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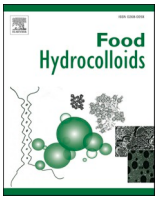

Ice recrystallization inhibition activity of chemically defined carrageenans

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ABSTRACT

Carrageenans are sulfated galactans from algae which are commonly used as thickeners, gelling agents, or stabilizers. It has been demonstrated that they also have significant ice recrystallization inhibition (IRI) activity. Previous studies mainly focused on κ-carrageenan, but recent studies suggested that other carrageenans such as ι-carrageenans or carrageenans with multiple structural elements also have this functionality. Therefore, the aim of our study was to analyze and compare the IRI activity of carrageenans with defined chemical structures and associated cations. For this purpose, κ- and ι-carrageenans as well as several hybrid carrageenans showing broad heterogeneity with regards to the molecular structure and the cations present were investigated. The selected commercial samples were subsequently converted into their potassium, calcium and (in part) sodium forms. Chemical characterization of the modified carrageenans demonstrated that the molecular structure was unaltered by the applied procedures and that the carrageenans were successfully converted into the different cation forms. The analysis of the IRI activity demonstrated that both molecular structure and associated cations had an influence on carrageenan functionality. The κ-carrageenan and hybrid carrageenans with consecutive κ-units showed a high IRI activity, whereas ι-carrageenan was less active. For κ-carrageenan, the potassium form showed a clearly higher activity than the calcium form, whereas the calcium form was more active for hybrid carrageenans and ι-carrageenans. Our results significantly expand the knowledge on the relationship between the molecular composition and the IRI activity of carrageenans. Furthermore, they can be used to optimize carrageenan production to obtain an enhanced IRI activity.

1. Introduction

Carrageenans are sulfated galactans which are extracted from red algae (Ruiter & Rudolph, 1997). These polysaccharides consist of alternating 1,3-linked β-D-galactopyranose units (G-unit) and 1,4-linked α-D-galactopyranose units (D-unit). The D-unit may also be present as 3,6-anhydro-α-D-galactopyranose, which is then referred to as DA-unit. Both the G-unit as well as the D/DA-unit can be sulfated at different positions (Usov, 1998; Van de Velde & Ruiter, 2001). Depending on the structural composition of the polysaccharide, the sulfate content usually varies between 22 and 38 % (w/w) (Ruiter & Rudolph, 1997).

To describe the chemical structure of carrageenans, Knutsen, Myslabodski, Larsen, and Usov (1994) proposed a nomenclature including the descriptor for the monosaccharide unit and the position of the sulfate group in combination with the letter S. According to this nomenclature, a 1,3-linked β-D-galactopyranose that is sulfated at position *O*-4 is for example named 'G4S' (Knutsen et al., 1994). Moreover, the structures of carrageenans are often described by using repeating disaccharide motifs (consisting of one G-unit and one D-/DA-unit). Depending on the disaccharide unit present, carrageenans are categorized into different types, which are abbreviated with Greek letters. The commercially most important types are κ-carrageenan (G4S-DA), ι-carrageenan (G4S-DA2S), and λ-carrageenan (G2S-D2S,6S) (Usov, 1998; Van de Velde & Ruiter, 2001). Carrageenans containing a D-unit instead of a DA-unit (e. g. λ-carrageenan, ν-carrageenan, μ-carrageenan) are often referred to as natural precursors. The conversion into the DA-unit typically occurs under alkaline conditions or by the use of enzymes. For example, ν-carrageenan (G4S-D2S,6S) can be converted into

Abbreviations: IRI, ice recrystallization inhibition; HPSEC, high-performance size exclusion chromatography; RI, refractive index; MALLS, multi angle laser light scattering; ICP-OES, inductively coupled plasma optical emission spectrometry.

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ι-carrageenan (G4S-DA2S) (Usov, 1998).

Categorizing carrageenans into different types can be challenging because one sample can contain more than one type of disaccharide unit. When multiple units are present within one polymer chain, the corresponding carrageenans are referred to as hybrid carrageenans. However, it is also possible that mixtures are present, which contain multiple non-covalently linked carrageenans (Van de Velde, 2008). Accordingly, carrageenans show highly heterogeneous chemical structures that determine the physicochemical properties of the polysaccharides.

