations (**I**, **II**). This is obviously caused by the high protein content of these seaweeds, usually remaining in the range of 13–25% in dry weight for *F. lumbricalis* (Krasil'nikova et al. 1972; Indergaard, Knutsen 1990) and of 16–20% for *C. truncatus* (Mathieson et al. 1984). It has been reported that the proteinic substances cannot be separated from the furcellaran matrix by ordinary techniques, e.g. re-precipitation, gel filtration or electrophoresis (Krasil'-nikova, Medvedeva 1975). Taking into account the high nitrogen content of *C. truncatus* galactan preparations, possibly the same tendency takes place for the polysaccharides from this species. 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 from Sigma. It was established that the amount of galactan-bound nitrogen does not depend significantly on the extraction conditions (**I**, **II**).

The principal storage glucan of red algae, floridean starch, has been reported to remain in the crude furcellaran preparations in relatively high quantities (Knutsen, Grasdalen 1987) attributable to the high content of this polysaccharide in the tissues of *F. lumbricalis* (Bird et al. 1991). Nevertheless, this glucan was not detected in the studied furcellaran preparations or in the galactan sample from *E. cottonii* (**I**). On the other hand, a significant amount of floridean starch was found in the *C. truncatus* galactan preparation extracted in pure water (**II**), whereas lesser quantities were observed in the alkali-extracted sample and in the commercial κ-carrageenan preparation from Sigma.

4.2.2. Inorganic part composition

The mineral composition of carrageenans depends greatly on the ionic binding between certain metallic cations and on the negative charge of ester sulfate groups. Ash content in the water-extracted furcellaran preparation is relatively low (12.3%) for the κ-type carrageenan. Using low concentration (0.02 mol/L) alkali metal hydroxides as extracting media resulted in products with ash contents increasing in row 13.2, 15.4, 17.3, 22.9 and 26.8% for Li^+, Na^+, K^+, Rb^+ and Cs^+-forms of furcellaran, respectively. The ash content for κ-carrageenan preparation from Sigma (24.9%) remained in the typical range for κ-type carrageenan.

Both the dominant species from Kassari algal stratum are characterized by low Na^+ and K^+ contents, whereas the level of these cations in the tropical seaweed *E. cottonii* remains significantly higher (**I**, **II**). An opposite tendency is observed for divalent cations Mg^{2+} and Ca^{2+}, which are more abundant in the tissues of *F. lumbricalis* and *C. truncatus* inhabiting the brackish Baltic Sea. Among these elements, the typical gel promoting cations for carrageenans K^+ and Ca^{2+} are of lower occurrence in the Baltic seawater (**IV**) (Table 2).

F. lumbricalis and *C. truncatus* have a diverse inorganic part in their composition. Both of the species contain notable amounts of iron and manganese in levels of about 50–800 times higher than is found in *E. cottonii* (Table 3). The microelement composition of algae varies strongly by samples originating from different habitats. Regardless of the great variation of concentrations, the elements may be determined equally well by AAS and ICP-OES (**IV**). Since there is a poor correlation between the content of microelements in seawater and algal biomass, the trustworthy characterization of the marine environments on the basis of algal material is complicated (**IV**).

Considering the ratio of element content in seaweed to its level in water-extracted polymer preparation (enrichment factor from alga to galactan), the galactan's affinity to bind certain cations becomes evident. Also, some information can be obtained whether the certain elements are localized mainly in the galactan or are present in the other parts of the alga. The ionic affinity is a characteristic strongly connected with the structure type of the galactan. For κ-type carrageenans from *F. lumbricalis* and *E. cottonii*, notable enrichment of the galactan matrix is evident for K^+-ions, whereas ι-carrageenans from *C. truncatus* are characterized by considerable affinity for divalent cations Mg^{2+}, Ca^{2+} and Ba^{2+} (Tables 2 and 3). On the other hand, the affinity for some typical toxic elements like cadmium and arsenic is very low; the content of arsenic in *E. cottonii* is nearly 60 times higher than in its galactan preparation; for *F. lumbricalis*, the same characteristic is 8.8. In the case of *E. cottonii*, some microelements were found in substantially higher concentrations in the galactan preparation than was detected in the algal raw material. This can be attributed to the heterogeneous distribution of inorganics in this seaweed.

Table 2. Content of some elements in the studied algae, carrageenan and seawater samples

Preparation	Element content (%)						
	Na	**K**	**Mg**	**Ca**	**N**	**P**	**S**
F. lumbricalis[*]	0.050	0.32	0.76	1.73	4.18	0.23	4.09
C. truncatus[*]	0.020	0.09	0.55	3.44	3.23	0.09	4.99
E. cottonii[*]	1.04	3.40	0.66	0.89	1.32	0.08	5.95
GW *F. lumbricalis*[**]	0.045	0.62	0.87	1.82	0.70	0.07	5.22
GA *F. lumbricalis*[**]	0.049	4.98	0.24	0.83	0.66	0.03	5.37
GL *F. lumbricalis*	0.040	0.72	0.55	3.32	0.76	0.02	5.26
GW *C. truncatus*	0.021	0.11	0.87	4.33	0.73	0.11	8.44
GA *C. truncatus*	0.041	6.19	0.22	2.86	0.82	0.09	8.66
GW *E. cottonii*	1.10	4.11	0.75	0.94	0.32	0.02	7.50
GA *E. cottonii*	0.55	10.37	0.10	0.66	0.50	0.02	8.24
κ-carrageenan (Sigma)	0.59	6.15	0.16	2.71	0.10	0.01	7.19
	Element content (mg/L)						
Baltic seawater[***]	1571	64	210	83	x	x	x

For indications, see Table 3.

Considering the actual sulfur content in the structure, the extracting media containing KOH at a concentration as low as 0.02 mol/L already saturate the galactan matrix with K^+-ions up to 80% for furcellaran and up to 60% for *C. truncatus* polysaccharides during the hot alkaline extraction (**I**, **II**). The treatment also affects the mineral heterogeneity of carrageenans. For both of the studied Baltic species the

hot alkaline extraction yields products with higher nickel, copper and cadmium levels (compared to the preparations extracted in pure water), whereas the contents of magnesium, calcium and manganese decrease markedly. Additionally, there was a notable decrease in furcellaran's iron content; for *C. truncatus* galactans the increase in sodium, cobalt, zinc and molybdenum concentrations occurs.

Table 3. Content of some minor elements in the studied algae, carrageenan and sea-water samples

Preparation	Element content (mg/kg)									
	Fe	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**Mo**	**Ba**	**Cd**	**As**
F. lumbricalis[*]	2465	3448	2.22	20.73	20.4	37.5	<0.5	34.6	0.343	6.25
C. truncatus[*]	1744	493	2.29	9.43	25.2	28.4	0.64	50.0	0.21	<0.06
E. cottonii[*]	32	4.2	0.18	<0.1	0.6	2.80	0.22	x	0.902	11.8
GW *F. lumbricalis*[**]	903	2317	1.05	3.27	3.69	14.9	<0.5	31.4	<0.05	0.71
GA *F. lumbricalis*[**]	481	352	0.67	5.74	14.6	14.8	<0.5	30.2	0.244	0.30
GL *F. lumbricalis*	509	592	0.71	6.11	10	9.68	<0.1	x	0.015	0.69
GW *C. truncatus*	1506	577	1.45	1.30	4.3	9.2	1.52	73.3	<0.05	<0.06
GA *C. truncatus*	1600	162	2.35	6.05	29.2	16.8	2.07	88.1	0.37	<0.06
GW *E. cottonii*	50	7.3	0.73	0.88	3.1	8.86	0.12	x	0.063	0.20
GA *E. cottonii*	68	1.6	0.50	0.48	3.2	3.67	<0.5	5.8	1.307	x
κ-carrageenan (Sigma)	166	9.5	0.40	0.19	10	4.97	0.20	x	0.114	0.68
	Element content (μg/L)									
Baltic seawater[***]	172.1	16	0.23	2.2	14	12	1.3	24	0.1	1.3

[*] Algae washed thoroughly with tap water and distilled water; [**] additionally purified by alcohol precipitation; [***] an average of the samples collected from seven places on the seashore around Tallinn City. GW – water-extracted (4 hours) galactan; GA – alkali-extracted (in 0.02 M KOH, 4 hours) galactan; GL – algae treated with 0.4 M KOH solution for 7 days, extracted 4 hours in water; x – not measured.

Interestingly, for both *F. lumbricalis* and *C. truncatus*, the hot alkaline extraction of seaweeds affords products with somewhat higher sulfur contents than those obtained by the isolation procedures not involving alkali (**I**, **II**). Generally, the hot alkaline medium reduces the sulfur content of carrageenans containing **D6S** or **D2S,6S** residues by conversion (cyclization) of these precursor units to **DA** or **DA2S**, respectively (Ciancia et al. 1993). The 4-hour extraction time and low alkali concentrations employed for carrageenan extraction should be insufficient for the complete cyclization reaction (Ciancia et al. 1997a). Higher sulfur contents in the case of alkali-extracted preparations are likely to be caused by the better solubility

of the precursor rich polymeric chains in alcoholic solution with low K^+-ion concentration, thus making the separation of these molecules more difficult. Another probable reason for such differences is the better extractability of the sulfur rich galactan chains (containing more κ-carrageenan segments) by the alkaline medium. Concerning the galactans originating from *F. lumbricalis*, there is a contradiction between these assumptions and the hybrid molecular structure of the furcellaran molecule. In spite of the hybrid nature of the furcellaran, some successful attempts have been made to separate the sulfur rich component from the fraction containing more non-sulfated β-carrageenan segments (Yu et al. 2007). Thus it is evident that *F. lumbricalis* contains a heterogeneous mixture of galactans in which the proportion of the κ/β-carrageenan segments considerably varies among the different polysaccharide chains (Knutsen, Grasdalen 1987). This allows slight variances in the sulfur contents of the preparations isolated under different extraction conditions.

