Proc. Estonian Acad. Sci. Chem., 2006, **55**, 1, 40–53
https://doi.org/10.3176/chem.2006.1.04

Extraction and quantification of hybrid carrageenans from the biomass of the red algae *Furcellaria lumbricalis* and *Coccotylus truncatus*

Rando Tuvikene[a*], Kalle Truus[a], Merike Vaher[b], Tiiu Kailas[b], Georg Martin[c], and Priit Kersen[c]

[a] Department of Natural Sciences, Tallinn University, Narva mnt. 25, 10120 Tallinn, Estonia
[b] Institute of Chemistry, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia
[c] Estonian Marine Institute, University of Tartu, Mäealuse 10a, 12618 Tallinn, Estonia

Received 26 August 2005, in revised form 2 November 2005

Abstract. The main isolation procedures (extraction, alkali modification, and precipitation) of hybrid carrageenan blend from seaweeds of the Kassari algal stratum (the Baltic Sea, Estonia) were quantitatively investigated. In the case of all extracting agents (water, KOH and NaOH solutions) used, a sharp rise occurred in the extracting rate during the first 2 h. Pure water was the most efficient extracting medium for carrageenans from the biomass of *Furcellaria lumbricalis*–*Coccotylus truncatus* with regard to yield but certainly not gelling properties (gel strength) of polysaccharides extracted. Long-time alkali extraction causes great losses due to degradation of carrageenans; however, alkali treatment is an obligatory step for the isolation of high-quality gelling galactans from the Kassari algal stratum, resulting in a more than 3-fold increase in gel strength. In the case of a typical algal blend from this stratum (69% *F. lumbricalis* and 10% *C. truncatus*), the most effective medium for the isolation of carrageenan mixture is 0.05 M KOH solution and the preferred duration of extraction is 3–4 h.

Key words: carrageenan, furcellaran, *Furcellaria lumbricalis*, *Coccotylus truncatus*, sulphated polysaccharide, gelling galactan, alkali treatment.

INTRODUCTION

Carrageenans [1, 2] are a family of linear sulphated polysaccharides, obtained by extraction from certain species of red seaweeds (Rhodophyta). They have a backbone built up of alternating 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose residues. A substantial part or even all the α-galacto-

* Corresponding author, rtu@akvaarium.com

pyranose residues may exist in the form of 3,6-anhydro derivative. The residues may be substituted by some groups in various positions (Fig. 1).

Natural carrageenans are mixtures of different sulphated galactans; they occur as very versatile hydrocolloids [3]. The galactans have a certain molecular mass distribution and contain a considerable amount of inorganic cations (usually about 10% or more of the mass). The gelling properties characteristic of carrageenans depend on the composition of the polysaccharic part as well as on the presence of counterions. Carrageenans from particular seaweed species and geographic districts differ considerably in their structure and rheological properties of solutions and gels. They are natural ingredients representing one of the major texturizing additives in the food industry [4, 5].

From about 15 principal structure types of carrageenans [6], two galactans, called kappa and iota carrageenan (Fig. 1), are the most widely distributed in algae and used in the food industry. To form strong and stable gels, they need a presence of some light cations such as potassium (for kappa carrageenan) or calcium (for iota carrageenan). Diverse natural mixtures of kappa and iota carrageenans are often formed directly by extraction of algal biomass issued from a variety of seaweed communities. Wide distribution and use of such carrageenan blends are the major reasons for extensive investigations [7–9] in recent years. Various gelation properties influenced by light cations and alkali extraction processes are also studied [10–12].

Red algae contain often non-stoichiometrically sulphated carrageenans. Part of them are known historically as furcellarans, polysaccharide blends from *Furcellaria lumbricalis* (Huds.) Lamour. (Furcellariaceae, Gigartinales) [13, 14]. This species was widely distributed in both the eastern and western North Atlantic and in brackish waters of the Baltic Sea still in the 1970s [15]. During the last two decades, however, natural sources of furcellarans have been strongly damaged, especially in the Baltic region. For example, the harvesting of *F. lumbricalis* in Denmark (the main producer of furcellaran) has drastically decreased since the 1980s [16] and the resources in Puck Bay (Poland) were completely destroyed by 1987 [17]. For that reason, the red algal stratum in Kassari Bay, Estonia (Fig. 2), is the greatest community of this seaweed species in Europe and probably in the world [18]. The shape and total biomass (about 150 thousand tonnes in 2005) of the stratum vary slightly from year to year.

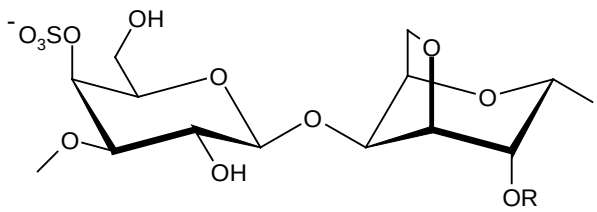

Fig. 1. Disaccharide repeating units of some principal carrageenans. Kappa carrageenan (R is H) and iota carrageenan (R is SO_3^-).