It has been demonstrated that different carrageenans have a significant ice recrystallization inhibition (IRI) activity. While κ-carrageenan showed a strong IRI activity in all studies, the results for ι-carrageenan were somewhat ambiguous (Gaukel, Leiter, & Spieβ, 2014; Kamińska-Dwórznicka, Janczewska-Dupczyk, Kot, Łaba, & Samborska, 2020; Kiran-Yildirim & Gaukel, 2020; Kiran-Yildirim, Hale, Wefers, & Gaukel, 2021; Leiter, Emmer, & Gaukel, 2018; Leiter, Ludwig, & Gaukel, 2017; Leiter, Mailänder, Wefers, Bunzel, & Gaukel, 2017). However, commercial carrageenans were used in previous studies and a structural characterization has not been conducted in all cases. An analysis of IRI activity of carrageenans characterized by NMR spectroscopy suggested that ι-carrageenan exhibited a lower IRI activity than κ-carrageenan. Furthermore, carrageenans with different structural units (κ, ι and, in part, ν) showed different levels of IRI activity (Kiran-Yildirim et al., 2021). However, NMR spectroscopy cannot distinguish between carrageenan mixtures and hybrid carrageenans. In a previous study, we demonstrated that commercial carrageenans show considerable structural heterogeneity and that carrageenan types specified by the manufacturer do not necessarily reflect the actual structural composition (Hale, Gerhäuser, Gaukel, & Wefers, 2024). Several samples specified as λ-carrageenans were actually κ-ι or κ-ι-ν hybrid carrageenans. Our investigation also revealed that commercial carrageenans contain different types of cations. Different types of monovalent and divalent cations are known to influence aggregation of carrageenan chains in solution which may also impact the interaction with the ice crystal surface (Funami et al., 2007; Hale et al., 2024; Mangione et al., 2005; Piculell, 2006; Schefer, Adamcik, Diener, & Mezzenga, 2015). Leiter, Ludwig, and Gaukel (2017) already demonstrated that the addition of different salts influences the IRI activity of κ-carrageenan. Therefore, it is highly likely that cations which are already present in carrageenans influence carrageenan functionality as well. However, this aspect has not been investigated yet.

Altogether, the IRI activity of carrageenans is well established but its dependence on the molecular structure and the cations present in carrageenans is poorly understood. Therefore, the aim of our study was to produce chemically defined carrageenans and investigate them for their IRI activity. For this purpose, we used commercial carrageenans which were previously characterized in detail and converted them into their monocationic forms.

2. Experimental

2.1. Materials

The carrageenans used in this study were obtained from various manufacturers, including food and chemical suppliers (Eurogum (Denmark), CP Kelco (USA), Tate & Lyle (United Kingdom), Merck (Germany), Alfa Aesar (USA), Biosynth (Switzerland), and Dextra Laboratories (United Kingdom)). From a total of 16 samples, seven carrageenans were selected for the preparation of monocationic forms and an analysis of IRI activity. The chemical structure of the selected carrageenans was investigated in detail in a previous study (Hale et al., 2024). Calcium chloride dihydrate (≥99 %), sodium hydroxide (≥99 %), dimethyl sulfoxide (≥99.5 %), and sucrose (≥99.5 %) were purchased from Carl Roth (Germany). Hydrochloric acid (37 %) and isopropanol were obtained from VWR International (Germany). Deuterium oxide (≥99.9 % D) was obtained from Deutero (Germany). All other chemicals were purchased from Merck (Germany). If necessary, Milli-Q water was used for the experiments.

2.2. Cation exchange and chemical modification

The monocationic carrageenans were prepared as described by Polowsky and Janaswamy (2015). Briefly, 400 mg of carrageenan were dissolved in 150 mL of 800 mM sodium chloride, 800 mM potassium chloride, or 100 mM calcium chloride. The solution was treated in a water bath at a temperature of 80 °C for 2 h. The polysaccharide was then precipitated with two volumes of cold isopropanol (0 °C). The precipitate was washed with 80 % and 100 % isopropanol, and dried at a temperature of 50 °C.

To convert ν-units into ι-units, 250 mg of carrageenan were mixed with 125 mL of a 1 M sodium hydroxide solution. The sample was treated in a water bath at a temperature of 80 °C for 35 min (Ciancia, Noseda, Matulewicz, & Cerezo, 1993; Doyle, Giannouli, Rudolph, & Morris, 2010). The solution was then cooled to room temperature in an ice bath and neutralized with 1 M hydrochloric acid. After neutralization, the volume of the solution was reduced to 100 mL by rotary evaporation. To remove excess salt, the sample solution was dialyzed for 20 h against Milli-Q water (dialysis solution was changed twice). Finally, the sample was freeze-dried. By using sodium hydroxide for the structural modification, the sodium form of the modified carrageenan was obtained. In order to prepare other monocationic forms of the modified carrageenan, the sample was subjected to the cation exchange process described above.

2.3. NMR spectroscopy

NMR experiments were carried out on an Avance Neo 400 MHz spectrometer (Bruker, Germany) equipped with a temperature-controlled 5 mm probe head. The spectra were recorded at a temperature of 65 °C. A standard ^{1}H pulse program ('zg90') from Bruker was used with a relaxation delay (d_1) of 25 s and an acquisition time of 2 s. The number of scans was either 16 or 32. For sample preparation, 5 mg of carrageenan were mixed with 1 mL of deuterium oxide and treated in a water bath at 70 °C until the polysaccharide was completely dissolved. Dimethyl sulfoxide (0.5 μL) was used as an internal reference (2.696 ppm according to Van de Velde, Pereira, and Rollema (2004)). The structural units of the different carrageenan types can be identified by the signals of the anomeric protons of the D-/DA-unit (Van de Velde et al., 2004).