The long-term room temperature modification of furcellaran by alkali (0.4 M KOH) increases 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 (**I**). At that, no substantial rise in potassium concentration was observed.

It becomes evident that the amount of positively charged cations in the studied galactan preparations slightly exceeds the quantity needed for the complete neutralization of the negative charge of the polysaccharide backbone, revealing the presence of a small amount of free salts (Figure 9). The overall neutralization capacity for the different water-extracted galactan preparations remains fairly stable, being in the range of 111–112%. Somewhat higher percentages (114–129%) were found for the preparations obtained through a hot alkaline extraction procedure. It appears that the long-term room temperature alkali treatment of *F. lumbricalis* (in 0.4 M KOH solution) followed by the hot water-extraction process yields product with a notably high salt content (neutralization capacity of 141%), primarily attributable to the high calcium level of the sample. The commercial κ-carrageenan preparation from Sigma also contains a substantial quantity of free salts (neutralization capacity of 148%), probably introduced for standardization purposes. As both of the dominant species of Kassari algal stratum are characterized by notably low Na^+ and K^+ contents, the neutralization capacity of their galactans is predominantly attributed to Mg^{2+}- and Ca^{2+}-ions. However, the same characteristic for the polysaccharides from *E. cottonii* is primarily accounted for the monovalent alkali metal ions.

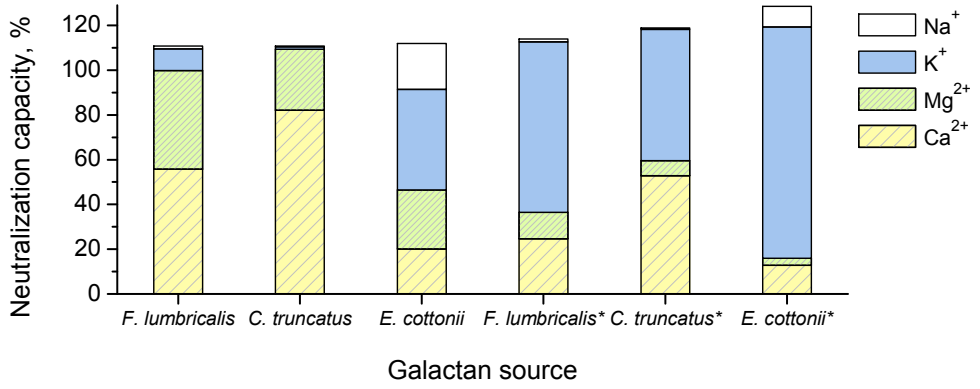


Figure 9. Capacity to neutralize the sulfate ester negative charge for the major cations bound to the galactan preparations of different origin. Polysaccharides extracted 4 hours in pure water or * in 0.02 M KOH solution.

4.3. Structural stability

4.3.1. Thermal stability of carrageenans

The thermal stability of the water-extracted *C. truncatus* galactans in dry state and the commercial ι-carrageenan preparation from Fluka were compared (**II**). The susceptibility to polysaccharide cleavage by elevated temperatures was also studied for the commercial κ-carrageenan sample from Sigma and for the water-extracted furcellaran preparation. The extent of galactan degradation was estimated by the decomposition of AG residues and on the basis of the data obtained by SEC. In addition to the M_w distribution, SEC coupled with refractive index detection gives information about the sulfate content in sample solution (Karlsson, Singh 1999), thus being a useful tool for the analysis of the degradation products of the highly sulfated polysaccharides.

It was evidenced that the release of sulfate from the galactan matrix is connected with the formation of poligeenan fraction (**II**). In the case of ι-carrageenan the removal of sulfate groups begins at the degradation temperature of 110 °C; for κ-carrageenan and furcellaran the same characteristics is 100 °C. The galactans from *C. truncatus* are characterized by notably labile properties, with the sulfate liberation process beginning already at the temperatures above 80 °C; for the preparation treated at 85 °C, already about 15% of the total sulfur was released. It was noted that during the heating process in dry state, the sulfur released from the galactan matrix is converted into the acidic products (obviously, $NaHSO_4$ and $KHSO_4$) that further promote the polysaccharide cleavage. The acidic nature of the degradation products was evidenced by the low pH values of their water solutions (**II**). Thus, it is obvious that the overall stability of the heterogeneous polysaccha-

ride preparations (galactan blends and hybrids) is determined by the most labile sulfate bearing structural units present in the polymer.

The intensive sulfate releasing process is also associated with the decomposition of AG residues present in the polymer. Whereas in the case of *C. truncatus* galactans, these two concomitant processes start at the same degradation temperature (85 °C), for ι-carrageenan, the sulfate liberation process begins slightly before the siqnificant decomposition of AG (**II**). The substantial decrease in the AG content was noted for ι-carrageenan preparation degraded at 120 °C, at this temperature virtually all the high-molecular component was decomposed. The complete decomposition of the highly polymerized fraction of κ-carrageenan and furcellaran takes place at much higher temperatures (150–160 °C).

Low thermal stability of the galactan from *C. truncatus* becomes also evident from the coloration of the samples treated at different temperatures. Visible darkening of the carrageenan powder was clearly evidenced for the preparation treated at 85 °C, whereas the same characteristic for the commercial ι-carrageenan from Fluka was 110 °C. Higher susceptibility to thermal degradation of *C. truncatus* polysaccharides can be accounted for the specific structural irregularities (pyruvate acetal substitution, galactan bound glycoproteins), the composition of the inorganic part and to a smaller polymerization degree of its native preparation.

4.3.2. Degradation products of ι-carrageenan

To investigate the degradation products of carrageenans, the water-extracted galactan from *C. truncatus* was thermally treated (as a dry powder) for seven days at 85 °C; the resulting partially degraded polysaccharides were subjected to ^{13}C-NMR analysis (**II**). The chosen degradation temperature (85 °C) was considered to prevent the excessive destruction of the monosaccharide residues, nevertheless, allowing substantial depolymerization of the polysaccharide chains.

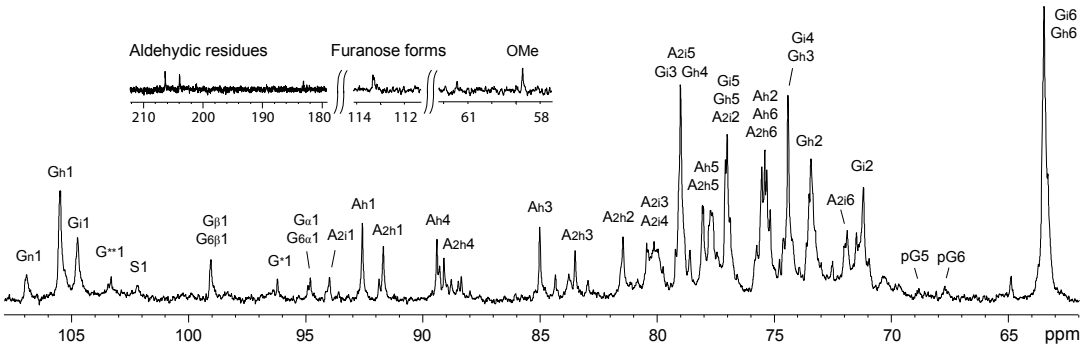


Figure 10. ^{13}C-NMR spectrum of the low-molecular fraction of thermally degraded (7 days at 85 °C) water-extracted *C. truncatus* galactan preparation recorded at 40 °C (26,000 transients collected). For the major signal marks and chemical shift values, see Figure 11.

It was evidenced that thermal treatment considerably affects the solubility properties of carrageenans. Although the degraded product under investigation was readily solubilized in water as 2% solution, the high-molecular fraction with M_w over 10 kDa completely precipitated within ten hours at 4 °C. The resulting clear, low-viscous solution allowed more detailed characterization of the low-molecular component by ^{13}C-NMR spectroscopy (Figure 10). The impaired solubility properties were also observed in the case of agarose preparations (Serva) treated at temperatures of 130–150 ºC.

The ^{13}C-NMR spectrum of the thermally treated *C. truncatus* galactan preparation indicated substantial changes in the polysaccharide structure rising mainly from the desulfation of the polysaccharic chains. The products formed during the degradation process are summarized in Figure 11. It was evidenced that during the thermal treatment the 2-sulfated glycosidic linkages are cleaved at a higher rate than the nonsulfated ones yielding products with terminal AG units. The main components of the low-molecular fraction formed at elevated temperatures are carrabiose

