DOCTORAL THESIS

Impact of Short-term Heat Treatment on the Structure and Functional Properties of Carrageenans

Kairit Eha

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Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology, has not been submitted for any other doctoral or equivalent academic degree.

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Lühiajalise termilise töötluse mõju karragenaanide struktuurile ja funktsionaalsetele omadustele

KAIRIT EHA

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Contents

List of publications

These publications form the basis of the thesis and are reproduced in the appendix with permission from the publishers:

I. Friedenthal, M., **Eha, K.**, Viitak, A., Lukas, A., and Siimer, E. (2001). Effects of drying on the gel strength and cation mobility of furcellaran. Innovative Food Science & Emerging Technologies 1, 275-279. doi: 10.1016/S1466-8564(00)00027-8.

II. Friedenthal, M., **Eha, K.**, Kaleda, A., Part, N., and Laos, K. (2020). Instability of low-moisture carrageenans as affected by water vapour sorption at moderate storage temperatures. SN Applied Sciences 2, 243. doi: 10.1007/s42452-020-2032-9.

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Author's contribution to the publications

Contribution to the papers in this thesis are:

I. The author performed the experiments, analysed and interpreted the results, and wrote the paper (co-author).

II. The author performed the experiments, analysed and interpreted the data, and wrote the paper (co-author).

III. The author performed the experiments, analysed and interpreted the data, and wrote the paper (corresponding author).

IV. The author performed the experiments, analysed and interpreted the data, and wrote the paper (corresponding author).

Introduction

Carrageenans are among the most important algae polysaccharides originating from various Florideophyceae species. The use and the number of scientific publications related to carrageenans has grown exponentially in recent decades and, in addition to the food industry, they are also being used in medical, pharmaceutical and biotechnological research. There are three main types of carrageenans: kappa (κ-), iota (ι-), and lambda (λ-) carrageenans. Furcellaran is a type of κ-carrageenan whose major structural difference is a smaller degree of sulphation.

Furcellaran is produced in Estonia from the local alga *Furcellaria lumbricalis*. It is a unique product that has been produced for over 60 years. The gels made by furcellaran are more elastic than carrageenans. The special properties of excellent gel texture and flavour release lead to furcellaran application in milk puddings and flans, as well as in such traditional confections as marmalade, zephyr, “bird's milk” and melt-in-the-mouth type sweets.

However, stability of quality of the final product, furcellaran, has been difficult to achieve. Major problems include a decrease in gel strength over time, occasional self-ignition of batches, and discolouration. One hypothesis for these problems is that carrageenans can decompose at the high temperatures used in production, leading to the formation of degradation products and changes in their properties. The onset of pyrolytic degradation at moderate temperatures is generally attributed to the pronounced exothermic behaviour. This can promote the development of hotspots due to self-heating and can even cause spontaneous ignition. However, there is no clear evidence of exothermic heat flow for galactans. It is known that amorphous sugars may sorb large amounts of water from their surroundings, resulting in crystallisation during storage above a critical, temperature-dependent relative humidity.

As a result, the aim of this dissertation is to examine the effect of heat treatment at the molecular level on commercial furcellaran and to compare it with other commercial carrageenans. The work describes the structure, physico-chemical and rheological properties of carrageenans and their changes as a result of heat treatment using NMR, a texture analyser, rheometry and SEC.

The study of sorption behaviour was undertaken in order to determine the moisture absorption capacity of initial and heat-treated samples from the environment as one of the important factors in ensuring the stability of the product. Isothermal microcalorimetry was used to study the exothermic heat flow caused by the absorption of water into furcellaran and into other carrageenans, leading to their thermodynamic instability under storage conditions.

A better understanding of the structural and functional changes in carrageenans after thermal processing at elevated temperatures is expected to ultimately lead to better process optimization and higher final product quality, valorisation and acceptance.

Abbreviations

EAAS	electrothermal atomic absorption spectrometry
FAAS	flame atomic-absorption spectrometry
FTIR	Fourier-transform infrared
HPAEC	high-performance anion-exchange chromatography
MSI	moisture sorption isotherm
NMR	nuclear magnetic resonance
SEC	size exclusion chromatography
SEM	scanning electron microscopy

1 LITERATURE REVIEW

1.1 Carrageenans

Carrageenans are a class of high molecular weight sulphated polysaccharides present in many red algae as a cell wall and intercellular component and comprising up to 50% of the dry weight (McHugh, 1987). Carrageenans have many multifunctional qualities (Pacheco-Quito et al., 2020), including biocompatibility (Feng et al., 2017; Grenha et al., 2010; Popa et al., 2014), biodegradability (Mokhtari et al., 2019; Popa et al., 2014; Sánchez-Sánchez et al., 2015; Yu, et al., 2018), non-toxicity (Cohen & Ito, 2002) and low immunogenicity (Hebar et al., 2015), which is why they have been widely used in the food, pharmaceutical and cosmetic industries as thickening (Blakemore & Harpell, 2010; Ito & Hori, 1989; Kılınç et al., 2013), stabilising (Kaminska-Dworznicka et al., 2020; Klojdová et al., 2018; Parvar et al., 2013) and gelling agents (Farahnaky et al., 2013; Nieto et al., 2016; Widjanarko et al., 2018). Carrageenans have no nutritional energy value and the major uses are in the food industry, approximately 80% of carrageenans' world production, particularly in meat and dairy products (Arltoft et al., 2007; Marchetti et al., 2014; Quemener et al., 2000). When used in food products, carrageenans have the EU additive E-number E407 (refined carrageenan, furcellaran) or E407a (semi-refined carrageenan). E407a has a slightly different composition; it also contains a considerable amount of cellulose (Commission Regulation (EU) No 231/2012). In recent years, diverse bioactive qualities of carrageenans, such as antiviral (Abu-Galiyun et al., 2019; Chen et al., 2020; Diogo et al., 2015; Eccles et al., 2015; Frediansyah, 2021), antioxidant (Barahona et al., 2011; Prasetyaningrum et al., 2021; Souza et al., 2018; Sun et al., 2010), immunomodulating (Chan et al., 2017; Liu et al., 2019; Yuan et al., 2011), antibacterial (Madruga et al., 2020; Wang et al., 2011; Zhu et al., 2017) and anticoagulant effects (Dos Santos-Fidencio et al., 2019; Pereira et al., 2005), have been reported.

1.1.1 Production

The main red seaweed species used in the commercial production of carrageenans are *Kappaphycus alvarezii* (formerly known as *Eucheuma cottonii)* for the production of kappa carrageenan*,* and *Eucheuma denticulatum* (formerly known as *Eucheuma spinosum)* for the production of iota carregeenan. These seaweeds grow along the islands of the Far East, and the main producers are South-east Asia and Tanzania (Blakemore & Harpell, 2010; Valderrama et al., 2013). Other commercially important species are *Chondrus crispus,* which consists of a mixture of kappa and lambda carrageenans, from the coasts of the North Atlantic and *Sarcothalia crispata* from the waters of South America. The red seaweed *Furcellaria lumbricalis* is a source of furcellaran, structurally related to kappa carrageenan (Knutsen et al., 1990; van de Velde et al., 2002). As the algae stocks in Denmark's Kattegat Bay have been destroyed, seaweed is mainly collected from Canadian or Estonian waters. The most abundant community of loose-lying *F. lumbricalis* in the world is known as the Kassari algal stratum, located on the west coast of Estonia. Although the attached form of *F. lumbricalis* is widely distributed in Estonia, the lack of suitable algae harvesting techniques means that only its beach wrack deposits have been utilised for commercial furcellaran production (Kersen et al., 2017; Tuvikene et al., 2010).

The specific production methods of carrageenans are considered to be trade secrets by their manufacturers, but generally follow similar processes (Cunha & Grenha, 2016; Guan et al., 2017). The seaweed is harvested, sorted by species, dried and shipped to a processing plant. Prior to extraction, the seaweed is washed and ground. Several methods can be used to extract carrageenan from seaweed. In the original method, the carrageenan is extracted from the seaweed in an aqueous solution (McHugh, 2003). Nowadays, for the production of high-value food and pharma carrageenans, the extraction protocol of carrageenans predominantly involves an alkaline pre-treatment/extraction (Al-Nahdi et al., 2019) and alcohol precipitation recovery (Mustapha et al., 2011). Tuvikene et al. (2006) found that the best extracting medium for carrageenans from the biomass of *Furcellaria lumbricalis–Coccotylus truncatus* was 0.05 M KOH. The aim of the alkaline treatment of carrageenan is to catalyze the cyclization reaction with hydroxide (OH^-) to generate the 3,6-anhydro-bridge (*Figure 1*). Thus alfa-D-galactose 6-sulphate residues are converted into 3,6-anhydro-alfa-D-galactose units in κ- and ι-carrageenan that allows the formation of a helicoidal secondary structure, which is essential for the gel-forming properties (Ciancia et al., 1993). The choice of a specific alkali, for example sodium, potassium or calcium hydroxide, depends on the carrageenan salt to be produced.