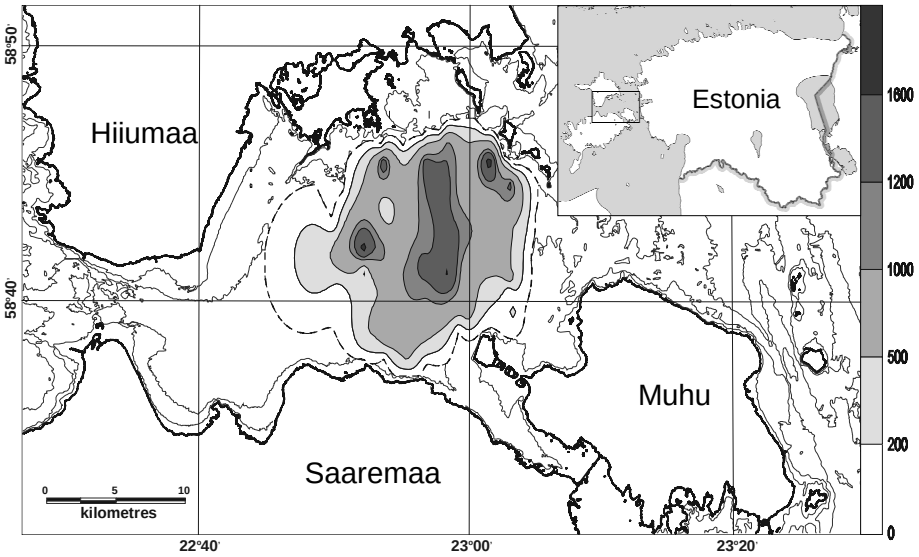

Fig. 2. Algal biomass distribution (scale on the right, g/m^2) and the location of the Kassari stratum in the Baltic Sea (summer 2004).

For the hybrid galactan mixture extracted from the biomass of this algal community containing *F. lumbricalis* with another red seaweed, *Coccotylus truncatus* (Phyllophoraceae, Gigartinales), some structure-property regularities have been researched [18, 19]. However, various aspects of alkali modification, extraction, and quantitative functionalities concerning this raw material have not been studied so far.

For sustainable utilization of the biomass from the Kassari algal stratum it is obligatory to solve problems connected with rational and economical isolation of seaweed galactans. The aim of this study was to elucidate suitable processing conditions for extracting carrageenans from the biomass of the Kassari Bay algal community. Great importance was attached to quantitative aspects of extracting media and isolation processes.

MATERIAL AND METHODS

Algae and carrageenan samples

Algae samples were collected in Kassari Bay (58°40.40′ N; 23°01.56′ W, the Baltic Sea, Estonia) using SCUBA diving from a depth of 6–8 m in August 2004. The samples were washed thoroughly with tap water and dried at outdoor temperature.

The average moisture content of the air-dried algal biomass was 6.51% (measured after drying 14 days in an air thermostat at 60°C). The species composition of the biomass is given in Table 1. In addition to dominant red algal

Table 1. Average composition of the algal biomass investigated

Species	Content, %
Furcellaria lumbricalis (Huds.) J. V. Lamour.	69
Coccotylus truncatus (Pall.) M. J. Wynne & J. M. Heine	10
Other marine organisms	21

species, the biomass contained also species from at least three algal genera (*Ceramium*, *Ectocarpus*, *Pylaiella*) and some other marine organisms.

Commercial carrageenan preparations were purchased from Sigma. Kappa carrageenan (from *Eucheuma cottonii*) contained 6.8% K^+, 0.6% Na^+, and 2.4% Ca^{2+} and iota carrageenan (from *Eucheuma spinosum*) 5.4% K^+, 1.3% Na^+, and 4.2% Ca^{2+}.

Extraction, modification, and precipitation

High-temperature extraction and modification

Air-dry algal mixture was refluxed in an extracting medium (distilled water, KOH or NaOH solutions of various concentrations). As a rule, the mass ratio of algae/extracting agent was 1 : 20 or 1 : 27; the time of extraction was counted in the boiling state.

Extraction and precipitation procedures were carried out according to Fig. 3. The hot extract was filtered under rarefying through a porous glass filter (porosity No. 2) into cold (7 °C) ethanol (95% v/v, 3-fold volume per extract), which causes precipitation of polysaccharides.

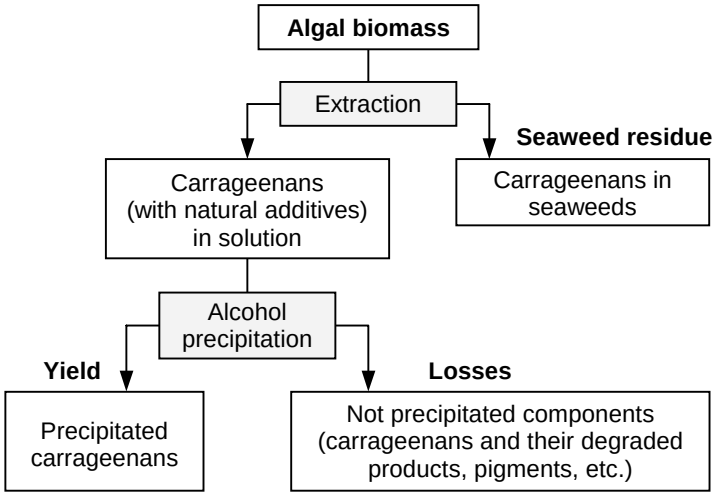

Fig. 3. General isolation process of carrageenans from algal biomass.