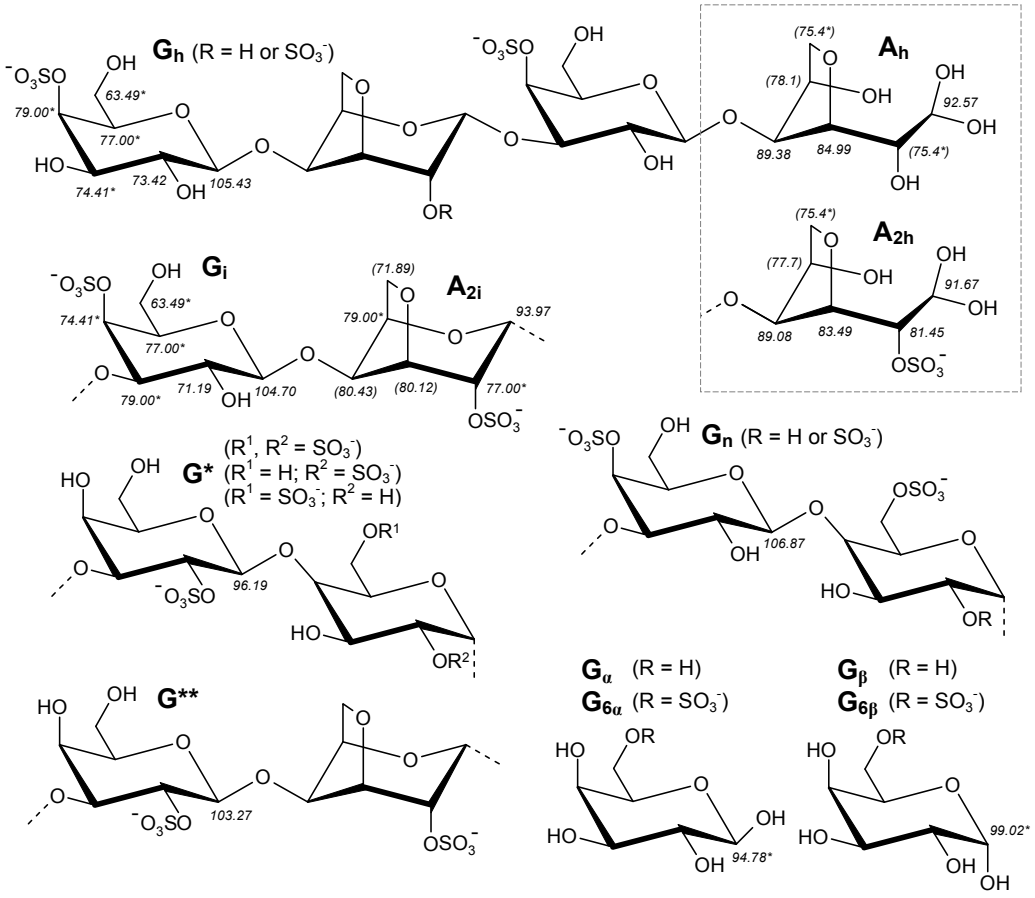


Figure 11. Low-molecular degradation products of thermally treated (7 days at 85 °C) water-extracted *C. truncatus* galactan preparation. The numbers in italic indicate ^{13}C-NMR chemical shift values; an asterisk after the value refers to coincident signals; the approximate values are given in brackets.

disaccharides **G-DA** and **G-DA2S**; the level of tetrasaccharides and longer oligosaccharides is substantially lower (estimated on the basis of the signal ratio of internal and terminal **G** units). Also, a noticeable amount of monosaccharides (galactose or galactose-6-sulfate) was detected in the degraded preparation, being indicative of the hydrolysis of β-1,4-glycosidic linkages. It was evidenced that significant amounts of **D2S,6S** and **GP** units remained in the high molecular fraction. Thus, the labile properties of the galactan preparation are not attributable to the presence of these units. Indicative of the monosaccharide cleavage, a small proportion of furanose forms derived from AG and minor amounts of aldehydic residues were detected in the preparation.

4.4. Rheological characteristics and gel structure

4.4.1. Gelation mechanism of red algal galactans

Sol-gel transition processes of furcellaran and agarose preparations were comparatively investigated using the cryofixation method in combination with freeze-drying and SEM (**III**). The structures formed in different stages of the gelling process upon cooling were rapidly frozen at predefined temperatures and observed by SEM. To protect fragile SEM specimens, the gel was inserted into metal capillary tubes before cryofixation. From the gradual elaboration of the preparation techniques it was concluded that the results were not affected by the tubing material (stainless steel or copper) used.

From the investigation of the gelling processes based on SEM images, some observations were made. The sequence of the steps leading to final gel formation can be divided into the following steps (proposed "compartment model", Figure 12).

(a) At high temperatures the galactans are severely water deficient in sols of moderate polysaccharide concentration (2%). Under such conditions, the polymeric chains are present as small dynamic particulates ("compartments") with polysaccharide rich zones in the outer region of the compartments. In cryofixated SEM preparations these particulates appear as specific honeycomb structures filled with tight polysaccharide networks. The thickness of these finely reticulated structures as well as the overall homogeneity of the gel pattern is highly dependent on the gelling state (temperature). Due to the conglomeration of the gel particulates during freeze-drying process, the walls of the honeycomb structure units appear as two-layered films under SEM (**I**, **III**). The honeycombs as the potential and dynamic precursor structures of the final gel network reflect the association tendencies of this type of macromolecules.

(b) During the cooling process, with increasing water deficiency, further aggregation of the polysaccharide chains inside the compartments takes place.

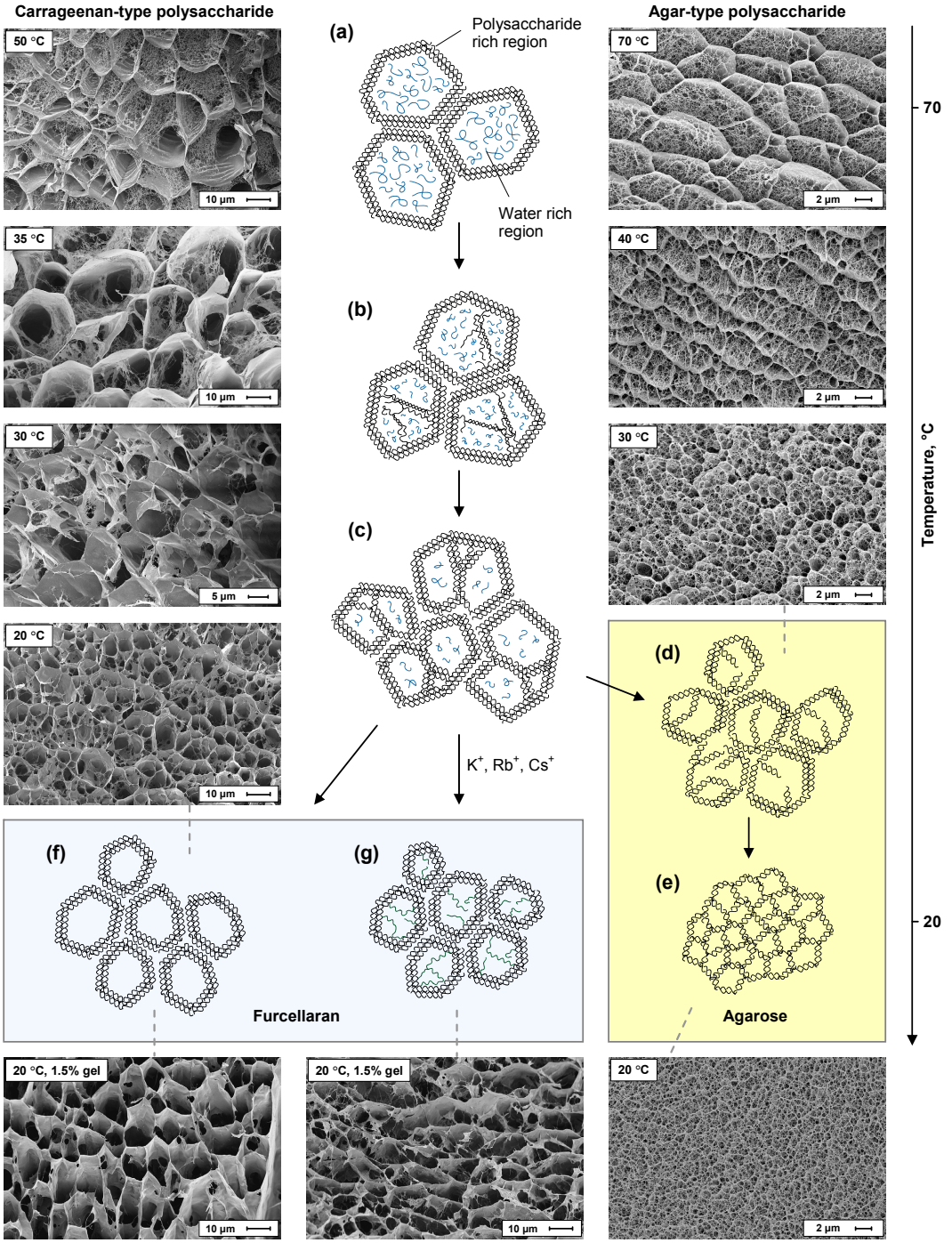


Figure 12. Schematic model of gel formation for red algal galactans and SEM images of cryofixated gelling states (upon cooling) of 2% water-extracted furcellaran and agarose (Serva) preparations. Micrograph of cation-mediated gel structure represents 1.5% water-extracted furcellaran gelled in 0.01 M RbCl solution. For letter mark meanings see the text.

(c) Near the gelling temperature of the sol, intensive wall formation inside the honeycomb structure units occurs, leading to the tightening of the final gel pattern. The process is accompanied by the decreasing amount of subtle network structures, as shown by SEM investigations. The formation of the tightly packed structures is related to an increase in the optical density of the sols (**III**). The latter takes place considerably before reaching the gelling temperature.

(d) For agar-type polysaccharides the additional structure tightening results in thinning of the honeycomb walls accompanied by more homogenous distribution of the resulting gel forming elements and the formation of new junction zones. The process is followed by the sharp increase in the optical density characteristics that expresses well the gelling temperature of the system.

(e) The final spongy structure of agar-type polysaccharide gels is more than 10 times tighter (estimated on the basis of pore size) than the respective state observed at 70 °C.

(f) In furcellaran gels, fine honeycomb pattern persists at the temperatures below the gelling point, at that the dimensions of the compartments are nearly half of those observed at 70 °C.

(g) The presence of gel-promoting cations in the furcellaran sol induces the formation of subtle tentacle-like constituents responsible for the tightening of its final structure. The shape and arrangement of the tentacle-like units is also affected by the presence of anionic substances (**I**). While in the gels co-soluted with salt these structure units are more subtle and arranged less regularly, alkali-extracted furcellaran affords gels whose tentacle-like constituents are characterized by more pronounced constructional function.