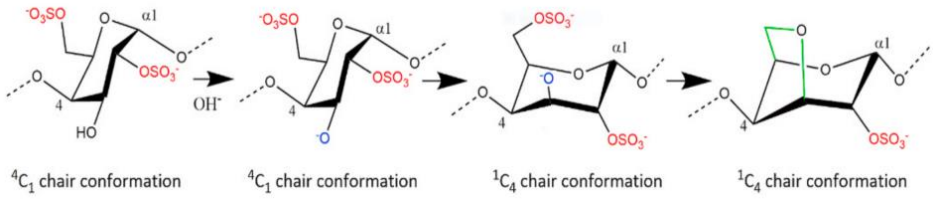

Figure 1. Proposed mechanism for alkali modification of D-galactose 2,6- sulphate to 3,6-anhydro-D-galactose 2-sulphate, i.e. ν-carrageenan to ι-carrageenan (adapted from Rhein-Knudsen & Meyer, 2021), to be presented with the permission of Elsevier.

Recently, more complex methods, such as extraction using enzymes (Blanco-Pascual et al., 2014; Rhein-Knudsen et al., 2015; Varadarajan et al., 2009) or deep eutectic solvents (Das et al., 2016; Zainal-Abidin et al., 2017), ultrasound- or microwave-assisted extraction, photobleaching and reactive extrusion processes, have been developed (Abdul Khalil et al., 2018; Boulho et al., 2017; Vázquez-Delfín et al., 2014; Youssouf et al., 2017) to improve the physico-chemical and bioactive properties of carrageenans. Also, the extraction process conditions, such as duration, temperature, pH and alkaline treatment have major impacts on the structure and gelling properties of the final product (Azevedo et al., 2015; Hilliou et al., 2006). After extraction, the liquid extract is purified by centrifugation and/or filtration. The liquid extract may be converted into flakes by simple evaporation of water to yield “drum-dried” carrageenan. A small amount of mono- and diglycerides is used to facilitate the removal of the dried material from the dryer roll. However, these emulsifiers cause turbidity in water solutions and results limited use of drum-dried carrageenans in aqueous gel systems. The presence of soluble salts from the extract may also affect the properties of the drum-dried carrageenans (CP Kelco, 2002). Djaeni et al. (2012) found that successful method to enhance carrageenan drying is to apply air dehumidification. This process allows to reduce the drying time at low temperature drying and to improve product quality.

Most of the carrageenans used in foods are isolated from the liquid extract by selective precipitation of the carrageenans with alcohol, commonly isopropanol (Hoffmann et al., 1995). This gives a fibrous mass that is squeezed to remove the alcohol and dried to obtain pure and concentrated product. Instead of using alcohol, KCl can be used for precipitation for the production of kappa type carrageenans. De-watering is achieved via a freeze-thaw process or by pressing the formed gel (Hernández-Carmona, 2013; Hotchkiss et al., 2016).

In Estonia, two different methods are used for the production of furcellaran. The traditional method is hot water extraction followed by filtering and drying on roller-driers (*Figure 2*). After drying, furcellaran is washed, bleached and treated with KCl solution to improve the gel strength of the final product. Finally, the furcellaran is dried for a second time using food oil for the proper release of dried material from the roller. It is then milled and packed (Est-Agar AS, n.d.). The obtained product is flaky and oily smelling, with fluctuating quality (Eha, 2001). This technology is used due to its low price and customer base.

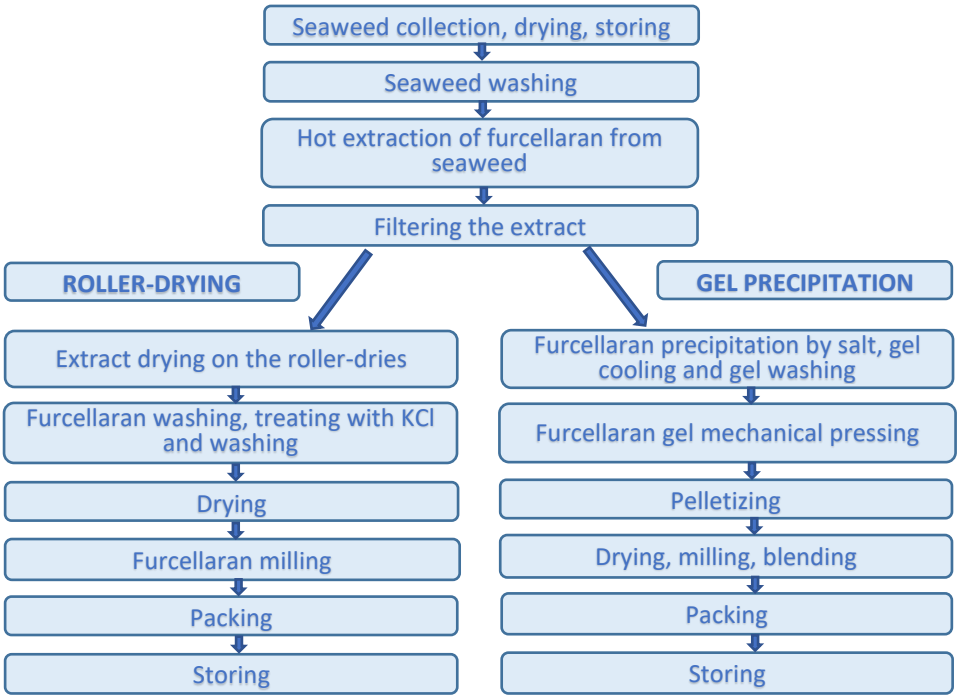

Figure 2. Furcellaran production process in Est-Agar AS (Estonia), to be presented with the permission of company Est-Agar AS.

The second production technology (*Figure 2*) involves gel precipitation after the stages of furcellaran aqueous extraction and extract filtering. The gelling agent is isolated from the liquid extract by the precipitation of the product with salt (KCl). After precipitation the product is pressed and dried. This process gives a high-quality and more expensive product (Saareleht, 2010).

1.1.2 Structure

Carrageenans are a mixture of linear polymers of sulphated galactans with an average relative molecular mass well above 100 kDa (Barbeyron et al., 2000; Uno et al., 2001). They differ due to the number and position (arrangement) of ester sulphate groups in the repeating galactose units and/or the presence of 3,6-anhydrogalactose units (Knutsen et al., 1994; Usov et al., 1992). Three commercially important carrageenans are kappa (κ-), iota (ι-), and lambda (λ-) carrageenans, although several other types have also been reported (Knutsen et al., 1994). Carrageenans are often a mixture of different types, for example κ/ι-carrageenans or furcellaran (Anastyuk et al., 2011; Correc et al., 2012; Saluri et al., 2021; van de Velde 2008). Kappa carrageenan consists of (1,3)-β-galactopyranose-4-sulphate and (1,4)-α-3,6-anhydrogalactopyranose residues (*Figure 3*). It has an ester sulphate content of 25–30% and an anhydrogalactose content of 28–35%.

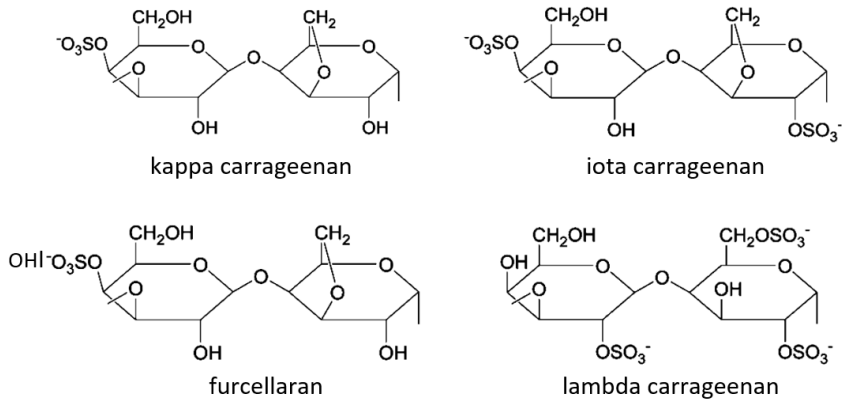

Figure 3. Chemical structure of main carrageenans (adapted from Imeson, 2009 with some modifications), to be presented with the permission of Elsevier.