2.4. HPSEC-RI/MALLS

An HPSEC-RI/MALLS system was used to determine the weight average molecular weight (M_W) of the carrageenans. The system was equipped with a refractive index detector (L-7490, Hitachi, Merck, Germany) and a multi angle laser light scattering (MALLS) detector (SLD7100, PSS Polymer Standards Service, Germany). The polymers were separated on a TSKgel G6000PW$_{XL}$ column (Tosoh Bioscience, Japan) at 60 °C. As described by Lecacheux, Panaras, Brigand, and Martin (1985), 0.1 M lithium chloride was used as the eluent to suppress aggregation of the carrageenans. The analysis was conducted at an isocratic flow rate of 0.5 mL/min. To calculate the M_W of the carrageenans, a refractive index increment of 0.115 g/mL was used (Lecacheux et al., 1985). For sample preparation, 2 mg of carrageenan were mixed with 1 mL of 0.1 M lithium chloride and treated in a water bath at a temperature of 60 °C until complete dissolution of the sample.

2.5. ICP-OES

For the analysis of associated cations and sulfur, a Varian 715-ES (Agilent Technologies, USA) or an iCAP 7000 instrument (Thermo Fisher Scientific, USA) was used. The external calibration covered a

range from 0.25 to 50 mg/L and was prepared using standard solutions of potassium, sodium, calcium and sulfur. For ICP-OES analysis, carrageenan solutions with a concentration of 1 mg/mL were used. The solutions were prepared in 1 % (v/v) nitric acid and had a total volume of 10 mL.

2.6. Ice recrystallization inhibition activity

The IRI activity of the carrageenan samples was determined in a 49 % (w/w) sucrose solution to which 1 mg/mL carrageenan was added. For sample preparation, sucrose, carrageenan and Milli-Q water were weighed and mixed. The solution was treated in a water bath at a temperature of 60 °C until complete dissolution of the samples (1–2 h). As a control, 49 % (w/w) sucrose solution without the addition of carrageenan was used. 10 μL of the sample solution were placed on a microscopic slide between two cover slips that had been previously glued to the slide at a distance of 8–10 mm. The sample was covered with a third cover slip and the edges were then sealed with silicone to prevent the solution from evaporating. After drying of the silicone, the microscopic slides were rapidly frozen in liquid nitrogen to ensure that the sample solution reached a glassy state. The samples were stored for 72 h in three small cooling chambers at a temperature of −12.0 °C ± 0.1 °C. The temperature of the cooling chambers was controlled by an external cryostat (FP50, Julabo, Germany). The three cooling chambers were placed inside a glove box, which maintained a temperature of −12.0 °C ± 1.0 °C. Over storage time, the temperatures of the cooling chambers and the glove box were recorded by thermocouples.

Microscopic images of each sample were taken with a polarizing microscope (BX41, Olympus, Japan) equipped with a camera (Mikrocam II 5 MP HIS, Bresser, Germany). To determine the mean ice crystal diameter of a sample, the area of at least 200 ice crystals per microscopic slide was manually analyzed using either ImagePro 9.3 (Media Cybernetics, USA) or ImageJ (National Institutes of Health (NIH), USA) software. The equivalent diameter of each ice crystal was determined by calculating the diameter of a circle with the same area. Based on the equivalent diameters of the individual crystals, the mean ice crystal equivalent diameter of the sample was calculated. Four microscopic slides were prepared for each carrageenan and the mean ice crystal diameter was derived from the mean equivalent diameters of the four slides.

3. Results & discussion

3.1. Production of chemically defined, monocationic carrageenans

Based on the detailed structural and compositional characterization of commercial carrageenans (Hale et al., 2024), 7 samples were selected for the production of chemically defined carrageenans. These samples were selected because they show a high structural diversity and are thus well-suited for an evaluation of structure-function relationships. The selected carrageenans included a κ- and ι-carrageenan (previously KC1 and IC1, subsequently referred to as κC and ιC) and five carrageenans with different structural units. In our previous study, NMR spectroscopy and partial enzymatic hydrolysis suggested that two carrageenans (IC3 and C2) were ι/κ and κ/ι hybrid carrageenans which are mainly composed of consecutive blocks of ι- and κ-units. These samples will be referred to as ικC (IC3) and κιC1 (C2). In contrast, the κ-/ι-carrageenan κιC2 (previously LC2) contains long blocks of κ-units and only single ι-units or very small sections thereof. The carrageenans LC1 and C1 were hybrid carrageenans with long blocks of κ-units and small blocks of ι- and ν-units. Therefore, these samples will be referred to as κιν C1 and κιν C2. In our previous study, we also demonstrated that the commercial carrageenans show significant variation of the associated cations (potassium, calcium, and sodium) (Hale et al., 2024). As described above, this may significantly influence IRI activity, thus, monocationic carrageenans were produced. The focus was on potassium and calcium because these cations are most common in carrageenans due to the manufacturing process. However, the κ- and ι-carrageenan samples κC and ιC as well as two hybrid carrageenans (κιν C1 and κιC2) were also converted to their sodium form and analyzed for their IRI activity. In addition, κιν C1 was also subjected to alkaline treatment to convert ν-units to ι-units (sodium and potassium form). By comparing the IRI activity before and after conversion, the influence of ν-units can be evaluated. The structural composition of all carrageenans was fully characterized to exclude significant changes which could affect the IRI activity.

3.2. Characterization of the chemically defined, monocationic carrageenans

3.2.1. Structural composition

To evaluate if the molecular structure of the carrageenans was modified, ^{1}H NMR spectroscopy was applied (Table 1).