4.4.2. Influence of extraction parameters

The extraction parameters can have a notable effect on the rheological properties of galactans by influencing the polysaccharide cleavage, its inorganic part composition and the purity of the product. For the carrageenans containing precursor structures (e.g. μ-, ν-, γ-carrageenan units), appropriate treatment with strong alkalis can also result in chemical changes in the polysaccharic part with enhanced gelling ability of the preparations.

The gelling properties of furcellaran are generally similar to those of κ-type carrageenans, giving characteristically strong gels in the presence of K^+-ions. The hot alkaline extraction of the seaweeds (*F. lumbricalis*) in 0.02 M KOH solution resulted in products with more than 11 times higher gel strength values compared to those obtained by the extraction in pure water (increase from 75 to 862 g/cm^2 for 1.5% gels) (**I**). It was evidenced that the greater stiffness for the alkali-extracted preparation was caused not only by the presence of greater amount of gel-promoting cations, but was also derived from the higher M_w of the sample (Figure 13).

Severe destruction of galactan chains in the extracting media containing KOH less than 0.01 mol/L is connected with the lower pH values (considerably below the optimum for furcellaran stability) of the extracting agent. At the alkali concentration range of 0.02–0.05 mol/L, with increasing alkali content, notable drop in gel strength values was observed. At that no substantial decrease in the M_w characteristic was noted, revealing that the gelation is primarily affected by the variable inorganic part composition of these preparations. Higher alkali contents in extracting media resulted in products with substantially declined M_w characteristics and impaired gelling abilities.

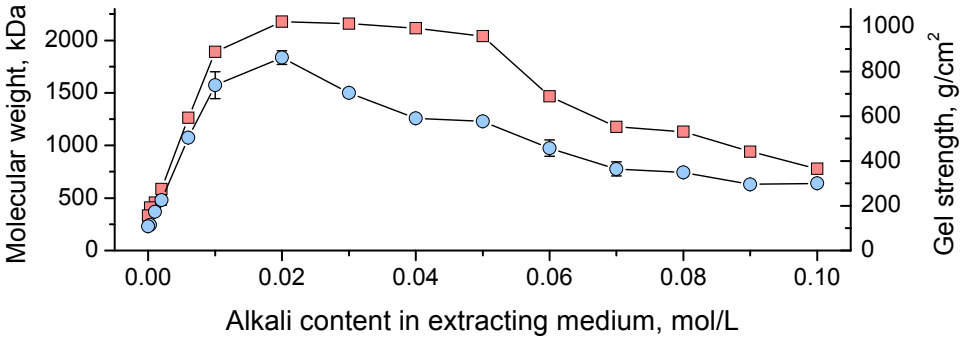


Figure 13. Dependence of furcellaran (○) gel strength and (□) molecular weight characteristic on KOH content in the extracting medium.

Unlike furcellaran, no marked effect on the M_w characteristics of the galactans from *C. truncatus* by the lower pH values of the extracting media was observed (**II**). This is obviously the effect of the sulfate groups at the *O*-2 position of **DA** units which is known to reduce the polysaccharide susceptibility to hydrolytic degradation. Thus, the gelling ability of the polysaccharides from this species (gel strength of 30–40 g/cm^2 for 2% gels) was found not to be considerably dependent on the extraction conditions (**II**). In the case of the most structure-preserving extracting media used (0.02 M NaOH solution), no significant polymeric chain cleavage was observed even for the furcellaran preparations obtained by the 9-hour extraction process (**I**); the M_w values of the products obtained by the extraction of 1 and 9 hours in 0.02 M NaOH solution were 2400 kDa and 1920 kDa, respectively.

The long-term room temperature alkaline modification followed by the hot extraction in pure water resulted in furcellaran gels with reduced optical density and more than two times higher strengths compared to the preparations obtained through the conventional water-extraction process. As potassium contents in these preparations were almost coincident (Table 2), the higher gel strength values for the treated furcellaran were evidently caused by the mutual action of Ca^{2+}-ions and the higher polymerization degree (M_w 870 kDa) of the sample.

In addition to rheological properties, the transparency of the gels is highly dependent on the extracting media used (**I**). Although the optical density of the water-extracted furcellaran was considerably lower than that of the preparation extracted in hot alkaline media, the nitrogen content in both preparations was quite similar (Table 2). Thus, the coloration of the samples can be mainly attributed to hot alkali modification of the proteinic substances present in both preparations.

Rheological properties of the gels are also reflected in the gel structure characteristics observed by SEM. It becomes evident that tighter and more homogenous gel network structures are responsible for the strong and brittle (agarose-type) gels of high light absorbance, whereas honeycomb-type gel patterns enhance the elastic properties common to carrageenan gels. Depending on the structural characteristics of agarose, the macroscopic gel structure can vary considerably even in the case of the preparations gelled under the same conditions; samples of lower gel strengths (e.g. *A. tobuchiensis* galactan gels) were characterized by sparser structural patterns (**III**). The structural peculiarities of carrageenan gels are mainly influenced by the presence of various gelation promoting counter-ions.

4.4.3. Effect of counter-ions

It is known that ionic co-solutes affect the solubility properties of algal galactans usually causing notable changes in the physical properties of the gels (Villanueva et al. 2004). In this study, the effect of various alkali metal ions on furcellaran rheology was investigated (**I**, **VI**). The cations were added as chlorides into the sols of the water-extracted polysaccharide prior to gelling or introduced by the extraction of seaweeds in hot alkali metal hydroxide solutions.

By means of gel stiffness, the most favourable salt concentrations in gelling sols were found to remain around 0.05 mol/L for gelation promoting cations (K^+, Cs^+, Rb^+), whereas for the non-selective alkali metal ions (Li^+, Na^+) within the concentration range under study, a positive correlation was observed to exist between gel strength values and salt concentration (**I**). The relatively weak gelling ability of the water-extracted furcellaran preparation with the gel strength value of 75 g/cm^2 was enhanced more than six times in the presence of RbCl (gel strength of 490 g/cm^2); the addition of KCl or CsCl resulted in somewhat lower gel strengths (380 g/cm^2). For the gels containing chlorides of the non-selective alkali metals (Li^+, Na^+), stiffness characteristics remained below 170 g/cm^2 in the investigated salt concentration range.

Compared to water-extracted furcellaran samples gelled in salt solutions, nearly two times higher gel strength values were observed for the preparations obtained by the extraction process involving alkali metal hydroxides (KOH, RbOH or CsOH). This is mainly caused by the lower M_w values of the water-extracted galactans (Figure 13), the higher content of divalent cations (Mg^{2+}, Ca^{2+}) preserving their composition (Figure 9), and the effect of chloride ions.

For the investigated furcellaran backbone, the maximum gel strength (970 g/cm^2) was achieved in the case of the Rb^+-form of the polymer obtained through alkaline extraction of the seaweeds in 0.02 M RbOH solution. As is typical for κ-type carrageenans, the formation of a characteristic gel network is not favoured in the presence of either Li^+- or Na^+-ions (Figure 5). While in the case of the water-extracted furcellaran, both KCl and CsCl showed a similar effect on the gel strength, the alkaline extraction in the CsOH solution resulted in a noticeable decrease in this characteristic (compared to that observed for KOH treatment). This is evidently connected with the lower quota of organics in the gelling solution caused by the abundant Cs^+-rich inorganic part (Figure 5). Thus, it can be predicted that the gelling ability of both the K^+ and Cs^+-forms of furcellaran, containing equal amounts of organics, is more similar. Based on these studies, it was established that the ability to promote furcellaran gel formation for various alkali metal ions follows the sequence observed also for κ-carrageenan gels: Li^+, $Na^+ << K^+$, $Cs^+ < Rb^+$.

In the presence of gel promoting cations, κ-carrageenan gels show a tendency to synerese (Dunstan et al., 2000). For the furcellaran preparations obtained through extraction in alkali metal hydroxide solutions (LiOH, NaOH, KOH, RbOH, CsOH), a good positive linear correlation exists between syneresis values and the following characteristics: gel strength (r = 0.9981), gel melting temperature (r = 0.9974) and gelling temperature (r = 0.9963) (Figure 14). Compared to alkali-treated preparations, 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) (**I**). Hysteresis loop for furcellaran is relatively high for κ-type carrageenan and increases in the row of Li^+, Na^+, K^+, Rb^+, Cs^+-forms of galactan with values remaining in the range of 13.7–24.4 °C (Figure 14).

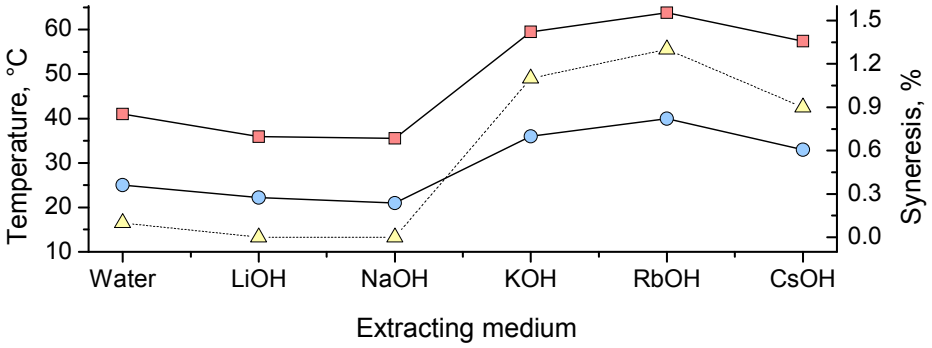


Figure 14. Characteristics of 1.5% furcellaran gels in the presence of different counterions. Galactans extracted 4 hours in 0.02 M alkali metal hydroxide solutions or in pure water. (■) Gel melting temperature; (○) gelling temperature; (△) syneresis value.