Iota carrageenan differs only in that its residue is sulphated at the C2 position; hence, they have a higher ester sulphate content of about 28–30% and a lower anhydrogalactose content of about 25–30%. Lambda carrageenan is further sulphated (32–39%) and have no anhydrogalactose content. It consists of (1,4)-α-galactopyranose 2,6 disulphate and (1,3)- β-galactopyranose, which is 70% substituted at the C2 position (Barbeyron et al., 2000; Campo et al., 2009). Furcellaran is a low sulphated κ-carrageenan consisting of one sulphate group per three or four sugar units (Knutsen et al., 1990; van de Velde et al., 2002).

1.1.3 Physicochemical properties

The physico-chemical properties of carrageenans strictly depend on their structural differences, whereas the functionality of carrageenans in various applications depends largely on their rheological properties (*Table 1*) (Campo et al., 2009; Yousefi & Jafari, 2019). All carrageenans are soluble in hot water and the solubility depends on the content of sulphates, which lowers the solubility temperature and the presence of potential associated cations (Necas & Bartosikova, 2013). Potassium and calcium salts make kappa and iota type carrageenans insoluble at low temperatures, but will swell; however, all sodium salts of these carrageenans, as well as lambda carrageenan, are also soluble in cold water (Imeson, 2009).

Table 1. Charateristics of carrageenan (adapted from Tobacman, 2001 with some modifications), to be presented with the permission of corresponding author.

Solubility	λ-carrageenan is readily soluble in cold or hot aqueous solution; κ- carrageenan is soluble in hot solution; treatment of aqueous solution with potassium ion precipitates κ-carrageenan
Gel formation	λ-carrageenan does not form gels; λ- and ι-carrageenan form right-handed helices, KCl promotes gel formation of κ-carrageenan; calcium ion promotes gel formation of ι-carrageenan
Metabolism	hydrolysis of glycosidic linkages at lower pH, especially pH≤3.0, also desulpation by sulphatases
Properties	λ- and κ-carrageenan combine easily with milk proteins to improve solubility and texture; serve as thickening agent, emulsifier, stabilizer
Synergistic effects	with locust bean gum, increase in gel strength; other hydrocolloids may also affect gel strength and cohesiveness
Concentration in food products	0.005 – 2.0% by weight
Major uses	milk products, processed meats, infant formula, toothpaste, cosmetics, skin preparations, pesticides

Carrageenans hydrate and typically form highly viscous sols, which depends on the type, molecular weight and concentration of the carrageenans, the presence of other solutes and the temperature (Lai et al., 2000). The viscosity increases with the molecular weight and the concentration of carrageenans and decreases with temperature (Bui, 2019).

Gelling

Carrageenans have the ability to form strong thermo-reversible gels at very low concentrations (around 1% w/w) (Glicksman, 1983). Their gels, as "molecular gels", are formed by heating. The process of gelling usually includes three steps: the hydration of the carrageenan, the heating of a mixed "sol" and the cooling of the "sol" in moulds (Shanthilal & Bhattacharya, 2015).

In solution, carrageenans have a disordered random-coil conformation (*Figure 4*) (Goff & Guo, 2019). Upon cooling, κ-carrageenan and ι-carrageenan molecules pass through a double helix transition, with the sulphate groups pointing outwards, while the higher sulphate content λ-carrageenan inhibits the formation of the helicoidal structure and does not gel, but forms viscous solutions and is used as a thickener (Yuguchi et al., 2002). The positive cations in solution neutralise the charge of the sulphate groups (Makshakova et al., 2020; Thanh et al., 2010; Zhang et al., 1991), leading to a self-association of the helices and the formation of a three-dimensional gel network (Du et al., 2016; Rochas & Rinaudo, 1980).

For the gelling of carrageenans, cations, typically potassium for κ-carrageenans and calcium for ι-carrageenans, stabilise the junction zones between the two helices by binding to the negatively charged sulphate groups to cross-link with the two helices through ionic salt bridges (Wu & Imai, 2012). Among the monovalent cations that induce the gelation of κ-carrageenans are K^+, Rb^+, Cs^+ and high concentrations of Na^+ and Li^+ (MacArtain et al., 2003; Mangione et al., 2005). However, the gels formed with K^+ are the strongest and most stable (Chen et al., 2002). The charged sulphate esters of ι- carrageenans encourage

conformation via the repulsive effect of the negative SO_3 groups and prevent gelation while increasing viscosity in the solution (Montero & Pérez-Mateos, 2002).

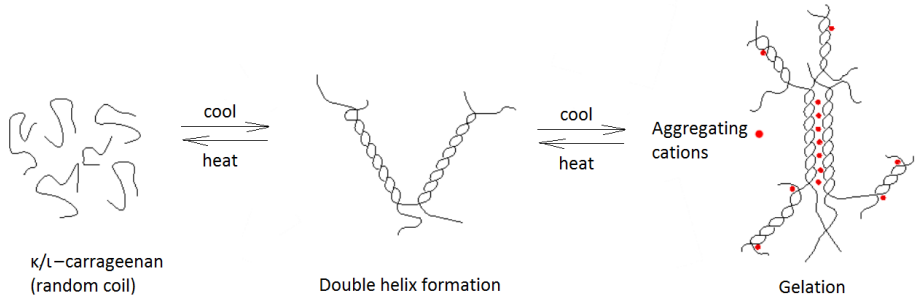

Figure 4. The mechanism of gelation of kappa and iota carrageenans (adapted from Dea, 1993 with some modifications), to be presented with the permission of Elsevier.

Another important indicator of the gel properties is the number of molecules forming the junction zone. The smaller the number of molecules involved in the junction zone, the more flexible the structure and gel obtained. In κ-carrageenans, junction zones are formed by 6–10 molecules through hydrogen bonding, whereas in ι-carrageenan only two molecules are involved. This is the reason why κ-carrageenan gels are rigid while elastic gels are produced by ι-carrageenan (Saha & Bhattacharya, 2010). The thermal history also greatly influences the junction zone formation. It has been shown that at slow cooling large junction zones form in κ-carrageenan systems, whereas many small junction zones appear at fast gelling rates (Iijima et al., 2007).

1.1.4 Sorption processes

Water is an important basic ingredient affecting the quality of biopolymers since the physical and chemical properties are strongly dependent on their temperature and moisture history. It has been suggested that reduced-moisture food products (e.g. low and intermediate) may be nonequilibrium systems, and that most of them are in the amorphous metastable state, which is very sensitive to changes in moisture content and temperature. A considerable number of studies have shown that product stability depends not only on the water content but also on the water activity (Aguirre et al., 2021; Ocieczek & Ruszkowska, 2011; Rahman, 2009). Water activity (a_w) is the ratio of the partial water vapour pressure of the food sample to the partial vapour pressure of pure water at equilibrium at the same temperature and pressure.

The function representing the relationship between water content and water activity at constant temperature and pressure is called the “moisture sorption isotherm” (MSI) of a food (Barbosa-Cánovas & Vega-Mercado, 1996). MSI is a graphic representation of the process in which water molecules are progressively and reversibly released from all kinds of hygroscopic forces in a food system, caused by colligative effects, capillary effects and direct bonding (Caballero-Cerón et al., 2015). The knowledge and understanding of water sorption characteristics among biopolymers and atmosphere are important in food science and technology for the design and optimisation of drying equipment, the design of packages, predictions of quality, stability and shelf-life, and for calculating moisture changes that may occur during storage (Bazardeh & Esmaiili, 2014; Bispo et al., 2015; Noriega et al., 2014; Yang et al., 2015). MSIs are notably influenced by

the biopolymer structure or state (i.e. crystal, an ordered molecular lattice, or amorphous, lacking in molecular order) (Torres et al., 2018). Due to the complexity of the sorption processes, the isotherm cannot be determined by calculations but must be experimentally recorded for each product. Several moisture sorption isotherm measurement techniques, such as vapour pressure manometric (Ajibola et al., 2003; Labuza et al., 1976) and hygrometric (Cervenka et al., 2007; Crapiste & Rostein, 1982; Demarchi et al., 2013; Pollatos et al., 2013) are available, although the most widely used and recommended method is the static gravimetric technique (Arslan-Tontul, 2021; Getahun et al., 2020; Kaleemullah & Kailappan, 2004; Santalla & Mascheroni, 2003; Stepien et al., 2020; Swami et al., 2005). It is convenient to measure the isotherm of a specific material by measuring the weight gained by the product compared to the equilibrium when it is confined in an atmosphere of controlled relative humidity, which is mostly achieved by using different salt solutions (Aguirre-Loredo et al., 2016; Goneli et al., 2013; Hawa et al., 2020).