For all carrageenan samples except κιC1, the same structural elements were detected in similar portions before and after modification. Minor variations could be derived from inaccuracies during the integration of the diagnostic NMR signals. Therefore, the cation exchange does not lead to a structural modification. However, the potassium and calcium forms of κιC1 showed 42 %/40 % κ-units and 58 %/60 % ι-units and thus a clearly different structural composition than the unmodified carrageenan (59 % κ-units and 41 % ι-units). This variation is caused by the presence of sucrose (added for standardization by the manufacturer) in the unmodified carrageenan: The sucrose-derived signal at 5.40 ppm interferes with the integration of the signal derived from the DA-unit of the ι-units at 5.28 ppm. Sucrose is removed during the precipitation step of the cation-exchange, thus, the results obtained for the monocationic carrageenans better reflect the structural composition of κιC1. For the modified versions of κιν C1 (κιν C1m), ν-units were not detected which confirms the successful modification.

3.2.2. Molecular weight

To investigate if the carrageenans were partially hydrolyzed during the treatment with strong salt solutions or NaOH, the molecular weight was analyzed by HPSEC-RI/MALLS (Table 2).

Most of the monocationic carrageenans showed a similar molecular weight than the unmodified samples, except for the calcium forms of κC, ιC, and κιC2. However, because the molecular weights of other calcium forms were unaltered, these changes could be derived from a selective precipitation due to the different ionic environment. Because the molecular weights were still high (491–838 kDa), an influence on the IRI activity is highly unlikely: Leiter, Mailänder, et al. (2017) showed that a

Table 1
Structural composition of the carrageenans used in this study before and after cation exchange and alkaline modification (κιν C1m). The characteristic signals of the D-/DA-units were assigned according to Van de Velde et al. (2004) and signal integrals were used to calculate the portions of the individual structural elements. NMR spectra are shown in Fig. S1.

Sample	Structural unit (portion, %)	Sample	Structural unit (portion, %)
κC	κ	κιC2	κ (72), ι (28)
κC K	κ	κιC2 K	κ (67), ι (33)
κC Na	κ	κιC2 Na	κ (68), ι (32)
κC Ca	κ	κιC2 Ca	κ (67), ι (33)
ιC	ι	κιν C1	κ (47), ι (31), ν (22)
ιC K	ι	κιν C1 K	κ (51), ι (29), ν (20)
ιC Na	ι	κιν C1 Na	κ (47), ι (32), ν (21)
ιC Ca	ι	κιν C1 Ca	κ (46), ι (30), ν (23)
ικC	κ (27), ι (73)	κιν C1m K	κ (53), ι (47)
ικC K	κ (27), ι (73)	κιν C1m Na	κ (52), ι (48)
ικC Ca	κ (25), ι (75)	κιν C2	κ (37), ι (33), ν (30)
κιC1	κ (59), ι (41)	κιν C2K	κ (37), ι (34), ν (29)
κιC1 K	κ (42), ι (58)	κιν C2 Ca	κ (38), ι (32), ν (30)
κιC1 Ca	κ (40), ι (60)		

Table 2
Molecular weight (determined by HPSEC-RI/MALLS) of the carrageenans used in this study before and after cation exchange and alkaline modification (κιν*C1m). Chromatograms of the individual carrageenans are shown in Fig. S2.

Sample	M_w, kDa
κC	1013 ± 35
κC K	919 ± 8
κC Na	901 ± 42
κC Ca	644 ± 14
ιC	999 ± 16
ιC K	924 ± 15
ιC Na	925 ± 91
ιC Ca	838 ± 6
ικC	523 ± 61
ικC K	519 ± 2
ικC Ca	514 ± 0
κιC1	886 ± 30
κιC1 K	890 ± 0
κιC1 Ca	825 ± 16
κιC2	641 ± 8
κιC2 K	639 ± 15
κιC2 Na	613 ± 0
κιC2 Ca	491 ± 12
κιν*C1	923 ± 8
κιν*C1 K	860 ± 4
κιν*C1 Na	831 ± 5
κιν*C1 Ca	868 ± 37
κιν*C1m K	540 ± 27
κιν*C1m Na	686 ± 2
κιν*C2	1158 ± 21
κιν*C2 K	1068 ± 55
κιν*C2 Ca	1163 ± 14

Table 3
Contents of K^+, Ca^{2+}, Na^+, and sulfate (determined by ICP-OES) of the carrageenans used in this study before and after cation exchange and alkaline modification (κιν*C1m). +++ = > 1 %, ++ = 0.5–1 %, + = 0.1–0.5 %, - = ≤ 0.1 %. The individual contents can be found in Table S2.