5. CONCLUSIONS

In this thesis the relationships between polysaccharide structures from the seaweeds of the Baltic Sea origin, their chemical composition, rheology and gel microstructures were studied in comparison with some other galactan preparations. Special attention was given to the polysaccharides originating from *F. lumbricalis*, the only algal species of economic value in the Baltic Sea, in which cases some structure-property relations were investigated in connection with the extraction and chemical treatment conditions.

The primary results of this thesis can be summarized as follows.

- The main components of galactan from *F. lumbricalis* of the Baltic Sea (M_w of 160 kDa) are **G** (15 ± 1%), **G4S** (36 ± 1.5%) and **DA** (29 ± 1%), **D6S** (6 ± 0.5%), **G6S** and **G6M** residues. Polysaccharides from *C. truncatus* (M_w of 460 kDa) were found to contain **G4S** (44 ± 2%), **DA2S** (30 ± 1.5%), **D2S,6S** (12 ± 2%) and **GP** (1.4%) units. The main structural difference between the galactans originating from the loose-lying and attached forms of *F. lumbricalis* is the higher **G6S** content of the latter. The galactans from Far-Eastern species *A. tobuchiensis* (M_w of 97 kDa) belong to the agar family and are characterized by the main constituents of **G** (58%), **LA** (30 ± 3.5%), **LA2M** (8 ± 1%) and **G6S** residues.
- Even relatively low temperatures can induce the cleavage of galactan chains in dry state; the overall stability of the heterogeneous polysaccharide preparations is determined by the most labile sulfate bearing structural units present in the polymer. In the case of the thermally labile *C. truncatus* polysaccharides, the intensive depolymerization process begins already at 80 °C. The low-molecular degradation products initially formed during the thermal treatment of carrageenans in dry state were found (after solubilization) to be similar to those obtained by the acid hydrolysis of these polysaccharides; for ι-carrageenan the favourable cleavage of 1,3-linked glycosidic bonds leads to the formation of oligosaccharides with the terminal AG residues. The solubility properties of algal galactans depend notably on the extent of polysaccharide cleavage, allowing the fractionation of samples on the basis of thermal stability of their constituents. In some cases it is possible to employ the thermal depolymerization as an aid for the structure elucidation of the minor constituents of red algal galactans.
- The extraction parameters have a marked effect on the gelling ability of red algal galactans mainly by influencing the composition of their inorganic part and molecular weight distribution. For *F. lumbricalis* galactans, the maximum gel strength value of 970 g/cm^2 (for 1.5% gels) is attainable in the case of optimal extraction conditions in the alkaline medium containing Rb^+-ions (duration of 4 hours in 0.02 M RbOH solution). The galactans from *C. truncatus* are characterized by a weak gelling ability that does not depend notably on the conditions of extraction.

- Unpurified carrageenan preparations isolated from the natural seaweed sources usually contain cationic substances (primarily alkali metal ions) in relatively constant levels considerably exceeding the amount needed for the neutralization of the negative charge of the sulfate ester groups of the polysaccharide (excess positive charge of 11–12% for water-extracted preparations and 14–29% in the case of alkali-extracted samples). Extraction of furcellaran in the presence of various alkali metal hydroxides yields products with similar amounts of organics; the quota of inorganics varies considerably and is primarily connected with the atomic weight of the introduced alkali metal. The microelement composition of seaweed samples may be elucidated equally well by AAS and ICP-OES methods.
- Depending on the configuration of AG (D- or L-isomer) in the macromolecular chain, the gelation process of algal galactans follows different pathways. Carrageenans (AG in D configuration) form gels mainly by tightening of the characteristic honeycomb network; the subtle structure units disappear in the course of the final gel formation. In contrast, agar-type galactans (AG in L configuration) gradually lose their honeycomb structure during gelation, resulting in tight and homogeneous spongy gel networks. The presence of gel promoting cations (K^+, Rb^+, Cs^+) in κ-type carrageenan sols induces the formation of specific subtle tentacle-like structure units responsible for the tightening of the final gel structure.

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KEEMILISE KOOSTISE JA STRUKTUURIDE FUNKTSIONAALSÕLTUVUSED LÄÄNEMERE VETIKAKOOSLUSTES

Kokkuvõte

Läänemeri on unikaalne ökosüsteem, mille riimveeline keskkond avaldab seal elavatele organismidele tugevat survet. Elu vee madalast soolsusest tingitud keskkonnastressi tingimustes võib punavetikate puhul ilmneda muutustena nende morfoloogias, arengutsüklis või polüsahhariidkoostises. Vetikapolüsahhariidide geelistumisvõime on punavetikate elutegevuse aluseks, aga ka eelduseks nende loodusliku makromolekulide kasutamisele toiduainetööstuses ja mikrobioloogias. Geelide võrkjaid hüdraatunud struktuure kasutavad punavetikad oma elutegevuses omapärase molekulaarsõelana, samuti annavad need taimele vesikeskkonnas toimimiseks vajaliku elastsuse. Seega võivad nimetatud polüsahhariidide struktuur-omadus sõltuvused huvi pakkuda nii teaduslikust kui ka tööndusliku aspektist.

Käesoleva töö põhilise loodusliku objektina on Kassari lahe vetikakooslus tüüpiline näide iseloomustamaks ökoloogilisi tingimusi Läänemeres. Selle nimetatud riimveekogu üks suuremaid kompaktseid punavetikakooslusi on küllalt selgelt piiritletud liigilise koosseisuga ning kujutab endast piirkonna ainsat tööndusliku väärtusega loodusvara.

Doktoriväitekirja põhieesmärgiks oli välja selgitada seoseid Läänemere punavetikatest pärinevate galaktaanide molekulaarstruktuuri, keemilise koostise, geelide mikrostruktuuri ja reoloogiliste näitajate vahel ning hinnata nende parameetrite muutlikkust seoses ekstraktsioonitingimuste eripäradega.

Järgnevalt on esitatud doktoriväitekirja põhitulemused.

- *F. lumbricalis* galaktaanide (molekulmass 160 kDa) põhikomponendid on β-D-galaktoos (15 ± 1%), β-D-galaktoos-4-sulfaat (36 ± 1,5%) ja 3,6-anhüdro-α-D-galaktoos (29 ± 1%), väiksemates kogustes esinevad α-D-galaktoos-6-sulfaat (6 ± 0,5%), β-D-galaktoos-6-sulfaat ja 6-*O*-metüül-β-D-galaktoos. *C. truncatus* galaktaanide (molekulmass 460 kDa) peamised komponendid on β-D-galaktoos-4-sulfaat (44 ± 2%) ja 3,6-anhüdro-α-D-galaktoos-2-sulfaat (30 ± 1,5%), minoorsete komponentidena leiduvad α-D-galaktoos-2,6-disulfaat (12 ± 2%) ja 4',6'-püruvaaditud karrabioos-2-sulfaat (1,4%). Punavetika *F. lumbricalis* uuritud lahtise ja kinnitunud vormi galaktaanide ainsaks arvestatavaks struktuurierinevuseks on viimaste mõnevõrra suurem β-D-galaktoos-6-sulfaadi sisaldus. Kaug-Ida vetikaliigi *A. tobuchiensis* galaktaanid (molekulmass 97 kDa) on keemiliselt ehituselt agarid, mille koostisse kuuluvad β-D-galaktoos (58%), 3,6-anhüdro-α-L-galaktoos (30 ± 3,5%), 2-*O*-metüül-3,6-anhüdro-α-L-galaktoos (8 ± 1%) ning β-D-galaktoos-6-sulfaat.
- Kõrge väävlisisaldusega galaktaanahelate intensiivne lagunemine võib kuivas olekus aset leida suhteliselt madalatel temperatuuridel; heterogeensete polü-

sahhariidpreparaatide struktuurne stabiilsus on suuresti määratud polümeeri kõige labiilsemate sulfaaditud struktuurifragmentide poolt. Termolabiilsete *C. truncatus* galaktaanide märgatav depolümerisatsioon algab juba 80 °C juures. Karraginaanide termilise kuivlagunemise esmased produktid on sarnased happelisel hüdrolüüsil tekkivatega. ι-Karraginaani puhul on nendeks α-1,3-glükosiidsidemete katkemise tulemusel moodustunud terminaalsete 3,6-anhüdrogalaktoosi jääkidega oligosahhariidid. Kuumtöödeldud galaktaanide lahustuvus vees sõltub nende lagunemisastmest; see asjaolu võimaldab termiliselt töödeldud polüsahhariidide täiendavat fraktsioneerimist. Termilist depolümerisatsiooni saab teatud juhtudel rakendada abivahendina galaktaanide detailsema struktuuri uurimisel.

- Ekstraktsiooniparameetrite mõju vetikapolüsahhariidide geelistumisvõimele on seotud peamiselt muutustega eraldatud produktide anorgaanilise osa koostises ning molekulmassides. *F. lumbricalis* galaktaanide maksimaalne geelitugevus 970 g/cm^2 (1,5% geelide puhul) on saavutatav optimaalsete ekstraktsioonitingimuste juures Rb^+-ioone sisaldavas leeliselises keskkonnas (ekstraktsioonil 4 tundi 0,02 M RbOH vesilahuses). *C. truncatus* polüsahhariidide geelimoodustumisvõime on väike (geelitugevus 30–40 g/cm^2 2% geelide korral) ning ei sõltu oluliselt ekstraktsioonitingimustest.