Sorption isotherms can be generated from an adsorption process or a desorption process; the difference between these curves is defined as moisture sorption hysteresis (*Figure 5*) (Kapsalis, 1987). A variety of hysteresis loop shapes have been observed, depending on the type of material and the temperature. The principal factors affecting hysteresis are the composition of the product, its temperature, storage time, drying temperature, and the number of successive adsorption and desorption cycles (Sahin & Sumnu, 2006). It should be noted that the occurrence of hysteresis indicates that either the adsorption or desorption curve is not at true equilibrium or that some material in the product has undergone a change of state in the process, e.g. the crystallisation of sugars during adsorption (Iglesias & Chirife, 1978; Jouppila & Roos, 1994).

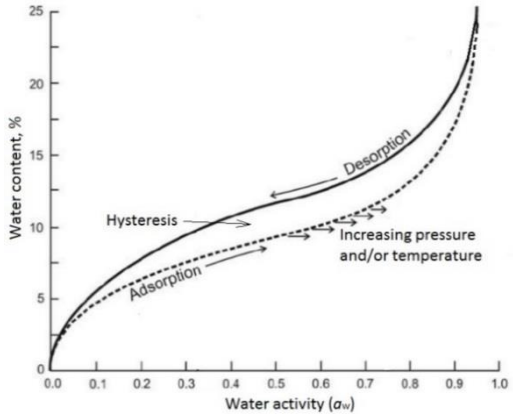

Figure 5. A schematic representation of a sorption isotherm with a hysteresis (adapted from Kapsalis, 1987 with some modifications), to be presented with the permission of Elsevier.

In the bibliography, numerous empirical and semi-empirical models can be found that make it possible to establish mathematical correlations between equilibrium moisture content and water activity, and that are commonly evaluated by the fitting of experimental data sets (Moreira et al., 2009). The most common equations used for describing sorption in food products are GAB (Guggenheim, Anderson & de Boer), Caurie, Henderson-Thompson, Oswin, Peleg and Smith (*Table 2*).

Table 2. Models applied to the experimetal water sorption data of furcellaran.

Name of the model	Model equation	Reference
GAB	$X_e = \frac{abc a_w}{(1 - c a_w)(1 - c a_w + bc a_w)}$	van der Berg and Bruin, 1981
Caurie	$X_e = \exp(a + b a_w)$	Castillo et al., 2003
Henderson-Thompson	$X_e = \left[\frac{\ln(1 - a_w)}{-a(T + c)}\right]^{1/b}$	Thompson et al., 1968
Modified Oswin	$X_e = (a - bT)\left(\frac{a_w}{1 - a_w}\right)^c$	Chen, 2000
Peleg	$X_e = a(a_w)^b + c(a_w)^d$	Peleg, 1993
Smith	$X_e = a - b\,(\ln(1 - a_w))$	Smith, 1947

Where X_e is the equilibrium moisture content (g g^{-1} d.b.), a_w is the water activity, a, b, c, d are adjustable parameters and T is temperature (°C).

The GAB model (Guggenheim, Anderson and De Boer) has viable theoretical background and is an improvement of BET and Langmuir physical adsorption theories. Langmuir adsorption model assumes that only a monolayer of adsorbate will be formed on the adsorbent surface (Bernal et al., 2021). BET model considers multilayer formation but usually fits only for small values of a_w, up to 0.4–0.5 (Staudt et al., 2013). GAB model postulates that the state of sorbate molecules in the second layer is identical to the one in superior layers, but different from those of the liquid state. This isotherm contains a third constant, c, which measures the difference of the chemical potential standard between the molecules of this second stage and those of the pure liquid state. However, GAB model underestimates the moisture content values at high water activity levels (a_w > 0.93) (Rao, 2015).

Henderson model is best known semi-empirical model for the sorption isotherms of biological materials and is derived from Gibbs adsorption theory. Thompson modified the Henderson equation by adding another constant into the temperature term to move the temperature of absolute zero to a higher temperature (Chen, 1990).

Caurie, Oswin, Peleg and Smith are all empirical models. Oswin model represents an expansion in series for sigmoid-shaped curves. Later this model was modified by including the temperature term in the equation (Chen, 1990). Peleg is a four-parameter model and can be used for both sigmoidal and non-sigmoidal isotherms and presents the same or even better suitability than the GAB model (Al-Muhtaseb et al., 2004). The use of Caurie's equation is important in modelling dehydrated food isotherms since it provides the parameter, which indicates the moisture content that results in the maximum stability of food during its storage (Zapata et al., 2014). The Smith model describes the final curved portion of water sorption isotherm of high molecular weight biopolymers. He proposed that two fractions of water are sorbed onto a dry surface; the first fraction have a higher condensation heat than the normal and it follows the Langmuir model. The second fraction can be formed only after the first fraction has been sorbed and it consists of multilayers of condensed water molecules, which prevent

evaporation of the initial layer. Smith proposed that the moisture content in the second fraction was proportional to the logarithm of the difference between the a_w of the sample and pure water (Andrade et al., 2011).

Factors affecting water activity are food components, processing treatment, temperature, pressure and relative humidity (Alhamdan & Hassan, 1999; Fennema, 1981; Lasekan & Lasekan, 2000; Okos et al., 1992; Weisser, 1985). The water activity shift caused by temperature is mainly due to changes in water binding, dissociation of water, the physical state of the water, or an increase in the solubility of a solute in water (Rahman & Labuza, 2007).

Carrageenans have less ability to hold water at high temperatures and at constant a_w. The effect of temperature is most pronounced at lower to intermediate water activities; above an a_w value of 0.8, no temperature influence is observed (Kapsalis, 1987; Labuza et al., 1970). In some products, at water activity values higher than 0.7, there is an inversion in the effect of temperature (i.e. equilibrium moisture content increases with temperature) mainly due to an increase in the solubility of sugars in water (Bell & Labuza, 2000; Saravacos et al., 1986). A sugar solution can bind water so that the water is less able to contribute to the partial pressure of water vapour. Consequently, as these sugar solutions increase in concentration, the resulting water activity will decrease. As the temperatures of sugar solutions increase, the water activity for a given equilibrium moisture content will also increase (Goula et al., 2008).

1.1.5 Degradation of carrageenans

Due to the frequent use of carrageenans in the food and pharmaceutical industry, extensive toxicological evaluation has been carried out. The major problem that has been identified involves degraded carrageenans (M_w = 20–40 kDa) and especially poligeenans (M_w = 10–20 kDa) formed through acid hydrolysis and extensive heating of food-grade carrageenan (M_w = 200–800 kDa) (McKim et al., 2018), which has caused deleterious effects in animal models, *e.g.* inducing inflammatory bowel disease, such as Crohn's disease (Liu et al., 1997; Miura & Hiwatashi, 1987) and ulcerative colitis (Watt & Marcus, 1969). On the other hand, the low-molecular weight degradation products of such polysaccharides are often important in medical applications in relation to inducing various kinds of bioactivities, including antioxidant, anticoagulant, antiviral and antitumour activity (Campo et al., 2009; Liu et al., 2015; Necas & Bartosikova, 2013).

The degradation of carrageenans can be hydrolytic (Karlsson & Singh, 1999; Singh & Jacobsson, 1994; Wu & Imai, 2012), thermal (Bradley & Mitchell, 1988; Lai et al., 2000; Robal et al., 2017) or oxidative (Chen et al., 2010; Prasetyaningrum et al., 2017; Sun et al., 2010). In recent years, the degradation of polysaccharides by radiation techniques has received a great deal of attention (Abad et al., 2009; Abad et al., 2016; Relleve et al., 2005). Polysaccharide degradation is mainly analysed by viscosimetry and size exclusion chromatography (SEC).