The seaweed residue was washed with boiling water, pressed on the filter to separate excessive liquid, and after that carefully removed and dried. Carrageenans precipitated were separated from the ethanol–water mixture by filtration under rarefying through a porous glass filter (porosity No. 3) and washed with cold (7 °C) ethanol. The isolated polysaccharide mixture was dried to a constant weight in a drying oven (60 °C, 14 days).

Long-term room-temperature modification

Air-dry algal mixture was stored in 25-fold mass of alkali solution (0.1 M KOH or NaOH) for 48 h at room temperature; after that the algal mass was separated from the solution and stored (in wet state) in an open vessel for 10 days. Then the algae mixture was thoroughly washed and used for the extraction of carrageenans (in 20-fold mass of boiling water).

Gel testing and chemical analysis

For gel strength assessments a simple gel tester equipped with a hemispherically tipped plunger (effective cross-section area 1 cm^2) was constructed. The gel strength measurements were done in triplicate for 1.5% gels (w/w) formed by dissolving dry carrageenan in hot water after gelling in an air thermostat at 20 °C for 4 h. The cylindrical samples were 36 mm in diameter and 17 mm in height. The force needed to rupture the gel by the plunger was expressed in g/cm^2; constant weight increase (234 g/min) was provided by water inflow.

The 3,6-anhydrogalactose (3,6-AG) content was determined colorimetrically using the resorcinol–acetal method [20] and fructose as the standard sugar in samples extracted in water or KOH solutions (mass ratio algae/ extracting medium 1 : 20) of various concentrations for 4 h.

FTIR spectra of the carrageenan samples were scanned with a PerkinElmer FTIR System Spectrum BX spectrometer (12 scans per spectrum; nominal resolution 4 cm^{-1}) from thin (0.015 mm) films obtained by slow evaporation of 1% solutions on the surface of Petri dish. The films were prepared from the carrageenans extracted in pure water and in 0.15 M KOH solution (extraction time 3 h). The spectra were recorded in the 4000–370 cm^{-1} region.

RESULTS AND DISCUSSION

Extraction dynamics and losses

Water and alkali (NaOH and KOH) solutions were comparatively used as extracting agents to separate hybrid carrageenans from the seaweed mixture. A blend of kappa and iota carrageenans formed by alkaline extraction has higher gel strength than a blend formed by water extraction [18, 19].

The dynamics of the process depends on the extracting agent used (Fig. 4). Water is the most efficient medium to isolate carrageenans from algal tissue. In

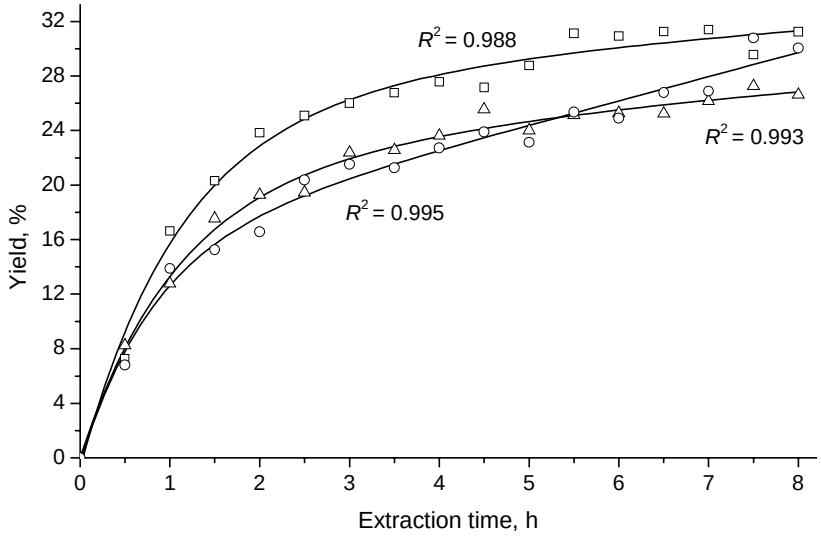

Fig. 4. Extraction dynamics of carrageenans from algal biomass in various media: water (□), 0.15 M KOH (○), and 0.15 M NaOH (△).

0.15 M KOH solution the process has the minimum rate but the final extraction yield is quite high.

In this study, 8-h extraction time was used as a maximum. Total extraction yields varied in the range 27–31% (of air-dry algal biomass) depending on the extracting agent. However, during the first 2 h already the bulk of carrageenans were extracted (of total yield, including impurities): 76.3% in water, 72.6% in 0.15 M NaOH, and 55.3% in 0.15 M KOH solution. Actually, the process could be interrupted after 4–5 h to prevent excessive destruction of carrageenans and to save energy but still achieving quite a high extraction yield.