Sample	K^+	Na^+	Ca^{2+}	Sulfate, % (w/w)
κC	+++	–	++	18.1
κC K	+++	–	–	16.3
κC Na	+	+++	–	20.0
κC Ca	++	–	+++	20.4
ιC	+++	–	+++	28.2
ιC K	+++	–	–	27.5
ιC Na	+	+++	–	30.2
ιC Ca	+	–	+++	26.4
ικC	+++	–	+++	21.7
ικC K	+++	–	–	26.9
ικC Ca	+	–	+++	26.0
κιC1	+++	+++	+	17.4
κιC1 K	+++	–	–	24.3
κιC1 Ca	+	–	+++	24.3
κιC2	+++	+	+++	21.9
κιC2 K	+++	–	–	18.9
κιC2 Na	+	+++	–	23.1
κιC2 Ca	++	–	+++	21.8
κιν*C1	++	+	+++	29.4
κιν*C1 K	+++	–	–	29.5
κιν*C1 Na	–	+++	–	30.3
κιν*C1 Ca	+	+	+++	27.8
κιν*C1m K	+++	–	–	22.9
κιν*C1m Na	–	+++	+	25.2
κιν*C2	+++	+++	+	28.9
κιν*C2 K	+++	–	–	29.2
κιν*C2 Ca	+	+	+++	29.9

reduction of the molecular weight of κ-carrageenan from 1420 kDa to 262 kDa did not result in a decreased IRI activity. Altogether, the results from the HPSEC analysis demonstrated that the conditions during cation exchange do not lead to significant changes in the molecular weight of carrageenans. The alkaline conditions which were applied to remove ν-units from κιν*C1 led to a decreased molecular weight from 923 kDa to 540 kDa. This may be the result of degradation due to base peeling or due to some hydrolysis during the short dialysis step in the purification. However, the molecular weight is still in an acceptable range, thus, the modified samples are suitable to evaluate the influence of ν-units on IRI activity.

3.2.3. Sulfate content and associated cations

The sulfate content as well as the type and content of cations were analyzed by ICP-OES (Table 3).

The results confirm that the cations in the carrageenans were successfully exchanged. The potassium and sodium forms of the carrageenans almost exclusively contain this cation and only trace amounts of the respective other cations. For the calcium forms of carrageenans containing a comparably high portion of κ-units, small amounts of potassium can be detected in addition to calcium. This is most likely caused by the strong affinity of κ-units to this cation. Nevertheless, the corresponding carrageenans are clearly dominated by the presence of calcium. The sulfate contents (calculated from the sulfur contents) of the cation-exchanged carrageenans were slightly different from the unmodified carrageenans. For ικC and κιC1, a clearly higher sulfate content was observed after cation-exchange. This can be explained by the presence of sucrose and glucose in the unmodified samples. These low molecular weight compounds are added for the standardization of the carrageenans and are removed by the precipitation step during the cation exchange. For all other samples, the sulfate content was comparable or in some cases even lower than in the unmodified samples. The partially lower contents can be explained by small amounts of salt in the samples. This is also supported by a comparison of the sulfate contents from ICP-OES and the theoretical sulfate content (calculation based on the structure). However, small amounts of salt are not expected to influence the determination of the IRI activity significantly: The salt excess in the monocationic carrageenans would lead to a salt concentration of about 0.2–2.4 mM in a 1 mg/mL carrageenan solution. Leiter et al. (2018) demonstrated that low concentrations of salt (0.3 mM KCl) do not significantly influence IRI activity, whereas high concentrations (30 mM KCl or 100 mM NaCl) lead to a significant increase in ice crystal diameter. Given that the salt concentration of the samples used in this study is notably lower than 30 mM, it can be assumed that the effect on IRI activity is minimal. The sodium and potassium forms of sample κιν*C1m contained almost exclusively the corresponding cation and showed a clearly lower sulfate content than the unmodified sample. A lower sulfate content was expected for this sample because the aim of the alkaline modification was the removal of the sulfate group at position *O*-6 of the 1,4-linked α-galactose units (part of ν-units) and the resulting formation of a 3,6-anhydrogalactose unit. Therefore, ICP-OES results also confirm the successful structural modification of κιν*C1.

Altogether, the selected carrageenans were successfully converted into monocationic carrageenans without an unwanted modification of the molecular structure. Therefore, they are ideal for an investigation of the relationship between structural composition and IRI activity.

3.3. Ice recrystallization inhibition activity of chemically defined carrageenans

3.3.1. Ice crystal morphology of selected samples

The IRI activity in the model system (see section 2.6) was analyzed by comparing the ice crystal morphology and mean ice crystal diameters after 4 h and 72 h in a carrageenan solution to a carrageenan-free control solution. For an initial evaluation of the effect of different cations on the IRI activity, exemplary pictures of the ice crystals obtained after 72 h in solutions prepared with the potassium, calcium, and sodium forms of κC, ιC, κιν*C1, and κιC2 were visually assessed (Fig. 1).

The comparison of the ice crystal images to a sucrose solution without carrageenans clearly demonstrated that all carrageenans