The degradation of carrageenan chains is a complex process that can be created during production processes and is affected by various factors: the pH of the medium, temperature, the presence of oxygen etc. The degradation of carbohydrate chains may be accompanied by the release of sulphate groups even in a slightly acidic medium and accelerates at pH values below 3–4, thereby changing the pH around the carrageenan environment (Karlsson & Singh, 1999). The acid-catalysed hydrolysis of algal galactans

occurs mainly at the 1,3-glycosidic linkages, producing carra-oligosaccharides, -(G-A)n-, with A at the reducing and G at the non-reducing terminus. These oligosaccharides are derived from two-step cleavages of the glycosidic bonds on either side of the 3,6-anhydrogalactose residues (Yang et al., 2009). Hydrolysis is increased by the presence of 3,6-anhydrogalactose residues and decreased by the presence of sulphate groups at the O-2 of galactose (Mangin, 2000).

The thermal degradation reaction occurs mainly through the breaking of glycosidic bonds, dehydration and loop opening. Masson (1954) has shown that the degradation of κ-carrageenan chains starts at temperatures above 60 °C. Robal et al. (2017) have thoroughly studied the thermal stability of red galactans. They found that the presence of sulphate groups at the O-2 of 3,6-anhydrogalactose and at the O-4 of β-galactose, but also methylation at the O-6 of β-galactose exhibit a structure stabilising effect on algal galactans. However, divalent metal ions as counter-ions increased the susceptibility to the thermal degradation of galactans in dry states, while among monovalent cations stability was more effectively enhanced by K^+-ions than Na^+-ions. They also noticed that, before the depolymerisation of the galactans, desulphation occurs. The thermal degradation products are relatively complex, and most of the degradation products are non-toxic, although furyl hydroxymethyl ketone, furfural and HFM have some toxicity for humans (Ouyang et al., 2018).

The oxidative degradation kinetics of κ-carrageenans at different pHs and times using ozone treatment has been studied by Prasetyaningrum et al. (2017). They found that the highest rate constants were achieved at lower pH, and depolymerisation occurs by random scission. Under similar conditions, the apparent value of rate constants of κ- carrageenan depolymerisation by ozone treatment is higher than depolymerisation by ultrasound methods and almost equal to thermal depolymerisation.

Upon irradiation, carrageenans can also be depolymerised to form shorter fragments with some biological activities. Oligomers obtained from radiolytically degraded carrageenans, as well as those from other marine polysaccharides, have been used as plant growth promoters for several medicinal and agricultural crops (Naeem et al., 2015). There is no difference in radiation degradation yield between different types of carrageenans in solid and sol states (Abad et al., 2009). Irradiation of κ-carrageenans in a solid or aqueous solution gives higher radiation yields of scission in the presence of O_2. The radiolytic products indicate increasingly reduced sugars, carbonyl, carboxylic acids and sulphates with increasing doses. The values are very much lower in solid irradiation (in vacuum and in air) than in aqueous irradiation (Abad, 2010).

2 AIMS OF THE THESIS

Traditionally, furcellaran has been produced in Estonia by hot water extraction followed by drying on the roller-driers. Due to the high temperatures used in production, furcellaran may decompose and deteriorate. Thermal decomposition may occur during non-uniform drying in the production process causing hotspots. One important parameter that is directly related to the decline in quality is the self-heating and formation of black pieces of furcellaran. Furcellaran damaged during drying process in combination with high environmental humidity may cause spontaneous combustion. In connection with the above, it became necessary to assess the effect of the water sorption on the possible self-heating process of the product.

Consequently, the objectives of the dissertation were as follows:

- to characterise commercial roller-dried furcellaran,
- to understand the effects of heat treatment on the furcellaran structure and functional properties,
- to study exothermic and endothermic phenomena of furcellaran caused by water sorption,
- to compare the obtained results with commercial carrageenans.

3 MATERIALS AND METHODS

3.1 Carrageenans

The studied commercial furcellaran was an industrial product from AS Est-Agar (Kärla, Estonia). It is a typical product extracted from *Furcellaria lumbricalis* (Gigartinales) with subsequent drum drying (surface temperature > +130 °C, steam pressure 196–392 kPa) in the final step *(Papers I, II and III)*. Commercial carrageenan preparations – κ- carrageenan, ι-carrageenan and a mixture of κ- and λ-carrageenans (kappa and lesser amounts of lambda carrageenans) – were purchased from Sigma Aldrich Co, Ltd., USA (product codes 22048, C1138 and C1013, respectively) *(Papers II and III).*

3.2 Methods

3.2.1 Drying

Optional air-drying was carried out on a Halogen Moisture Analyzer HR 73 (Mettler Toledo, Switzerland). The selected temperature range was 55–130 °C, and the drying duration 5–15 min. The sample was heated within 15 s to the drying temperature and held constant at this temperature. The dried samples were stored hermetically in plastic containers at room temperature (20 °C) until used for analysis.

3.2.2 Chemical analysis

The water content of each sample was determined by weight loss on drying (L_D, %) after heating at 105 °C to constant weight. The residual moisture content of the samples was expressed by L_D.

Monosaccharide contents were obtained by the hydrolysis of the polysaccharides in 2 M H_2SO_4 at 110 °C for 60 min followed by neutralisation with 1 M NaOH; the monosaccharides were quantified by high-performance anion-exchange chromatography (HPAEC-PAD) (Quemener & Lahaye, 1998) using the Shimadzu Prominence HPLC system (Shimadzu, Japan) equipped with an Antec II Decade electrochemical detector (ANTEC Leyden, The Netherlands). An analysis was carried out on a Dionex CarboPac MA-1 column, thermostated at 35 °C. Elution was performed using 450 mM NaOH at a flow rate of 0.4 ml min^{-1}, and the sample injection size was 10 µl (*Paper III*).

The sulphate content was quantified using the $BaCl_2$-gelatin turbidity method (Dodgson & Price, 1962) after hydrolysing the samples in 1 M HCl at 115 °C for 5 h (*Paper III).*

The ash content of polysaccharides, which represents the total inorganic materials, was quantified gravimetrically according to the method of You et al. (2010) at 600 °C for 8 h.

The nitrogen content of the carrageenans was determined using the Kjeldahl method. On the basis of nitrogen content, the protein content was calculated.

The crude fat content was determined by the Soxhlet extraction method proposed by the Association of Official Analytical Chemists (AOAC).

The pH of 1% (w/v) polysaccharide sols was determined using a Mettler Toledo SevenEasy pH meter (*Paper III*).

Conductivity measurements were carried out with a Hand-Held Conductivity Meter model handylab LF 1 (Schott Glaswerke, Germany). A weighed sample (1.0 g) was suspended in 100 ml of bidistilled water at 25 °C under intensive stirring for 5 min.

Then, the solution was filtered through a glass filter with a pore size of 160 µm and the conductivity in filtrate was registered at 25 °C (*Paper I*).

The concentrations of Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} in the furcellaran were determined by flame atomic-absorption spectrometry (FAAS) and emission spectrometry (EAAS) (SP9-700 Pye Unicam AA Spectrometer, Cambridge, UK). The dried sample (0.2–0.6 g) was mineralised by concentrated nitric acid and hydrogen peroxide (5:1) on a hot plate at 140 °C for 6–8 h, as described by Viitak and Treumann (1996). The mineralised sample was cooled and diluted up to 100 ml with bidistilled water. The conventional parameters of acetylene-air FAAS and EAAS were applied (Philips Scientific Atomic Absorption Data Book, 1988; Price, 1979) (*Paper I*).

3.2.3 Spectroscopy

NMR spectroscopy

^{13}C NMR spectra of 2.5% polysaccharide solutions in D_2O (w/w) were obtained at 60 °C on a Bruker AVANCE III spectrometer operating at 800 MHz. T 30000 transients were collected with an inter pulse delay of 1 s. The chemical shifts were calculated with reference to a C-6 signal from the galactose subunit having a constant value of 61.3 ppm for these carrageenans (Usov & Shashkov, 1985) (*Paper III*).

^{13}C CP-MAS NMR spectra were recorded on a Bruker AVANCE-II spectrometer at 14.1 T magnetic field using cross polarisation, a proton decoupling pulse sequence and a home-made double resonance probe with magic-angle-spinning for 4x25mm Si_3N_4 rotors. The spinning speed of the sample was 12.5 kHz, the duration of the ramped polarisation transfer pulse was 1 ms, and the relaxation delay was 5 s (*Paper III*).

FTIR spectroscopy

Powdered furcellaran and carrageenan samples were made into pellets using KBr for an FTIR analysis using a Fourier-transform infrared spectrophotometer (Bruker Tensor 27 FT-IR, Germany). The data from infrared transmittance was collected over a wave number ranging from 4000 cm^{-1} to 400 cm^{-1} with a 4 cm^{-1} resolution and 24 scans per spectrum.