The somewhat lower extraction yields in NaOH or KOH solutions were obviously caused by alkaline destruction of galactans; the low-molecular products cannot be precipitated by alcohol (do not constitute yield, see Fig. 3). The extent of alkaline destruction is expressed by losses of carrageenan extraction. A higher value of losses refers to two circumstances: the extracting agent has a greater ability to extract material (polysaccharides, pigments, proteins, etc.) from seaweeds and/or exerts a greater destructive effect on the galactans.

Losses (as well as yields) in alkaline media increase most rapidly in the initial stage of extraction and they also depend on extraction time (Fig. 5). Total losses (during 8-h extraction) are the largest in NaOH solution (33.4%) and somewhat lower in KOH solution (30.2%). In pure water these are minimal (21.7%) and nearly constant.

In the case of short extraction duration (3 h) even a very small alkali amount in the extracting medium exerts a great influence on the yield of carrageenan extraction. At alkali concentrations between 0 and 0.1 mol/L already small concentration differences lead to great changes in the yields (Fig. 6). The pheno-

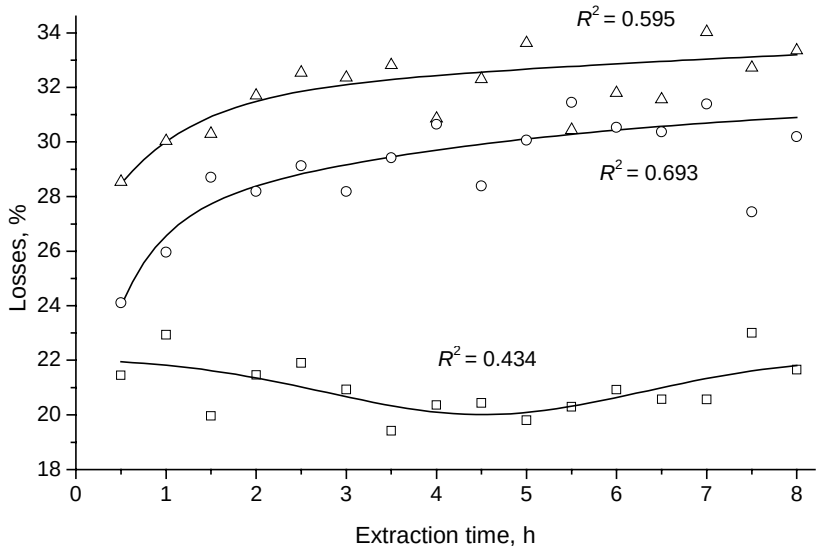

Fig. 5. Dependence of losses on extraction time in various media: water (□), 0.15 M KOH (○), and 0.15 M NaOH (△).

menon is probably connected with the pH jump that occurs when alkali is added to pure water. In the case of alkali solutions of the same concentrations, KOH solutions as the extracting media give greater yields.

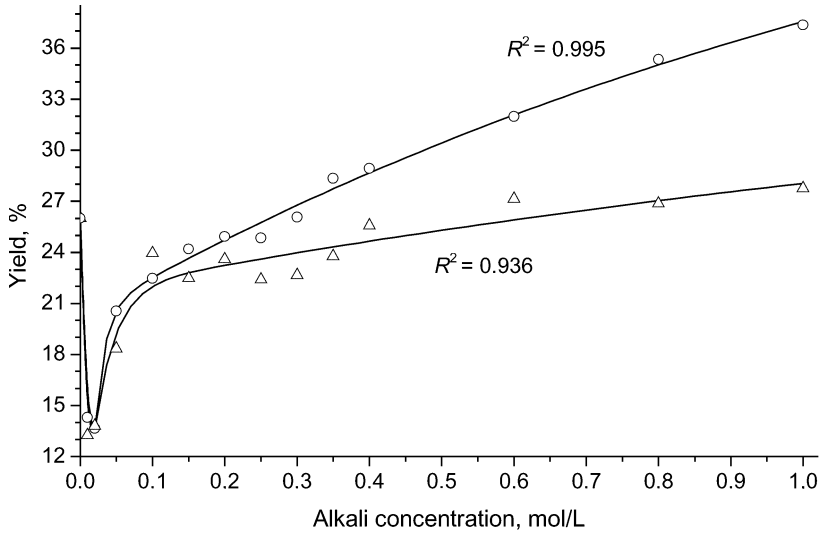

Fig. 6. Extraction differences of carrageenans by KOH (○) or NaOH (△) solutions at various concentrations. Extraction time 3 h. (Note the fluctuative influence of medium at very low alkali concentrations and in pure water.)