- Looduslikust vetikamassist ekstraktsiooni käigus eraldatud galaktaanpreparaadid sisaldavad katioonseid lisandeid (peamiselt leelismetallioone) kogustes, mis ületavad polümeeri negatiivsete sulfaatrühmade laengu tasakaalustamiseks vajaliku hulga küllaltki konstantses ulatuses (positiivse laengu ülehulk vesi-ekstraheeritud preparaatide puhul 11–12%, leelisekstraktsiooni korral 14–29%). Furtsellaraani ekstraktsioonil erinevates leelismetallhüdroksiidide vesilahustes saadavad produktid sisaldavad lähedase koguse orgaanikat, anorgaanilise komponendi osakaal aga varieerub oluliselt, sõltudes peamiselt preparaadi koostisse viidud leelismetalli aatommassist. Mikroelementide määramiseks vetikaproovides on võrdselt hästi rakendatavad induktiivsidestunud plasma optilise emissioonspektromeetria ja aatomabsorptsioonspektromeetria meetodid.

- Sõltuvalt galaktaani makromolekulaarse ahela koostises esineva 3,6-anhüdrogalaktoosi ruumilisest konfiguratsioonist (D- või L-isomeer), toimub vetikapolüsahhariidide geelistumine põhimõtteliselt erinevaid radu mööda. Agarite (anhüdrogalaktoos L-vormis) puhul saadab geelistumist iseloomuliku kärgstruktuuri kadumine ning võrkjate struktuuritüüpide tihenemine; karraginaanide (anhüdrogalaktoos D-vormis) geelimoodustamisprotsess seisneb aga võrkstruktuuride kadumises ning kärgstruktuuri üldises tihenemises. Geelistumist soodustavate katioonide (K^+, Rb^+, Cs^+) esinemine κ-tüüpi karraginaanide kuumades vesilahustes indutseerib niitjate, geeli mikrostruktuuri tihendavate lisaelementide moodustumise geelistumisprotsessi käigus.

REFERENCES

ABAD, L. V., NASIMOVA, I. R., RELLEVE, L. S., ARANILLA, C. T., DE LA ROSA, A. M., SHIBAYAMA, M. 2004. Dynamic light scattering studies of irradiated kappa carrageenan. – *International Journal of Biological Macromolecules*, 34, 81–88.

AGUILAN, J. T., BROOM, J. E., HEMMINGSON, J. A., DAYRIT, F. M., MONTAÑO, M. N. E., DANCEL, M. C. A., NIÑONUEVO, M. R., FURNEAUX, R. H. 2003. Structural analysis of carrageenan from farmed varieties of Philippine seaweed. – *Botanica Marina*, 46, 179–192.

ANDERSON, N. S., CAMPBELL, J. W., HARDING, M. M., REES, D. A., SAMUEL, J. W. B. 1969. X-ray diffraction studies of polysaccharide sulphates: double helix models for κ- and ι-carrageenans. – *Journal of Molecular Biology*, 45, 85–88.

ANDERSON, N. S., DOLAN, T. C. S., LAWSON, C. J., PENMAN, A., REES, D. A. 1968. Carrageenans: part V. The masked repeating structures of λ- and μ-carrageenans. – *Carbohydrate Research*, 7, 468–473.

ARMISÉN, R., GALATAS, F. 2000. Agar. – G. O. Phillips, P. A. Williams (eds.). *Handbook of hydrocolloids*. Boca Raton: CRC Press, 21–40.

BARABANOVA, A. O., YERMAK, I. M., GLAZUNOV, V. P., ISAKOV, V. V., TITLYANOV, E. A., SOLOV'EVA, T. F. 2005. Comparative study of carrageenans from reproductive and sterile forms of *Tichocarpus crinitus* (Gmel.) Rupr (Rhodophyta, Tichocarpaceae). – *Biochemistry (Moscow)*, 70, 350–356.

BIRD, C., SAUNDERS, G., MCLACHLAN, J. 1991. Biology of *Furcellaria lumbricalis* (Hudson) Lamouroux (Rhodophyta: Gigartinales), a commercial carrageenophyte. – *Journal of Applied Phycology*, 3, 61–82.

BONOTTO, S. 1979. List of multicellular algae of commercial use. – H. A. Hoppe, T. Levring, Y. Tanaka (eds.). *Marine algae in pharmaceutical science*. Berlin: Walter de Gruyter, 121–137.

BRADLEY, T. D., MITCHELL, J. R. 1988. The determination of the kinetics of polysaccharide thermal degradation using high temperature viscosity measurements. – *Carbohydrate Polymers*, 9, 257–267.

BRIAND, X. 1991. Seaweed resources in Europe. – M. D. Guiry, G. Blunden (eds.). *Seaweed resources in Europe: uses and potential*. Chichester: John Wiley & Sons, 259–308.

CAIRNS, P., ATKINS, E. D. T., MILES, M. J., MORRIS, V. J. 1991. Molecular transforms of kappa carrageenan and furcellaran from mixed gel systems. – *International Journal of Biological Macromolecules*, 13, 65–68.

CHAPMAN, V. J., CHAPMAN, D. J. 1980. *Seaweeds and their uses*, 3rd ed. London: Chapman and Hall.

CHEN, Y., LIAO, M.-L., DUNSTAN, D. E. 2002. The rheology of K^+-κ-carrageenan as a weak gel. – *Carbohydrate Polymers*, 50, 109–116.

CHEN, Y., LIAO, M. L., BOGER, D. V., DUNSTAN, D. E. 2001. Rheological characterisation of κ-carrageenan/locust bean gum mixtures. – *Carbohydrate Polymers*, 46, 117–124.

CHERONIS, N. D., MA, T. S. 1964. *Organic functional group analysis by micro and semimicro methods*. New York: Interscience.

CHOPIN, T. 1998. Seaweed resources of Eastern Canada. – A. T. Critchley, M. Ohno (eds.). *Seaweed resources of the world*. Japan: Japan International Cooperation Agency, 273–302.

CHRISTIANSEN, E. 1959. Danish agar. – R. L. Whistler (ed.). *Industrial gums*. New York: Academic Press, 51–54.

CIANCIA, M., MATULEWICZ, M. C., CEREZO, A. S. 1997a. Alkaline modification of carrageenans. Part III. Use of mild alkaline media and high ionic strengths. – *Carbohydrate Polymers*, 32, 293–295.

CIANCIA, M., MILAS, M., RINAUDO, M. 1997b. On the specific role of coions and counterions on κ-carrageenan conformation. – *International Journal of Biological Macromolecules*, 20, 35–41.

CIANCIA, M., NOSEDA, M. D., MATULEWICZ, M. C., CEREZO, A. S. 1993. Alkali-modification of carrageenans: mechanism and kinetics in the kappa/iota-, mu/nu- and lambda-series. – *Carbohydrate Polymers*, 20, 95–98.

CISZEWSKI, P., KRUK-DOWGIAŁŁO, L., ZMUDZINSKI, L. 1992. Deterioration of the Puck Bay and biotechnical approaches to its reclamation. – E. Bjørnestad, L. Hagerman, K. Jensen (eds.). *Proceedings of the 12th Baltic marine biologists symposium*. Fredensborg: Olsen & Olsen, 43–46.

CRAIGIE, J. S. 1990. Cell walls. – K. M. Cole, R. G. Sheath (eds.). *Biology of the red algae*. Cambridge: Cambridge University Press, 221–257.

DEMBITSKY, V. M., SMOUM, R., AL-QUNTAR, A. A., ALI, H. A., PERGAMENT, I., SREBNIK, M. 2002. Natural occurrence of boron-containing compounds in plants, algae and microorganisms. – *Plant Science*, 163, 931–942.

DININNO, V., MCCANDLESS, E. L., BELL, R. A. 1979. Pyruvic acid derivative of a carrageenan from a marine red alga (*Petrocelis* species). – *Carbohydrate Research*, 71, C1–C4.

DUARTE, M. E. R., CAUDURO, J. P., NOSEDA, D. G., NOSEDA, M. D., GONÇALVES, A. G., PUJOL, C. A., DAMONTE, E. B., CEREZO, A. S. 2004. The structure of the agaran sulfate from *Acanthophora spicifera* (Rhodomelaceae, Ceramiales) and its antiviral activity. Relation between structure and antiviral activity in agarans. – *Carbohydrate Research*, 339, 335–347.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. – *Analytical Chemistry*, 28, 350–356.

DUCKWORTH, M., YAPHE, W. 1970. Definitive assay for pyruvic acid in agar and other algal polysaccharides. – *Chemistry and Industry (London)*, 23, 747–748.

DUNSTAN, D. E., SALVATORE, R., JONSSON, M., LIAO, M.-L. 2000. Syneresis of κ-carrageenan gels at different KCl and LBG concentrations. – P. A. Williams, G. O. Phillips (eds.). *Gums and stabilizers for the food industry.* Vol. 10. Cambridge: Royal Society of Chemistry, 137–148.

ERIKSSON, B. K., JOHANSSON, G. 2005. Effects of sedimentation on macroalgae: species-specific responses are related to reproductive traits. – *Oecologia*, 143, 438–448.

ESTEVEZ, J. M., CIANCIA, M., CEREZO, A. S. 2000. The system of low-molecular-weight carrageenans and agaroids from the room-temperature-extracted fraction of *Kappaphycus alvarezii*. – *Carbohydrate Research*, 325, 287–299.

ESTEVEZ, J. M., CIANCIA, M., CEREZO, A. S. 2001. DL-Galactan hybrids and agarans from gametophytes of the red seaweed Gymnogongrus torulosus. – *Carbohydrate Research*, 331, 27–41.

ESTEVEZ, J. M., CIANCIA, M., CEREZO, A. S. 2004. The system of galactans of the red seaweed, *Kappaphycus alvarezii*, with emphasis on its minor constituents. – *Carbohydrate Research*, 339, 2575–2592.

ESTEVEZ, J. M., CIANCIA, M., CEREZO, A. S. (2008). The system of sulfated galactans from the red seaweed *Gymnogongrus torulosus* (Phyllophoraceae, Rhodophyta): location and structural analysis. – *Carbohydrate Polymers*, 73, 594–605.