Colourimetry

The lightness of the carrageenan samples was evaluated with a CM-700d spectrophotometer (Konica Minolta, Japan), CIE D65/11 mm/10° (*Paper III*).

3.2.4 Size exclusion chromatography

The molecular weight (M_w) determination was carried out in polysaccharides through size exclusion chromatography (SEC) analysis following the method of Saluri et al. (2019), using a Shimadzu LC-30AD liquid chromatograph equipped with a RID-10A refractive index detector, Shimadzu CTO-20AC column oven, OHpak SB-G guard column, and two Shodex OHpak SB-806MHQ columns in series. Elution was conducted using a 0.1 M $NaNO_3$ solution as the mobile phase at a flow rate of 0.8 mL min^{-1}. The temperature of the column oven was maintained at 60 °C. To estimate the peak-average M_w, a calibration curve was obtained from 12 pullulan standards (*Paper III*).

3.2.5 Water sorption behaviour

Sorption isotherms measurements

The experimental water adsorption and desorption isotherms of furcellaran were determined at the temperatures 20, 35 and 50 °C at different water activities within the range of 0.19–0.95 (*Paper IV*, *Table 1*), using a static gravimetric method (Lang et al., 1981). First, samples for desorption processes were placed into an environment of 100% (H_2O) relative humidity until equilibrium was achieved. The samples with the initial water activity (a_w = 0.235) were used to study adsorption. The samples (0.5 ± 0.0001 g) were placed in sealed jars, which were provided with different saturated salt solutions ($C_2H_3KO_2$, $Mg(NO_3)_2$*$6H_2O$, NaCl, KCl and KNO_3) to generate atmospheres of different relative humidity, until equilibrium was achieved. The samples were weighed at regular intervals until a constant weight (± 0.0005 g) was established. The average time of sample equilibration was around three months.

Modelling of sorption isotherms

The experimental data regarding the sorption isotherms of furcellaran were fitted to the GAB, Caurie, Henderson-Thompson, modified-Oswin, Peleg and Smith mathematical models at 20, 35 and 50 °C. *Paper IV, Table 2* shows equations for the used models in this study. The non-linear regression function from package "nls2" 0.2 for R 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria) was used to fit the equations to experimental results and to estimate the parameters of the models.

The goodness of fit of the different models to data was assessed by the mean relative percentage deviation modulus (P), the root mean square error (RMSE) and the coefficient of determination (R^2), determined by using equations 1–3 (Bahloul et al., 2008).

$$P = \frac{100}{N} \sum_{j=1}^{N} \left| \frac{Xe_{j\,cal} - Xe_{j\,exp}}{Xe_{jexp}} \right| \quad (1)$$

$$SE = \sqrt{\sum_{j=1}^{N} \frac{(Xe_{j\,cal} - Xe_{j\,exp})^2}{N - n_p}} \quad (2)$$

$$R^2 = \frac{S_t - SCE}{S_t} \quad (3)$$

where

$$S_t = \sqrt{\frac{\sum_{j=1}^{N} \overline{(Xe} - Xe_j)^2}{n-1}} \qquad \overline{Xe} = \frac{\sum_{j=1}^{N} Xe_j}{N}$$

$$SCE = \sum_{j=1}^{N} \left(Xe_{j\,cal} - Xe_{j\,exp}\right)^2$$

where $Xe_{j\ cal}$ and $Xe_{j\ exp}$ are calculated and experimental values of the equilibrium moisture content ($\overline{Xe}$), respectively. N is the number of data points and n_p is the number of free parameters in the model. SCE is the model sum of squares and S_t is the total sum of squares. Values of P below 10% are indicative of a good fit (Aguerre et al., 1989).

Net isosteric heat of sorption from isotherms

The net isosteric heat of sorption (Q_{st}) was determined from the moisture sorption data using an equation derived from the Clausius-Clapeyron equation (Jamali et al., 2006; Labuza et al., 1985) as follows:

$$\ln(a_w) = -\left(\frac{Q_{st}}{R}\right)\frac{1}{T} + K \quad (4)$$

where a_w is the water activity, Q_{st} is the net isosteric heat of sorption (J g^{-1}), R is the water characteristic gas constant (0.4615 J g^{-1} K^{-1}) and T the absolute temperature (K).

The net isosteric heat of sorption can be calculated from equation 4 by plotting the sorption isotherm as ln(a_w) versus T^{-1} for the fixed moisture content of the material and determining the slope which equals $-Q_{st} * R^{-1}$ (Staudt et al., 2013; Tsami, 1991). This procedure was repeated for all values of the equilibrium moisture content. The water activity values of the material for different temperatures were obtained using the equation that best fit the experimental moisture sorption data.

Heat flow measurement

The heat flows of the furcellaran and carrageenans were performed using a Thermal Activity Monitor TAM IV built up as an isothermal heat conduction microcalorimeter (TA Instruments, Delaware, US). A furcellaran or carrageenan sample (of 100 mg) was quantitatively transferred into a standard 3 ml glass ampule. Additionally, a small glass container (*micro-hygrostat*) with a saturated salt solution was placed inside the glass ampule immediately before sealing it. The heat flow due to any changes in the powder because of the humidity was recorded. The enthalpy of the endotherms and exotherms were determined from the peak areas. Four parallel experiments under various *RH* against blank with deionized water (2 ml) were run simultaneously at 35 °C. Power-time curves were registered using the data acquisition program Digitam 3.0 (TA Instruments, Delaware, US) (*Paper II*).

Various salt solutions were used to create certain humidity environments for heat flow measurements. High purity standards of sodium chloride NaCl 6.0 molal in H_2O (a_w = 0.76) and lithium chloride LiCl 8.57 molal in H_2O (a_w = 0.51) and LiCl 13.41 molal in H_2O (a_w = 0.26) were purchased from Decagon Devices, Inc. (USA). For a_w = 1.0 distilled water was used. The values of water activities are given at temperature 35 °C (*Paper II*).

The temperature change of furcellaran due to heat release during moisture absorption was determined from calorimetry data using equation 5.

$$Q = m * c * \Delta T \quad (5)$$

where Q is experimental net heat evolved (J kg^{-1}), m is mass (kg), c is specific heat capacity (J $kg^{-1}°C^{-1}$) and ΔT is the change of temperature (°C). Specific heat of capacity of dry carrageenan is 4000 J $kg^{-1}°C^{-1}$ (Kent et al., 1984).

3.2.6 Rheological characteristics of carrageenan sols and gels

Preparation of sols and gels

All hydrocolloid solutions were prepared by dissolving carrageenan powders in distilled water and they were left to swell for 1 h. After that, the mixtures were heated up to 80±5 °C and, maintaining the temperature, the mixtures were stirred until there was complete solubilisation of the polysaccharides. The sols were used for viscosity measurements and temperature sweep tests or poured into moulds

(diameter 28 mm x 20 mm length (*Paper I*) or (20x20x20 mm (*Paper II*)), cooled to +4±1 °C and stored at that temperature overnight for other rheological measurements (*Paper I and III*).

Polysaccharide concentrations were 2.5% (w/v) in all cases *(Papers I and III)*, except for the temperature sweep test where the polysaccharide concentration was 1.5% (w/v) (*Paper III)*.

Viscosity

The flow properties of 2.5% (w/v) polysaccharide sols at 75 °C were measured with a RheolabQC rotating viscometer (Anton Paar, Germany) fitted with a concentric cylinder measuring system set CC27, with a temperature-controlled water bath. The sample volume was 20 ml and the apparent viscosity η (Pa*s) was measured as a function of shear rate $\dot{\gamma}$ (10–50 s^{-1}) for 40 s (*Paper III*).

Hardness

The moulds with carrageenan gels were removed from the fridge and equilibrated at room temperature (22±2 °C) for 2 h before testing.

The gel strength, or hardness, was determined using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, England) *(Paper I and III)*. The gel specimen was placed upright in the centre of a Valenti probe to compress samples of their original height at a constant speed of 0.2 mm s^{-1} and subjected to force until it failed *(Paper I)*. A stainless steel cylindrical probe (25 mm in diameter) was used to compress samples to 50% of their original height at a constant speed of 1 mm s^{-1} (*Paper III)*.

Rheology

Dynamic rheological measurements were performed with an MCR 301 rheometer (AntonPaar GmbH, Germany) using a serrated parallel plate of 50 mm diameter (profile depth: 0.5 mm) to minimise slippage at the gel-geometry interfaces. The gap between the plates was kept at 1 mm. All measurements were performed in duplicate, and data points were recorded at steady state.