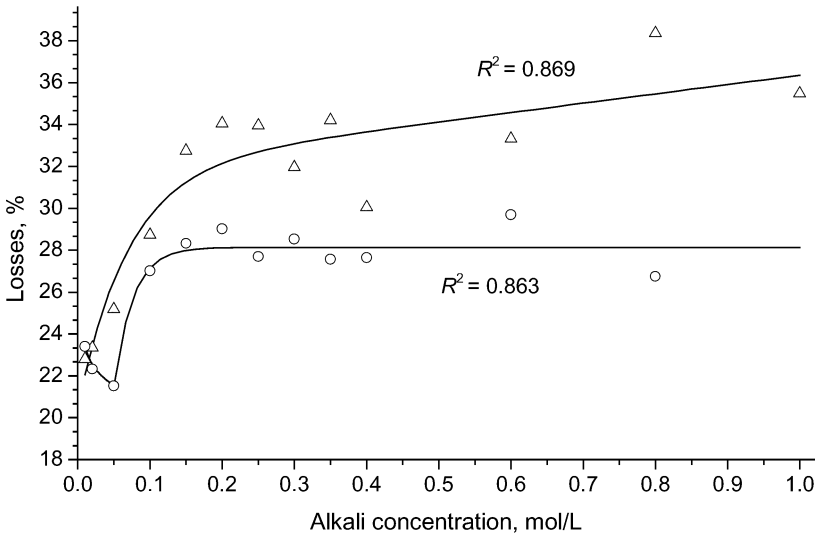

Fig. 7. Influence of alkali concentration in the extracting media on the losses of carrageenan extraction. Extraction time 3 h in KOH (○) or NaOH (△) solutions.

Losses were minimal when the algal biomass was extracted in 0.05 M KOH (21.5%) or 0.01 M NaOH solution (22.8%) (Fig. 7). Further increase in the alkali concentration in the extracting medium led to a sharp rise in losses.

NaOH in the extracting medium exerts a significantly greater destructive effect (greater losses) on carrageenans than KOH. Because of greater yields and lower losses KOH solutions show better properties as extracting media compared to NaOH solutions.

Dependence of gelling ability on alkali modification

High-temperature extraction and modification

In KOH extracting media, the alkali concentration has a great influence on gel strength, especially in the range 0.01–0.02 mol/L (increase 1.69 times at 3-h extraction, see Fig. 8). The highest gel strength (350 g/cm^2) of extracted carrageenans was achieved by boiling algae in 0.05 M KOH solution during 3–4 h. This value is 3.3 times higher than the maximum gel strength attainable in water. Such a great effect of potassium hydroxide treatment is caused by a favourable influence of an alkaline medium as well as potassium ions.

In NaOH extracting media, the higher gel strengths were achieved at low (0.02–0.05 mol/L) alkali concentrations; at higher values the gel strength did not depend significantly on alkali concentration (Fig. 9). The highest gel strength (280 g/cm^2) of extracted carrageenans was achieved by boiling in 0.02 M NaOH solution during 3 h.

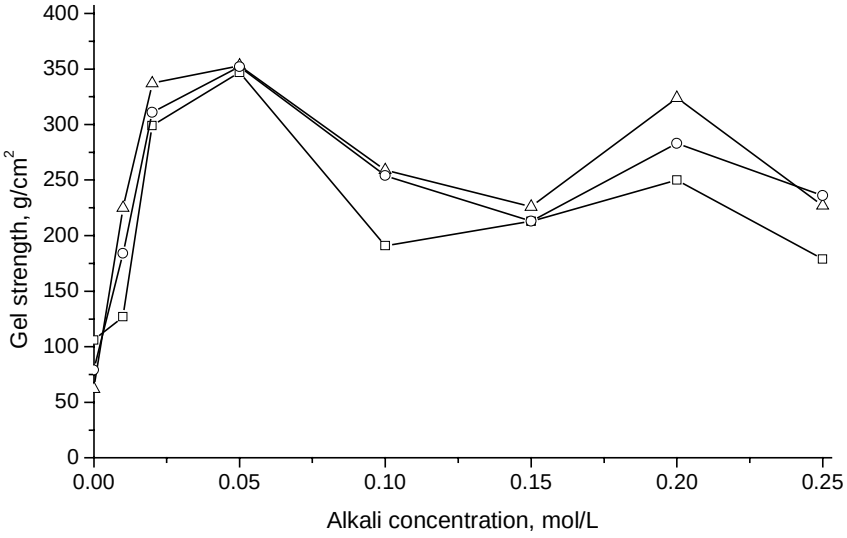

Fig. 8. Influence of extraction time and alkali concentration in the extracting media on the gel strength (for 1.5% gels) of extracted carrageenans. Extraction time 2 h (□), 3 h (○), or 4 h (△) in KOH solutions.

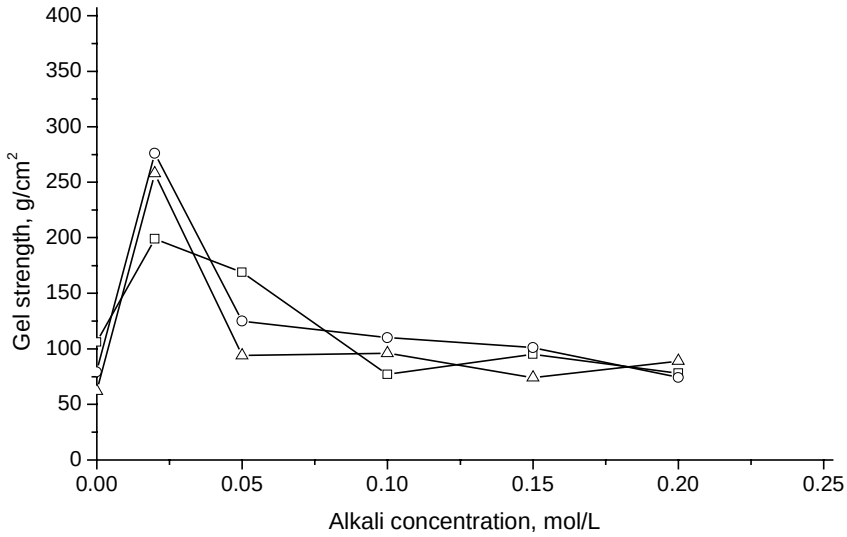

Fig. 9. Influence of extraction time and alkali concentration in the extracting media on the gel strength (for 1.5% gels) of extracted carrageenans. Extraction time 2 h (□), 3 h (○), or 4 h (△) in NaOH solutions.