FREILE-PELEGRÍN, Y., ROBLEDO, D., AZAMAR, J. A. 2006. Carrageenan of *Eucheuma isiforme* (Solieriaceae, Rhodophyta) from Yucatán, Mexico. I. Effect of extraction conditions. – *Botanica Marina*, 49, 65–71.

FRIEDENTHAL, M., EHA, K., VIITAK, A., LUKAS, A., SIIMER, E. 2000. Effects of drying on the gel strength and cation mobility of furcellaran. – *Innovative Food Science & Emerging Technologies*, 1, 275–279.

FURNEAUX, R. H., MILLER, I. J. 1986. Isolation and ^{13}C-NMR spectral study of the water soluble polysaccharides from four South African red algae. – *Botanica Marina*, 29, 3–10.

FURNEAUX, R. H., MILLER, I. J., STEVENSON, T. T. 1990. Agaroids from New Zealand members of the Gracilariaceae (Gracilariales, Rhodophyta) – a novel dimethylated agar. – *Hydrobiologia*, 204–205: 645–654.

GLICKSMAN, M. 1983. Red seaweed extracts. – M. Glicksman (ed.). *Food hydrocolloids.* Vol. 2. Boca Raton: CRC Press, 73–113.

GRAHAM, L. E., WILCOX, L. W. 2000. *Algae*. London: Prentice-Hall.

GUIBET, M., KERVAREC, N., BOULENGUER, P., MAZOYER, J., CRITCHLEY, A., HELBERT, W. 2006. Enzymatic analysis of κ/ι-hybrid carrageenan. – P. A. Williams, G. O. Phillips (eds.). *Gums and stabilisers for the food industry*. Vol. 13. Cambridge: The Royal Society of Chemistry, 52–60.

GUISELEY, K. B. 1970. The relationship between methoxyl content and gelling temperature of agarose. – *Carbohydrate Research*, 13, 247–256.

HOLMSGAARD, J. E., GREENWELL, M., MCLACHLAN, J. 1981. Biomass and vertical distribution of *Furcellaria lumbricalis* and associated algae. – T. Levring (ed.). *Proceedings of the Xth international seaweed symposium*. Berlin: Walter de Gruyter, 309–314.

HUMM, H. J. 1947. Agar – A pre-war Japanese monopoly. – *Economic Botany*, 1, 317–329.

INDERGAARD, M., KNUTSEN, S. H. 1990. Seasonal differences in ash, carbon, fibre and nitrogen components of *Furcellaria lumbricali*s (Gigartinales, Rhodophyceae), Norway. – *Botanica Marina*, 33, 327–334.

IZUMI, K. 1971. Chemical heterogeneity of the agar from *Gelidium amansii*. – *Carbohydrate Research*, 17, 227–230.

KARLSSON, A., SINGH, S. K. 1999. Acid hydrolysis of sulphated polysaccharides. Desulphation and the effect on molecular mass. – *Carbohydrate Polymers*, 38, 7–15.

KING, G. M., LAUTERBACH, G. E. 1987. Characterization of carrageenan nitrogen content and its susceptibility to enzymatic hydrolysis. – *Botanica Marina*, 30, 33–39.

KIRST, G. O. 1990. Salinity tolerance of eukaryotic marine algae. – *Annual Review of Plant Physiology and Plant Molecular Biology*, 41, 21–53.

KNUTSEN, S. H., GRASDALEN, H. 1987. Characterization of water-extractable polysaccharides from Norwegian *Furcellaria lumbricalis* (Huds.) Lamour. (Gigartinales, Rhodophyceae) by IR and NMR spectroscopy. – *Botanica Marina*, 30, 497–505.

KNUTSEN, S. H., MYSLABODSKI, D. E., LARSEN, B., USOV, A. I. 1994. A modified system of nomenclature for red algal galactans. – *Botanica Marina*, 37, 163–169.

KOSTAMO, K. 2008. *The life cycle and genetic structure of the red alga Furcellaria lumbricalis on a salinity gradient. Doctoral thesis*. Finland: University of Helsinki.

KOSTAMO, K., MÄKINEN, A. 2006. Observations on the mode and seasonality of reproduction in *Furcellaria lumbricalis* (Gigartinales, Rhodophyta) populations in the northern Baltic Sea. – *Botanica Marina*, 49, 304–309.

KRASIL'NIKOVA, S. V., BOIKO, L. I., PETRENKO, E. B., PANCHENKO, K. A., MEDVEDEVA, E. I., LAPIN, V. R., ARGUS, K. Y. 1972. Study of nitrogenous substances of the red alga *Furcellaria fastigiata*. – *Biologicheskie Nauki*, 15, 90–93. (in Russian).

KRASIL'NIKOVA, S. V., MEDVEDEVA, E. I. 1975. Some characteristics of the alkali-soluble protein from the red alga *Furcellaria fastigiata*. – *Khimiya Prirodnykh Soedinenii*, 11, 400–404. (in Russian).

KRUK-DOWGIAŁŁO, L. 1991. Long-term changes in the structure of underwater meadows of the Puck Lagoon. – *Acta Ichthyologica et Piscatoria*, 21, 77–84.

LAI, V. M.-F., LII, C.-Y., HUNG, W.-L., LU, T.-J. 2000. Kinetic compensation effect in depolymerisation of food polysaccharides. – *Food Chemistry*, 68, 319–325.

LAOS, K. 2005. *Interactions between furcellaran and the globular proteins (bovine serum albumin, β-lactoglobulin). Doctoral thesis*. Estonia: Tallinn University of Technology.

LAOS, K., BROWNSEY, G. J., RING, S. G. (2007). Interactions between furcellaran and the globular proteins bovine serum albumin and β-lactoglobulin. – *Carbohydrate Polymers*, 67, 116–123.

LAOS, K., RING, S. 2005. Characterisation of furcellaran samples from Estonian *Furcellaria lumbricalis* (Rhodophyta). – *Journal of Applied Phycology*, 17, 461–464.

LECACHEUX, D., PANARAS, R., BRIGAND, G., MARTIN, G. 1985. Molecular weight distribution of carrageenans by size exclusion chromatography and low angle laser light scattering. – *Carbohydrate Polymers*, 5, 423–440.

LEVRING, T., HOPPE, H. A., SCHMID, O. J. 1969. *Marine algae. A survey of research and utilization*. Hamburg: De Gruyter and Co.

LII, C.-Y., CHEN, C.-H., YEH, A.-I., LAI, V. M. F. 1999. Preliminary study on the degradation kinetics of agarose and carrageenans by ultrasound. – *Food Hydrocolloids*, 13, 477–481.

LÜNING, K. 1990. *Seaweeds: their environment, biogeography, and ecophysiology*. New York: John Wiley & Sons.

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. – *Proceedings of Polish-Swedish symposium on Baltic coastal fisheries. Resources and management.* 2–3 April 1996, Gdynia, Poland. Gdynia: Sea Fisheries Institute, 121–129.

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. – *Journal of Applied Phycology*, 18, 557–563.

MATHIESON, A. C., EMERICH PENNIMAN, C., TVETER-GALLAGHER, E. 1984. Phycocolloid ecology of underutilized economic red algae. – *Hydrobiologia*, 116–117, 542–546.

MATSUHASHI, T. 1990. Agar. – P. Harris (ed.). *Food gels*. London: Elsevier, 1–51.

MATULEWICZ, M. C., CEREZO, A. S. 1980. The carrageenan from *Iridaea undulosa* B.; Analysis, fractionation and alkaline treatment. – *Journal of the Science of Food and Agriculture*, 31, 203–213.

MCCANDLESS, E. L., WEST, J. A., GUIRY, M. D. 1982. Carrageenan patterns in the Phyllophoraceae. – *Biochemical Systematics and Ecology*, 10, 275–284.

MCCANDLESS, E. L., WEST, J. A., VOLLMER, C. M. 1981. Carrageenans of species in the genus *Phyllophora*. –T. Levring (ed.). *Proceedings of the Xth international seaweed symposium*. Berlin: Walter de Gruyter & Co, 473–478.

MEUNIER, V., NICOLAI, T., DURAND, D. 2001. Structure of aggregating κ-carrageenan fractions studied by light scattering. – *International Journal of Biological Macromolecules*, 28, 157–165.

MICHEL, A.-S., MESTDAGH, M. M., AXELOS, M. A. V. 1997. Physico-chemical properties of carrageenan gels in presence of various cations. – *International Journal of Biological Macromolecules*, 21, 195–200.

MIKULICH, D. V., KOPYTOV, Y. P. 2002. Carbohydrate composition and some technological properties of Baltic *Rhodophyta*. – *International Journal of Algae*, 4, 11–19.

MINICHEVA, G. G. 2007. Contemporary morpho-functional transformation of seaweed communities of the Zernov phyllophora field (Black Sea). – *International Journal on Algae*, 9, 1–21.

MONTOLALU, R. I., TASHIRO, Y., MATSUKAWA, S., OGAWA, H. 2008. Effects of extraction parameters on gel properties of carrageenan from *Kappaphycus alvarezii* (Rhodophyta). – *Journal of Applied Phycology*, 20, 521–526.

MORRIS, E. R., REES, D. A., ROBINSON, G. 1980. Cation-specific aggregation of carrageenan helices: domain model of polymer gel structure. – *Journal of Molecular Biology*, 138, 349–362.

MORRIS, V. J. 1986. Gelation of polysaccharides. – J. R. Mitchell, D. A. Ledward (eds.). *Functional properties of food macromolecules*. London: Elsevier, 121–170.