The storage modulus (G') and loss modulus (G'') of the gels at room temperature were determined with a modified method proposed by Chen et al. (2017). The study consisted of the following steps: time sweep (2 min) at 10 Hz and 0.1% strain (within the viscoelastic region), followed by a frequency sweep from 0.01 to 100 Hz at 0.1% strain. After another time sweep (2 min) at 0.1% strain 10 Hz measurement, amplitude sweeps at 0.01–100% strain 10 Hz, and a final time sweep (2 min) at 0.1% strain 10 Hz were recorded.

For temperature sweeps, the polysaccharide solutions were loaded onto a Peltier plate (pre-heated to 90 °C) and its periphery was coated with low-viscosity silicon oil to avoid water evaporation. After a conditioning step of 5 min, the gelation process of each sample was monitored by cooling down the solution to 25 °C at a rate of 1 °C min^{-1} at 0.1% strain and 10 Hz frequency. The melting process of each sample was followed during a heating ramp carried out from 25 to 90 °C, at a constant rate of 1 °C min^{-1} and under the same fixed conditions of strain and frequency (*Paper III*).

3.2.7 Scanning electron microscopy

2.5% (w/v) polysaccharide gels were frozen in liquid nitrogen and freeze-dried under a vacuum at –60 °C. Samples were mounted on aluminium sample holders and were transferred to a scanning electron microscopy (SEM) unit equipped with an EPSE detector (EVO LS15, Carl Zeiss, Milan, Italy), which was at ambient temperature and filled with water vapour at 70 Pa pressure (*Paper III*).

3.2.8 Statistical analysis

All of the data were analysed with Statistica Software and were expressed as mean ± S.D. (*Paper I, III*).

The statistical significance was checked with ANOVA and Tukey's HSD (honestly significant difference) test at 95% confidence level (*Table 4*).

The calorimetrical curve fitting was performed with a brute force nonlinear least-squares method from an R 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria) package "nls2" version 0.2 *(Paper II*).

4 RESULTS AND DISCUSSION

4.1 Characterisation of furcellaran and carrageenans

The aim of this study was to characterise the chemical composition and structure of commercial furcellaran and compare it with commercial carrageenans.

4.1.1 Chemical composition

The total carbohydrate content determined for all samples showed high variation (52.7 to 78.0%, reported in *Table 3*) and the results were statistically different. The analysis of the carrageenan monomeric composition (*Paper III, Table 1*) showed that the predominant sugars in all samples were galactose and 3,6-anhydrogalactose. High sucrose content (26.3% w/w) was found in the κ-carrageenan samples, probably added to improve the functional properties of the product. Small amounts of glucose were found in the ι-carrageenan and κ/λ-carrageenan samples, probably derived from Floridean starch and 6-O-methylgalactose in furcellaran samples.

Table 3. Chemical composition of commercial carrageenans (adapted from Paper III with some modifications).

Compositions, % w/w dry weight	Sample			
	furcellaran	κ-carrageenan	κ/λ-carrageenan	ι-carrageenan
Total carbohydrates	70.7 ± 0.5^{a}	78.0 ± 0.5^{b}	54.8 ± 0.4^{c}	52.7 ± 0.4^{d}
Sulphate ester	16.2 ± 0.4^{a}	15.7 ± 0.3^{a}	22.9 ± 0.3^{b}	36.1 ± 0.4^{c}
Total ash	11.9 ± 0.3^{a}	5.8 ± 0.2^{b}	21.8 ± 0.3^{c}	10.7 ± 0.2^{d}
Protein	0.82 ± 0.00^{a}	0.50 ± 0.00^{b}	0.50 ± 0.00^{b}	0.50 ± 0.00^{b}
Lipid	0.34 ± 0.00^{a}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}

The contents of the sulphate groups in the furcellaran and carrageenans were in agreement with their expected values (Truus et al., 2006; Yang et al., 2011), except for κ- carrageenan, which was lower. This low value can be explained by the added sucrose in the κ-carrageenan and the actual sulphur content in pure κ-carrageenan was expected to be about 1/3 times higher, which is in agreement with the literature (Tuvikene et al., 2009).

The ash content was the highest for κ/λ-carrageenan which is mainly influenced by the washing process (Mehta et al., 2008). Compared with other carrageenans, furcellaran had significantly higher protein and lipid contents. Lipid content of furcellaran is strongly influenced by processing techniques as during the drum-drying process, vegetable oil is added to the drums. Many factors, such as the growth conditions, seasonal variation and type of algae affect the protein content in seaweed. Compared with other types of seaweed*, Furcellaria lumbricalis* showed the highest protein content (Nazeri et al., 2019), which may explain the higher protein content in the studied furcellaran compared with the carrageenans.

4.1.2 Structure

FTIR

FTIR spectroscopy was used to describe the structural characteristics of the furcellaran and carrageenans. All of the spectra were recorded in the 4000–500 cm^{-1} region *Figure 6*.

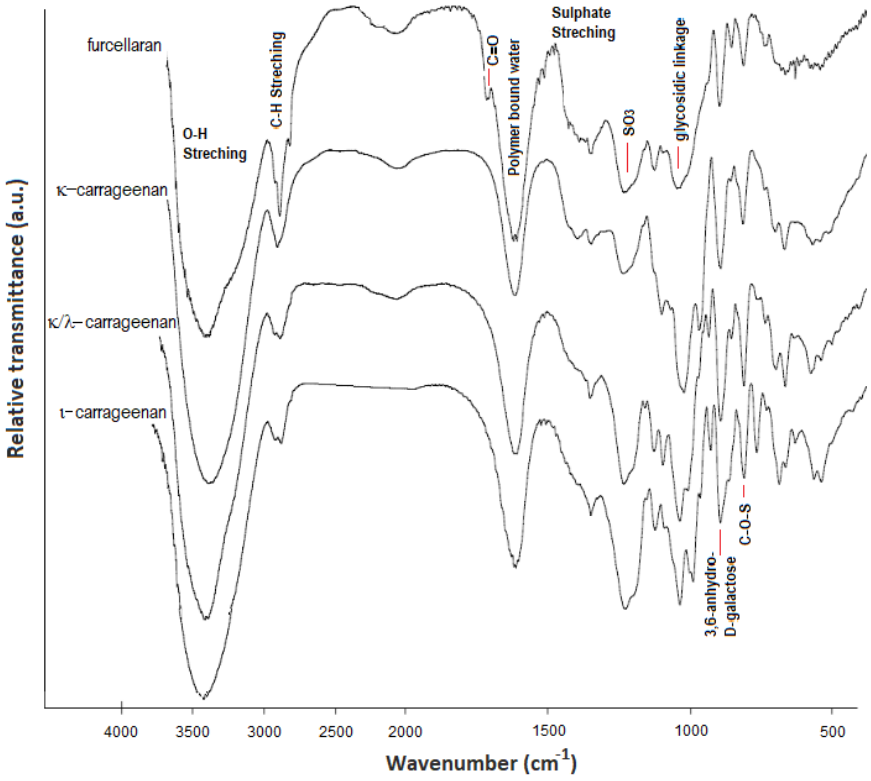

Figure 6. FTIR spectra of the furcellaran and carrageenans.

The absorption observed at 3500–3000 cm^{-1} was characteristic of the O–H stretching and the absorption at ~ 2925 cm^{-1} was due to the C–O groups and the interlayer C–H stretching (Wembabazi et al., 2015).

All of the polysaccharide preparations revealed absorption bands at 1375 and 1260 cm^{-1}, which is indicative of sulphate ester substitution (Prado-Fernández et al., 2003). The presence of a strong absorption band at 930 cm^{-1} is attributable to 3,6-anhydro-D-galactose residues (Pereira et al., 2003).

For the ι-carrageenans, the sharp absorption band and for the κ/λ-carrageenans the weak absorption band at 804 cm^{-1} indicated the presence of 3,6-anhydrogalactose-2-sulphate from ι-carrageenans (Tuvikene et al., 2010).

The absorbance of furcellaran at 847 and 1260 cm^{-1} was substantially weaker than in other commercial carrageenans. This indicates that furcellaran has the lowest sulphur content and correlates with the ascending order of sulphur content of κ-carrageenan, κ/λ-carrageenan and ι-carrageenan shown in *Paper III, Table 1.*

^{13}C NMR

The ^{13}C NMR spectra of the furcellaran and carrageenans are shown in *Paper III, Figure 1*. The signals assignments of these spectra (*Paper III, Table 2*) were interpreted using the chemical shifts data of the carrageenan structures as reported by Van de Velde et al., 2002.