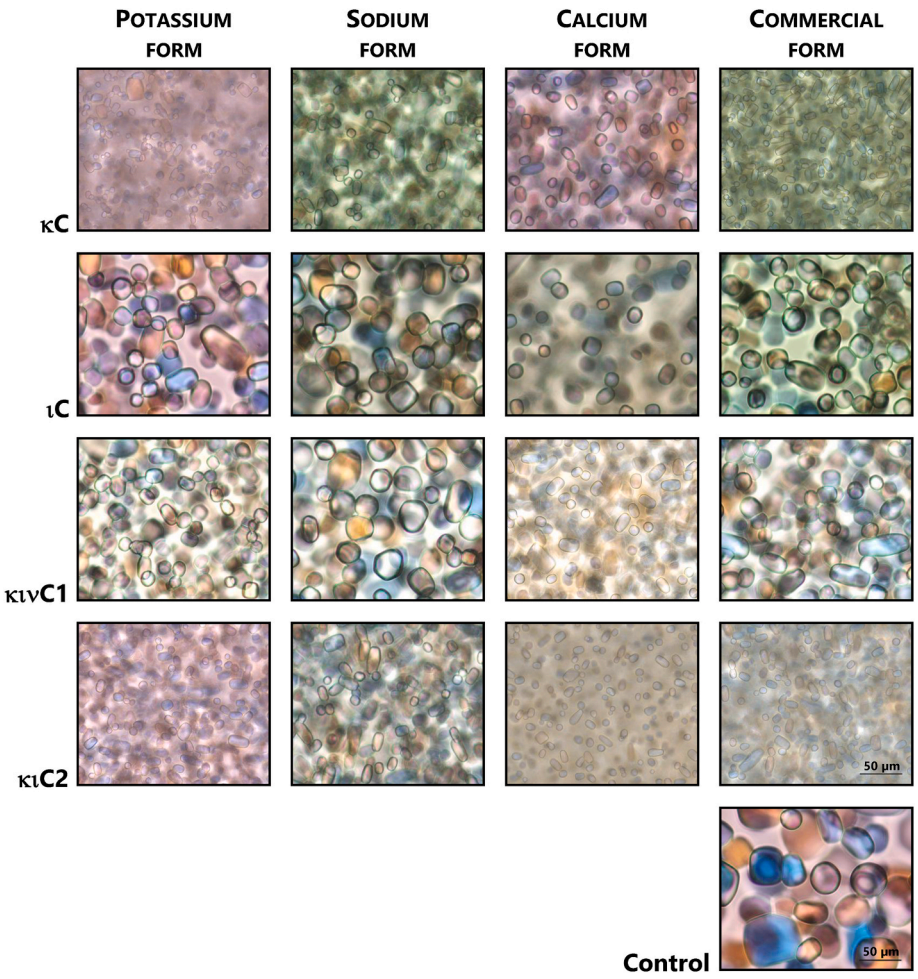

Fig. 1. Ice crystals grown in a 49 % (w/w) sucrose solution with and without the addition of carrageenans κC, ιC, κινC1, and κιC2 in their potassium, sodium, calcium, and commercial form after storage for 72 h at −12 °C.

exhibited some IRI activity. However, clear variations in the ice crystal size and morphology were observed. As it would be expected for κ-carrageenan, κC led to comparably small ice crystals with the characteristic rectangular ice crystal shape. This was observed for all ionic forms, but it was most evident for the potassium form which is also the predominant form of commercial κ-carrageenans (Hale et al., 2024). Although the sodium and calcium form showed a comparable morphology, the corresponding samples contained clearly larger ice crystals. The ice crystal images obtained for κιC2 suggest a comparably high IRI activity for all forms. In addition, a rectangular ice crystal morphology was observed, which was most noticeable for the calcium form. Therefore, this carrageenan has at least a comparable although not identical functionality than κ-carrageenan despite its clearly different molecular composition. The samples ιC and κινC1 showed rather circular ice crystals as well as a rather low IRI activity for all cation forms. Although the ice crystals obtained from the calcium form were smaller, they were still visibly larger than the ones obtained from κC and κιC2. A comparison of the monocationic carrageenans and the unmodified carrageenans showed that the cation-exchange is suitable to enhance the IRI activity of carrageenans: The ice crystal images indicated smaller ice crystals for the individual most active monocationic forms than for the unmodified carrageenans.

To assess the IRI activity in a more quantitative way, mean ice crystal diameters were determined for the monocationic carrageenans.

3.3.2. IRI activity of κC and ιC

The mean ice crystal diameters of the monocationic forms of κC and

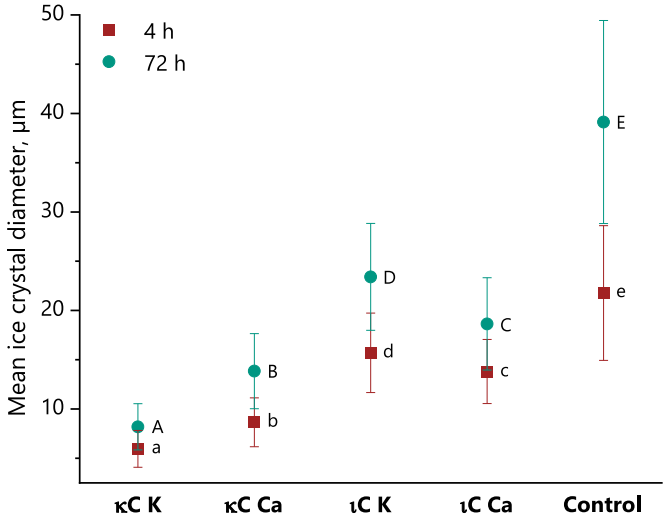

Fig. 2. Mean ice crystal diameter (n = 4) after 4 h and 72 h at −12 °C of a 49 % sucrose solution with 1 mg/mL of the potassium and calcium forms of the κ-carrageenan κC and the ι-carrageenan ιC. The control sample refers to a sucrose solution without carrageenan addition. The statistical analysis was conducted by using one-way analysis of variance (ANOVA) with post-hoc Tukey test (α = 0.05). In case of heterogeneity of variances, Welch ANOVA was applied.