Long-term room-temperature modification

The use of 0.1 M KOH solution as a modifying agent yields much higher gel strength (250 g/cm^2 if followed by 3-h water extraction) than NaOH solution of

the same concentration (80 g/cm^2, 3-h water extraction). In addition, the low-temperature alkali treatment yields a less coloured product than high-temperature treatment.

Thus, from the aspect of gelling ability, the use of KOH as the alkali in the extracting medium is preferable both at high and low temperatures.

Chemical composition and structure

The 3,6-AG content is higher in samples extracted in media of low alkali (KOH) concentrations (Fig. 10). Two maximums of 3,6-AG occur at KOH concentrations of 0.05 and 0.20 mol/L (24.1% and 22.2% respectively); the absolute maximum (24.6%) was achieved using pure water as the extracting agent. For reference the 3,6-AG content was determined in commercial carrageenan preparations (Sigma): kappa carrageenan 31.7% and iota carrageenan 26.3%.

Samples extracted under different conditions show a good positive correlation (r = 0.984) between the 3,6-AG content and gel strength: higher 3,6-AG content raises the gel strength (compare Figs 8 and 10). Such dependence does not occur at very low (0–0.02 M KOH) alkali concentrations when the galactan matrix is not saturated by potassium ions yet.

As it is seen from FTIR spectra (Fig. 11), only small changes occur in the sulphur concentration of hybrid carrageenans extracted by water or an alkali solution.

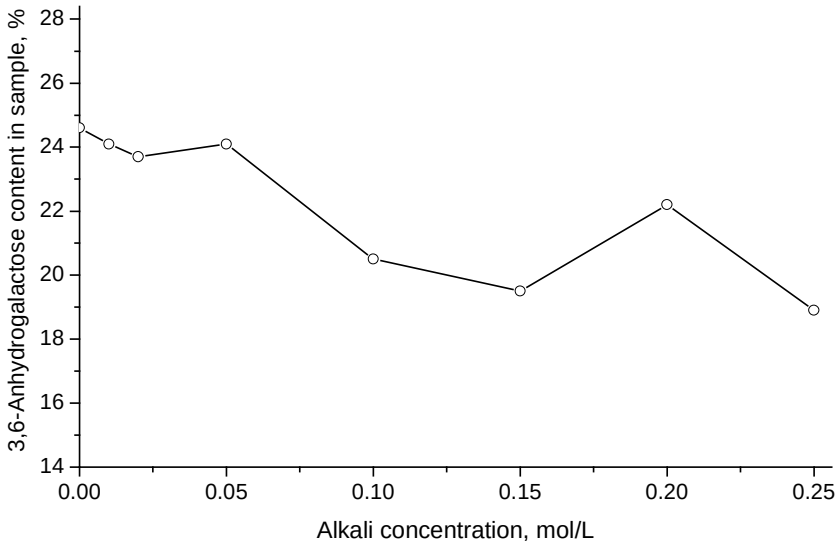

Fig. 10. Influence of alkali concentration in the extracting media on the 3,6-anhydrogalactose content of extracted carrageenans. Extraction time 4 h in KOH solutions.