MÄKINEN, A., KÄÄRIÄ, J., RAJASILTA, M. 1988. Factors controlling the occurrence of *Furcellaria lumbricalis* (Huds.) Lamour. and *Phyllophora truncata* (Pallas) Zinova in the upper littoral of the archipelago of SW Finland. – *Kieler Meeresforsch Sonderheft*, 6, 140–146.

MYSLABODSKI, D. E. 1990. *Red-algae galactans: isolation and recovery procedures – effects on the structure and rheology. Doctoral Thesis*. Norway: Norwegian Institute of Technology.

NIELSEN, R., KRISTIANSEN, A., MATHIESEN, L., MATHIESEN, H. 1995. Distributional index of the benthic macroalgae of the Baltic Sea area. – *Acta Botanica Fennica*, 155, 1–51.

NILSSON, S., PICULELL, L. 1991. Helix-coil transitions of ionic polysaccharides analyzed within the Poisson-Boltzmann cell model. 4. Effects of site-specific counterion binding. – *Macromolecules*, 24, 3804–3811.

PAINTER, T. J. 1966. The location of the sulphate half-ester groups in furcellaran and κ-carrageenan. – G. E. Young, J. L. McLachlan (eds.). *Proceedings of the Vth international seaweed symposium*. Oxford: Pergamon Press, 305–313.

PAINTER, T. J. 1983. Algal polysaccharides. – G. O. Aspinall (ed.). *The polysaccharides.* Vol. 2. New York: Academic Press, 195–285.

PAOLETTI, S., DELBEN, F., CESÀRO, A., GRASDALEN, H. 1985. Conformational transition of κ-carrageenan in aqueous solution. – *Macromolecules*, 18, 1834–1841.

PICULELL, L. 1995. Gelling carrageenans. – A. M. Stephen (ed.). *Food polysaccharides and their applications*. New York: Marcel Dekker, 205–244.

REES, D. A. 1969. Structure, conformation, and mechanism in the formation of polysaccharide gels and networks. – *Advances in carbohydrate chemistry and biochemistry*. Vol. 24. Academic Press, 267–332.

REES, D. A. 1972. Polysaccharide gels. A molecular view. – *Chemistry and Industry (London)*, 19, 630–636.

RELLEVE, L., NAGASAWA, N., LUAN, L. Q., YAGI, T., ARANILLA, C., ABAD, L., KUME, T., YOSHII, F., DELA ROSA, A. 2005. Degradation of carrageenan by radiation. – *Polymer Degradation and Stability*, 87, 403–410.

ROBERTS, M. A., ZHONG, H.-J., PRODOLLIET, J., GOODALL, D. M. 1998. Separation of high-molecular-mass carrageenan polysaccharides by capillary electrophoresis with laser-induced fluorescence detection. – *Journal of Chromatography A*, 817, 353–366.

ROBINSON, G., MORRIS, E. R., REES, D. A. 1980. Role of double helices in carrageenan gelation: the domain model. – *Journal of the Chemical Society, Chemical Communications*, 152–153.

ROCHAS, C., RINAUDO, M. 1984. Mechanism of gel formation in κ-carrageenan. – *Biopolymers*, 23, 735–745.

SCHRAMM, W. 1998. The seaweed resources of the Baltic Sea and the German coasts of the North Sea. – A. T. Critchley, M. Ohno (eds.). *Seaweed resources of the world*. Japan: Japan International Cooperation Agency, 226–232.

SLONEKER, J. H., ORENTAS, D. G. 1962. Pyruvic acid, a unique component of an exocellular bacterial polysaccharide. – *Nature*, 194, 478–479.

SLOOTMAEKERS, D., VAN DIJK, J. A. P. P., VARKEVISSER, F. A., VAN TRESLONG, C. J. B., REYNAERS, H. 1991. Molecular characterisation of κ- and λ-carrageenan by gel permeation chromatography, light scattering, sedimentation analysis and osmometry. – *Biophysical Chemistry*, 41, 51–59.

SMIDSRØD, O., GRASDALEN, H. 1982. Some physical properties of carrageenan in solution and gel state. – *Carbohydrate Polymers*, 2, 270–272.

SPICHTIG, V., AUSTIN, S. 2008. Determination of the low molecular weight fraction of food-grade carrageenans. – *Journal of Chromatography B*, 861, 81–87.

STANCIOFF, D. J., STANLEY, N. F. 1969. Infrared and chemical studies on algal polysaccharides. – R. Margalef (ed.). *Proceedings of the VIth international seaweed symposium*. Madrid: Dirección General de Pesca Marítima, 595–609.

STANLEY, N. F. 1990. Carrageenans. – P. Harris (ed.). *Food gels*. London: Elsevier, 79–119.

STEVENSON, T. T., FURNEAUX, R. H. 1991. Chemical methods for the analysis of sulphated galactans from red algae. – *Carbohydrate Research*, 210, 277–298.

STEYERMARK, A., ALBER, H. K., ALUISE, V. A., HUFFMAN, E. W. D., JOLLEY, E. L., KUCK, J. A., MORAN, J. J., OGG, C. L. 1956. Report on recommended specifications for microchemical apparatus. Alkoxyl. – *Analytical Chemistry*, 28, 112–115.

ZHANG, W., PICULELL, L., NILSSON, S. 1991. Salt dependence and ion specificity of the coil-helix transition of furcellaran. – *Biopolymers*, 31, 1727–1736.

TAKEMASA, M., NISHINARI, K. 2004. The effect of the linear charge density of carrageenan on the ion binding investigated by differential scanning calorimetry, dc conductivity, and kHz dielectric relaxation. – *Colloids and Surfaces B: Biointerfaces*, 38, 231–240.

THERKELSEN, G. H. 1993. Carrageenan. – R. L. Whistler, J. N. BeMiller (eds.). *Industrial gums*. San Diego: Academic Press, 145–180.

TRUUS, K., VAHER, M., USOV, A. I., 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. – *International Journal of Biological Macromolecules*, 21, 89–96.

TURVEY, J. R., WILLIAMS, E. L. 1970. The structure of some xylans from red algae. – *Phytochemistry*, 9, 2383–2388.

USOV, A. I. 1998. Structural analysis of red seaweed galactans of agar and carrageenan groups. – *Food Hydrocolloids*, 12, 301–308.

USOV, A. I., ARKHIPOVA, V. S. 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. – *Bioorganicheskaya Khimiya*, 7, 385–390.

USOV, A. I., SHASHKOV, A. S. 1985. Polysaccharides of algae. XXXIV: Detection of iota-carrageenan in *Phyllophora brodiaei* (Turn.) J. Ag. (Rhodophyta) using ^{13}C-NMR spectroscopy. – *Botanica Marina*, 28, 367–373.

VAN DE VELDE, F. 2008. Structure and function of hybrid carrageenans. – *Food Hydrocolloids*, 22, 727–734.

VAN DE VELDE, F., KNUTSEN, S. H., USOV, A. I., ROLLEMA, H. S., CEREZO, A. S. 2002a. ^{1}H and ^{13}C high resolution NMR spectroscopy of carrageenans: application in research and industry. – *Trends in Food Science & Technology*, 13, 73–92.

VAN DE VELDE, F., PEREIRA, L., ROLLEMA, H. S. 2004. The revised NMR chemical shift data of carrageenans. – *Carbohydrate Research*, 339, 2309–2313.

VAN DE VELDE, F., ROLLEMA, H. S., GRINBERG, N. V., BUROVA, T. V., GRINBERG, V. Y., TROMP, R. H. 2002b. Coil-helix transition of iota-carrageenan as a function of chain regularity. – *Biopolymers*, 65, 299–312.

VIANA, A. G., NOSEDA, M. D., DUARTE, M. E. R., CEREZO, A. S. 2004. Alkali modification of carrageenans. Part V. The iota-nu hybrid carrageenan from *Eucheuma denticulatum* and its cyclization to iota-carrageenan. – *Carbohydrate Polymers*, 58, 455–460.

VIEBKE, C., WILLIAMS, P. A. 2000. Determination of molecular mass distribution of κ-carrageenan and xanthan using asymmetrical flow field-flow fractionation. – *Food Hydrocolloids*, 14, 265–270.

VILLANUEVA, R. D., MENDOZA, W. G., RODRIGUEZA, M. R. C., ROMERO, J. B., MONTAÑO, M. N. E. 2004. Structure and functional performance of gigartinacean kappa-iota hybrid carrageenan and solieriacean kappa-iota carrageenan blends. – *Food Hydrocolloids*, 18, 283–292.

WICHMANN, J., CHRISTENSEN, T. M. I. E., DE VRIES, J. 2006. Distribution of kappa- and iota-structures in hybrid carrageenans. – P. A. Williams, G. O. Phillips (eds.). *Gums and stabilisers for the food industry*. Vol. 13. Cambridge: The Royal Society of Chemistry, 61–70.

YAPHE, W. 1960. Colorimetric determination of 3,6-anhydrogalactose and galactose in marine algal polysaccharides. – *Analytical Chemistry*, 32, 1327–1330.

YAPHE, W., ARSENAULT, G. P. 1965. Improved resorcinol reagent for the determination of fructose, and of 3,6-anhydrogalactose in polysaccharides. – *Analytical Biochemistry*, 13, 143–148.

YAROTSKY, S. V., SHASHKOV, A. S., USOV, A. I. 1978. Polysaccharides of algae. XXV. Application of ^{13}C-NMR spectroscopy for structural analysis of κ-carrageenan group polysaccharides. – *Bioorganicheskaya Khimiya*, 4, 745–751.

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*. – *Journal of Ocean University of China (English Edition)*, 6, 16–20.

YU, S. K., BLENNOW, A., BOJKO, M., MADSEN, F., OLSEN, C. E., ENGELSEN, S. B. 2002. Physico-chemical characterization of floridean starch of red algae. – *Starch-Starke*, 54, 66–74.

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