ιC are shown in Fig. 2.

The results clearly underline the observations made from the ice crystal images. The potassium form of κ-carrageenan κC showed the lowest mean ice crystal diameter after 72 h of storage, while the diameters observed for the potassium form of ιC were clearly higher. Exchanging potassium for calcium had a positive effect on the IRI activity of ιC, whereas larger ice crystals were observed for the calcium form of κC. However, κC still showed a significantly higher IRI activity than ιC. These results suggest that the stabilization of the helical conformation of carrageenans as well as κ structural elements are important for the IRI activity of carrageenans: Monovalent ions (especially potassium) were described to stabilize helices of κ-carrageenans (Piculell, 2006; Rochas & Rinaudo, 1980), whereas calcium ions stabilize the helices of ι-carrageenans (Nilsson, Piculell, & Joensson, 1989). In the literature, varying IRI activities were observed for different carrageenan types: In a previous study, we found a lower IRI activity for ι-carrageenan compared to κ-carrageenan (Kiran-Yildirim et al., 2021), while Kamińska-Dwórznicka et al. (2020) described a comparable activity of the two carrageenan types. However, the structure of the carrageenans used in the second study was not analyzed. As we also demonstrated in a previous study, the structural composition of commercial carrageenans shows considerable variation depending on the source (Hale et al., 2024). For example, ικC was declared as ι-carrageenan, although it also contains 27 % of κ structural elements. Furthermore, Kamińska-Dwórznicka et al. (2020) analyzed the IRI activity in a model food (sorbet) and not a defined model system which could also explain the different results (cations from the matrix may influence IRI activity).

3.3.3. IRI activity of hybrid carrageenans

The mean ice crystal diameters of the potassium and calcium forms of the hybrid carrageenans are shown in Fig. 3, comparisons of the two cation forms of all samples is shown in Fig. S3.

The results suggested that the IRI activity of the potassium forms is associated with the portion of structural elements of the κ-type. κιC2 and κιC1 (67 % and 42 % κ-units) showed low ice crystal diameters which were not significantly different from κC (Fig. S3). ικC yielded a significantly different mean ice crystal diameter than κιC1 and κιC2 after 72 h, which could be derived from the comparably low portion of κ-units (27 %). The ν-unit containing carrageenans κινC1 and κινC2 showed a significantly lower IRI activity which suggests a negative effect of structural elements with a higher degree of sulfation. However, the IRI activity of the carrageenans κιC2, κιC1, and ικC showed that a certain portion of ι structural elements does not significantly impede IRI activity.

The conversion into the calcium forms clearly improved the properties of κιC2 and ικC which showed the highest IRI activity among the calcium forms (8.92 ± 2.26 μm and 9.07 ± 2.36 μm after 72 h). Notably, the mean ice crystal diameters were lower than for the calcium form of κC (13.84 ± 3.81 μm after 72 h) and comparable to the potassium form of κC (8.19 ± 2.35 μm after 72 h). κινC1 and κινC2 also showed a higher IRI activity in the calcium forms although it was still comparably low. Notably, κιC1 showed a slightly although statistically significant lower IRI activity than ικC which has a comparable structure (consecutive blocks of κ- and ι-units) but a higher portion of ι-units. Nevertheless, the results demonstrate that the IRI activity of hybrid carrageenans is improved by exchanging the associated cation from potassium to calcium. In a previous study, carrageenans with more than one structural unit also showed a considerable IRI activity (Kiran-Yildirim et al., 2021). However, the samples used were inhomogeneous with regards to their associated cations. Thus, it was not possible to attribute the IRI activity to a specific structural feature. In contrast, the results from this study clearly demonstrate the influence of the associated cations as well as the structural composition of the carrageenans.

3.3.4. Influence of ν-units on IRI activity

To investigate the influence of the ν-units on IRI-activity, the potassium form of κινC1m (obtained after alkali-modification) was analyzed. Fig. 4 shows the ice crystal morphology and the mean ice crystal diameters after 4 h and 72 h.

Both the mean ice crystal diameter as well as ice crystal morphology were not significantly different between κινC1 and κινC1m. These results suggest that the presence of ν-units does not influence the IRI activity of this carrageenan. Because the IRI activity of κιC2 was clearly higher, the varying functionality must be derived from the structural composition. Based on the IRI activity of the other carrageenans, the distribution of κ-units or the length of blocks with κ structural elements may be an important factor: κιC2 was excessively degraded with κ-carrageenase while κινC1 was hydrolyzed to a lower extent. This is most likely the result of a higher portion of consecutive κ-units.