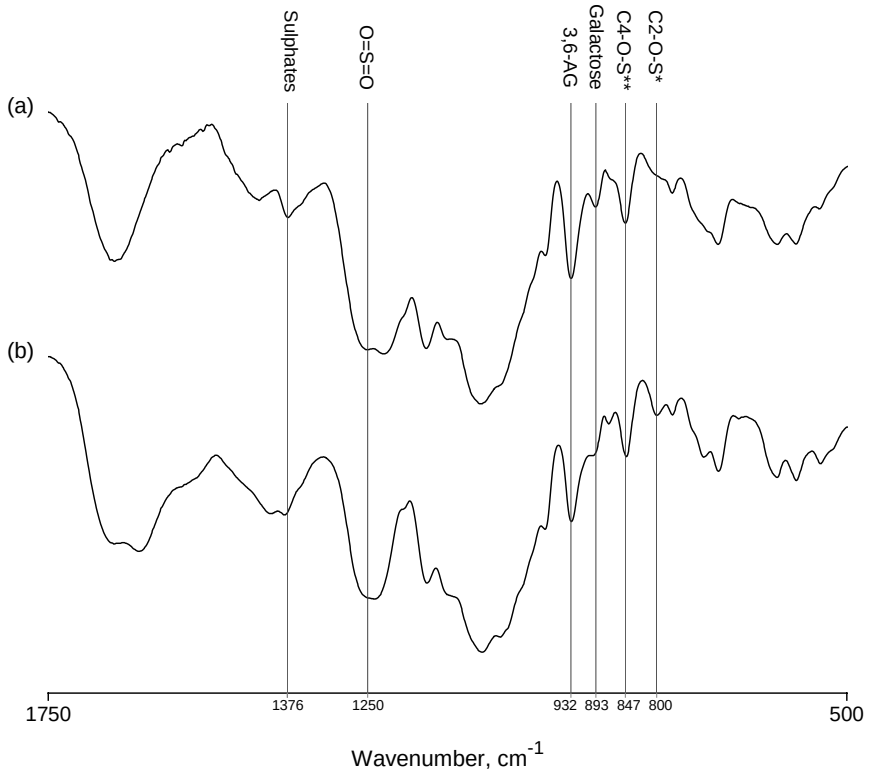

Fig. 11. FTIR spectra of carrageenans extracted 3 h in H_2O (a) and 0.15 M KOH (b). * in 3,6-AG; ** in galactose.

It is known from earlier studies [18] that the average sulphur content of galactans from *F. lumbricalis* is about 3% and it only slightly (±10%) varies depending on the alkali treatment. On the basis of these data and a recent concise study [8] it may be concluded that more detailed IR-investigations are needed for structural decisions about hybrid carrageenans from the Kassari algal stratum.

CONCLUSIONS

The main results of our study on the extraction and quantification of hybrid carrageenans from red algal biomass of the Kassari algal stratum may be summarized as follows.

Extraction yield and destruction. Especially intensive extraction proceeds during the first two hours of boiling. The greatest yields (31.2%) of carrageenans can be obtained using pure water or quite strong (0.4 mol/L and more) KOH solutions for extraction. The extent of carrageenans' destruction depends on the

alkali (being higher in NaOH solution) and its concentration, and greatly on extraction time.

Dependence of gel properties on extraction conditions. The rheological properties of carrageenan gels, particularly their gel strength, have a conclusive importance in practical application of these biopolymers. It is possible to increase gel strength more than 3 times by alkali treatment of algae. The greatest gel strength values of carrageenans achieved in different extracting media were as follows: 350 g/cm^2 in 0.05 M KOH solution (4 h), 280 g/cm^2 in 0.02 M NaOH solution (3 h), and 110 g/cm^2 in water (2 h). When KOH solutions are used as extracting agents, gel strength shows two maximums at alkali concentrations of 0.05 and 0.20 mol/L. The colour and transparency of the gel obtained depend greatly on the extraction method, agent, and duration. Pure water as a medium gives significantly lighter products (compared to samples extracted in alkaline solutions); greater alkali concentrations lead to darker and more pigmented products. Long-term alkali treatment of algae at room temperature yields less coloured and more transparent gels.

Influence of extraction conditions on the carrageenans composition. The 3,6-AG concentration in carrageenan samples varies according to extraction conditions (alkali concentration in media) from 18.9% to 24.6% and shows a good positive correlation to gel strength. In all cases the 3,6-AG contents of extracted samples were lower than those of the commercial carrageenan (kappa and iota forms) preparations. According to FTIR spectra and chemical analysis (resorcinol–acetal method), the alkali treatment causes a significant destruction of the monosaccharide components of carrageenan.

The most suitable way to extract carrageenans from the seaweeds of the Kassari stratum (considering yield and gel strength) is to use 0.05 M KOH solution as the extracting agent and extraction time of 3–4 h. In addition, the proposed extraction method has some advantages over traditional carrageenan isolation procedures: it is a more nature-preserving and technologically simpler method.

ACKNOWLEDGEMENT

The authors would like to thank Prof. Henn Kukk for useful discussions and identification of species composition of the algal biomass.

REFERENCES

1. Usov, A. I. Structural analysis of red seaweed galactans of agar and carrageenan groups. *Food Hydrocolloids*, 1998, **12**, 301–308.
2. Van de Velde, F., Knutsen, S. H., Usov, A. I., Rollema, H. S. & Cerezo, A. S. ^{1}H and ^{13}C high resolution NMR spectroscopy of carrageenans: application in research and industry. *Trends Food Sci. & Technol.*, 2002, **13**, 73–92.

3. Witt, H. J. Carrageenan, Nature's most versatile hydrocolloid. In *Biotechnology of Marine Polysaccharides* (Colwell, R. R., Pariser, E. R. & Sinskey, A. J., eds). Hemisphere Publishing Corp., Washington, 1985, 345–360.
4. Stanley, N. F. Carrageenans. In *Food Gels* (Harris, P., ed.). Elsevier, London, New York, 1990, 79–119.
5. Sandford, P. A. Applications of marine polysaccharides in the chemical industries. In *Biotechnology of Marine Polysaccharides* (Colwell, R. R., Pariser, E. R. & Sinskey, A. J., eds). Hemisphere Publishing Corp., Washington, 1985, 453–516.
6. Knutsen, S. H., Myslabodski, D. E., Larsen, B. & Usov, A. I. A modified system of nomenclature for red algal galactans. *Bot. Mar.*, 1994, **37**, 163–169.
7. Villanueva, R. D., Mendora, W. G., Rodrigueza, M. R. C., Romero, J. B. & Montaño, M. N. E. Structure and functional performance of gigartinacean kappa–iota hybrid carrageenan and solieriacean kappa–iota carrageenan blends. *Food Hydrocolloids*, 2004, **18**, 283–292.
8. Prado-Fernández, J., Rodríguez-Vázquez, J. A., Tojo, E. & Andrade, J. M. Quantitation of κ-, ι- and λ-carrageenans by mid-infrared spectroscopy and PLC regression. *Anal. Chim. Acta*, 2003, **480**, 23–37.
9. Tojo, E. & Prado, J. A simple ^{1}H NMR method for the quantification of carrageenans in blends. *Carbohydr. Polym.*, 2003, **53**, 325–329.
10. Mangione, M. R., Giacomazza, D., Bulone, D., Martorana, V., Cavallaro, G. & San Biagio, P. L. K^+ and Na^+ effects on the gelation properties of κ-carrageenan. *Biophys. Chem.*, 2005, **113**, 129–135.
11. MacArtain, P., Jacquier, J. C. & Dawson, K. A. Physical characteristics of calcium induced κ-carrageenan networks. *Carbohydr. Polym.*, 2003, **53**, 395–400.
12. Viana, A. G., Noseda, M. D., Duarte, M. E. R. & Cerezo, A. S. Alkali modification of carrageenans. Part V. The iota-nu hybrid carrageenan from *Eucheuma denticulatum* and its cyclization to iota-carrageenan. *Carbohydr. Polym.*, 2004, **58**, 455–460.
13. Painter, T. J. The location of the sulphate half-ester groups in furcellaran and κ-carrageenan. In *Proc. Intern. Seaweed Symp. 5* (Young, E. G. & McLachlan, J. L., eds). Pergamon, London, 1966, 305–313.
14. Christiansen, E. Danish agar. In *Industrial Gums. Polysaccharides and Their Derivatives* (Whistler, R. L. & BeMiller, J. N., eds). Academic Press, New York & London, 1959, 51–54.
15. Holmsgaard, J. E., Greenwell, M. & McLachlan, J. Biomass and vertical distribution of *Furcellaria lumbricalis* and associated algae. In *Proc. Intern. Seaweed Symp. 10* (Levring, T., ed.). Walter de Gruyter, Berlin, New York, 1981, 309–314.
16. Briand, X. Seaweed harvesting in Europe. In *Seaweed Resources in Europe: Uses and Potential* (Guiry, M. D. & Blunden, G., eds). John Wiley, Chichester, 1991, 259–308.
17. Kruk-Dowgiałło, L. & Ciszewski, P. *Zatoka Pucka. Możliwości rewaloryzacji*. Instytut Ochrony Środowiska, Warszawa, 1994, 178.
18. Truus, K., Vaher, M., Usov, A. I., Pehk, T. & Kollist, A. Gelling galactans from the algal community of *Furcellaria lumbricalis* and *Coccotylus truncatus* (the Baltic Sea, Estonia): a structure-property study. *Int. J. Biol. Macromol.*, 1997, **21**, 89–96.
19. Vaher, M., Truus, K., Taure, I., Leito, I., Lahe, L., Kallavus, U. & Pehk, T. Composition and structure of summary polysaccharide from the red alga *Furcellaria lumbricalis*. *Proc. Estonian Acad. Sci. Chem.*, 1998, **47**, 51–59.
20. Yaphe, W. & Arsenault, G. P. Improved resorcinol reagent for the determination of fructose and 3,6-anhydrogalactose in polysaccharides. *Anal. Biochem.*, 1965, **13**, 143–148.

Hübriidsete karraginaanide ekstraheerimine ja kvantifitseerimine punavetikate *Furcellaria lumbricalis* ja *Coccotylus truncatus* koosluse biomassist

Rando Tuvikene, Kalle Truus, Merike Vaher, Tiiu Kailas,
Georg Martin ja Priit Kersen

Kvantitatiivselt on uuritud Kassari vetikaplasti punavetikates sisalduvate hübriidsete karraginaanide peamisi eraldamisprotsesse (ekstraktsioon, leelistöötlus ja sadestamine). Kõigi kasutatud ekstrahentide (vesi, KOH ja NaOH vesilahused) puhul esines saagise kiire tõus ekstraktsiooni algetapil (esimese 2 tunni jooksul). Vesi on saagise (kuid mitte geelitugevuse) seisukohast kõige efektiivsem ekstraktsioonikeskkond karraginaanide eraldamiseks punavetikate *Furcellaria lumbricalis* ja *Coccotylus truncatus* koosluse biomassist. Kauakestva leelisekstraktsiooniga kaasnevad karraginaanide lagunemise tõttu suured kaod; kõrgekvaliteetsete geelistuvate galaktaanide saamisel on aga oluline leelistöötluse rakendamine, mille tulemusel suureneb nende geelitugevus enam kui kolm korda. Uuritud Kassari plasti punavetikasegust (69% *F. lumbricalis*'t ja 10% *C. truncatus*'t) on geelitugevuse ja saagise seisukohast karraginaane kõige otstarbekam ekstraheerida keetmisel 3–4 tundi 0,05 M KOH vesilahuses.