4. Conclusion

In the present study, we used well-characterized carrageenans and successfully converted them into their monocationic forms. The samples which contained only potassium, calcium, and, in part, sodium as

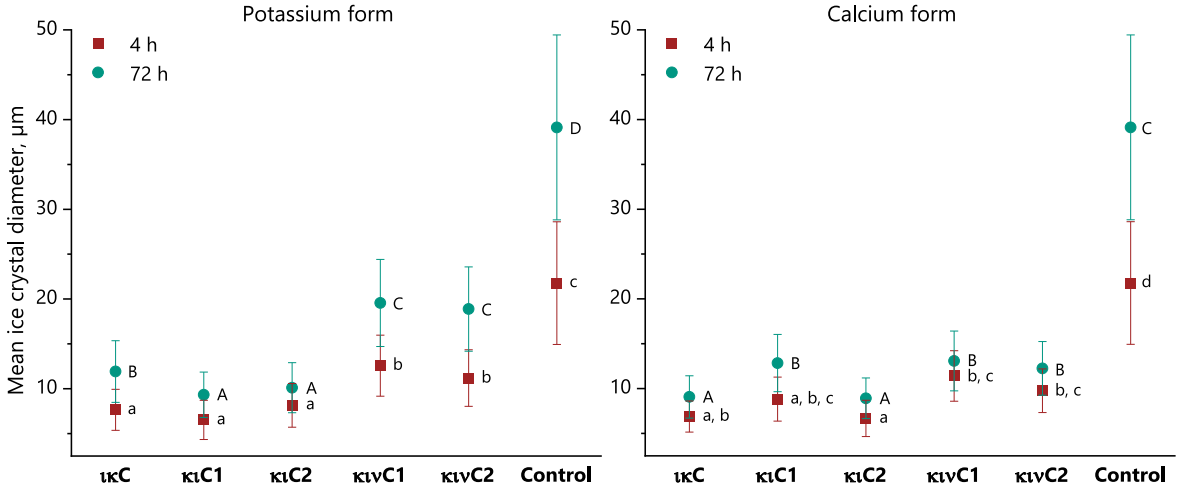

Fig. 3. Mean ice crystal diameter (n = 4) after 4 h and 72 h at −12 °C of a 49 % sucrose solution with 1 mg/mL of the potassium and calcium forms of the hybrid carrageenans used in this study. The control sample refers to a sucrose solution without carrageenan addition. The statistical analysis was conducted by using one-way analysis of variance (ANOVA) with post-hoc Tukey test (α = 0.05). In case of heterogeneity of variances, Welch ANOVA was applied.

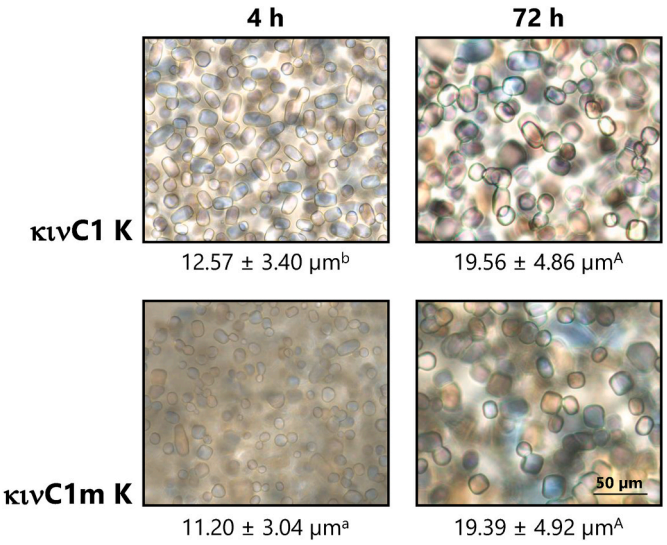

Fig. 4. Ice crystals grown in a 49 % (w/w) sucrose solution with the potassium form of κινC1 before and after alkaline modification after storage for 4 h and 72 h at −12 °C and their mean ice crystal diameters (n = 4).

associated cations were subsequently investigated for their IRI activity in a model system. Our results clearly demonstrated that both the molecular structure as well as the associated cations clearly influence IRI activity. The κ-carrageenan κC showed the overall highest IRI activity, but only in its potassium form. In contrast, the calcium form of the ι-carrageenan ιC was more active than its potassium form. Therefore, a higher IRI activity was observed for cations which lead to a strong helix stabilization for the individual carrageenans (Nilsson et al., 1989; Rochas & Rinaudo, 1980). These results suggest that helix formation or a rigid conformation is important for an interaction with the ice crystal surface. Generally, IRI activity was higher for κC than for ιC, independent from the cation form. Therefore, κ structural elements seem to have a positive influence on functionality. Carrageenans with both κ and ι structural units also showed significant IRI activity. A high IRI activity was observed for the potassium forms of carrageenans with a high portion of consecutive κ-units. However, conversion into the calcium form in part even improved the properties of these carrageenans. Our results also suggested that not only the portion of the individual structural elements are important but also the abundance and length of the corresponding blocks. The results of this study lay the basis for a targeted extraction of carrageenans to optimally exploit their functionality. Future work could focus on the application of structurally defined carrageenans in different food products.

CRediT authorship contribution statement

Julia Hale: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Alisa Furch:** Methodology, Investigation. **Julian Gerhäuser:** Writing – review & editing, Methodology, Investigation. **Volker Gaukel:** Writing – review & editing, Resources, Methodology, Conceptualization. **Daniel Wefers:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2024.110423.

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