The commercial furcellaran was found to be a hybrid of κ-, β- and ω-carrageenan (molar ratio approximately 5:4:1), with a structure consisting of (1→3) linked β-D-galactopyranose; (1→4) linked 3,6-anhydro-α-D-galactopyranose; (1→3) linked β-D-galactopyranose 4-sulphate and (1→3) β-D-galactopyranose 6-sulphate. Also, 3-linked 6-O-methyl-D-galactose residues were found, as these residues give specific signals for OMe at 59.0, for the substituted C–6 at 71.8, and for the neighbouring C–5 at 73.3 ppm in ^{13}C NMR. This complex hybrid structure of furcellaran has been reported before (Craigie et al., 1990; Tuvikene et al., 2010). The spectra of furcellaran also showed the presence of some additional peaks in the range of 10–35 ppm, indicating the presence of vegetable fats (Zamora et al., 2002).

The main components of the commercial κ-carrageenan were (1→4) linked 3,6-anhydro-α-D-galactopyranose and (1→3) linked β-D-galactopyranose 4-sulphate, indicating the presence of κ-carrageenan. Also, the addition of sucrose was confirmed. The mixture of κ/λ-carrageenan consisted of predominantly κ-carrageenan and small quantities of ι-carrageenan. However, contrary to expectations, there were no signals of λ-carrageenan. These results, together with the FTIR, prove that the studied κ/λ- carrageenan sample was actually κ/ι-carrageenan.

The ι-carrageenan was composed of a mixture of (1→3) linked β-D-galactopyranose 4-sulphate and (1→4) linked α-D-galactopyranose 2,6-disulphate. Additionally, peaks corresponding to amylopectin were registered, indicating the presence of starch in the product with branch-point residue C–6_b detectable at 65.5 ppm (Peng & Perlin, 1987).

4.2 Effect of short-term heat treatment on furcellaran and carrageenans

Drying is one of the most important operational methods in furcellaran production as it significantly influences the physico-chemical properties of the final product. Any single or recurrent drying step needs to be precisely controlled and optimised in order to produce a good quality product. Therefore the aim of the study was to understand the furcellaran drying behaviour and how the temperature affects the structure and physico-chemical properties of furcellaran compared with other carrageenans.

Drying curves

The different drying dynamics of furcellaran depend largely on the drying temperature expressed by the typical drying graphs shown in *Figure 7*.

The loss of drying of furcellaran *(L_D*, %) for drying curves at temperatures below 115 °C can be described by the equation 6:

$$L_D = a + b\tau + c\tau^2 + d\tau^3 + et \quad (6)$$

where

τ – time of drying (min);
t – temperature of drying (°C); and
a, *b*, *c*, *d*, *e* – empirical coefficients.

By applying multiregression analysis, the calculated values L_D correlated well with experimental data R^2=0.9976) when the following coefficients were used: *a*=–9.583; *b*=2.989; *c*=–0.249; *d*=0.00729; *e*=0.1034; S.D.=0.229.

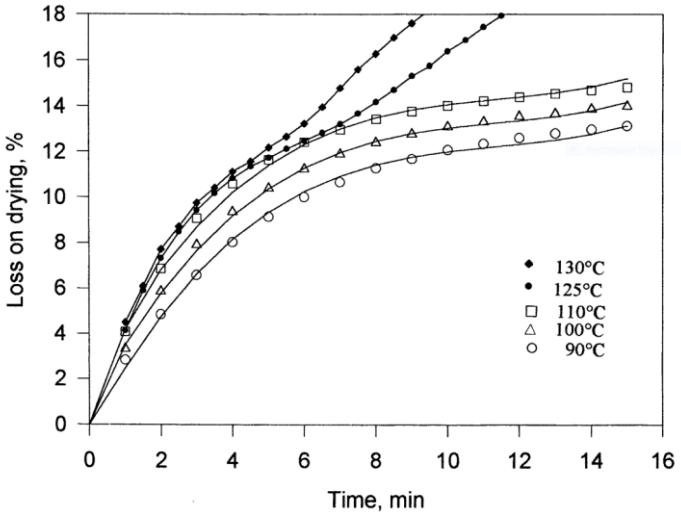

Figure 7. Loss on drying as a function of time at temperatures of drying 90–130 °C (symbols – experimental data; steady line with opened symbols – calculated curve) (adapted from Paper I).

However drying over 115 °C leads to a remarkable change of shape of the curve, the polynomial shape of the graph disappears, and the value of L_D rises sharply. It can be assumed that the increase in weight loss at high temperatures is probably due to the loss of chemically bound water and decomposition of carbohydrates (Bradley & Mitchell, 1988). This assumption is also supported by the decrease in gel strength as a function of temperature and time (5 or 15 minutes) (*Figure 8*) and discoloration of the samples (*Figure 9; Paper III, Figure 4*).

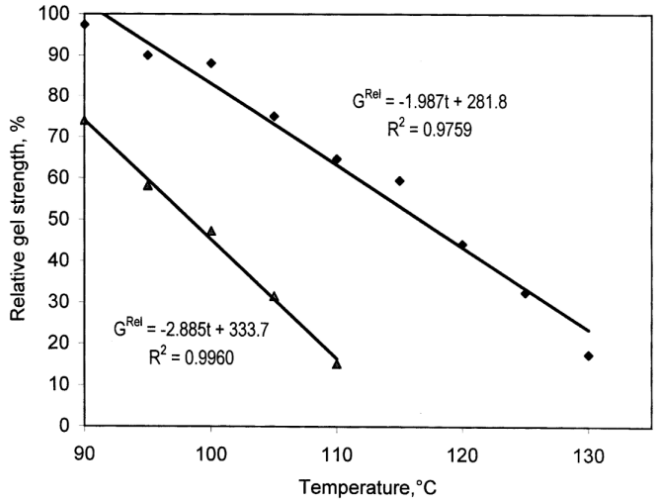

Figure 8. Relative gel strength (G^{Rel}) as a function of temperature of drying after 5 min (▲) and 15 min (■) processing (adapted from Paper I).

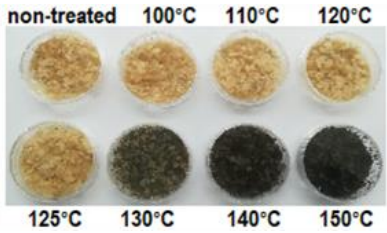

Figure 9. Effect of heat treatment on furcellaran samples (drying temperatures 0, 100, 110, 120, 125, 130, 140, 150 °C; drying time 15 min).

The corresponding relationships indicate that air-drying exerts an influence on gel strength under experimental conditions allowing for linear regression. The heat treatment at 115 °C 15 minutes caused furcellaran to lose its gelling ability, apparently due to the loss of the minimum polysaccharide chain length required for the establishment of stable, ordered self-associated structures. No changes in hardness values were observed for carrageenan gels after thermal treatment at 115 °C *(Paper III, Table 5)*. Also no discoloration of other carrageenans were observed. In view of the above results, a temperature of 115 °C and a drying time of 15 minutes were chosen for further experimental studies.

Structure and molecular weight

^{13}C CP-MAS NMR spectra (*Paper III, Figure 2*) showed no influence of heat treatment at 115 °C for 15 minutes on commercial carrageenans structure, except for furcellaran, where the intensity of the peaks from C3 to C6 (*Paper III, Table 3*) decreased, indicating the degradation of the polysaccharide. The ^{13}C NMR spectra of furcellaran (*Figure 10*A) showed minor changes after heat treatment (*Figure 10*B), which were more pronounced at a higher temperature (130 °C). Three new peaks at 90.4, 87.2 and 82.8 ppm appeared, due to the formation of oligosaccharides. Also coincident anomeric signals of β- D-galactopyranose at 70.1, 69.8 and 69.5 ppm and β-D-galactopyranose-4-sulphate at 69.5 ppm became resolved, with the latter appearing slightly more upfield in the spectrum. Another change of heat treatment was the decrease in the anomeric carbon of 3,6- anhydro-α-D-galactopyranose residue at 95.0 ppm, which can be explained by desulphation of the galactan.

The average molecular weight of non-treated carrageenans (*Paper III, Table 4*) shows a major difference between furcellaran and other carrageenans, which is likely related to the different temperatures, extraction conditions and drying times during their production (De Faria et al., 2014; Robal et al., 2017). Furcellaran has four to six times lower average molecular weight (253 kDa) than other carrageenans and was the most affected by heat treatment. While other carrageenans showed a slight decrease in molecular weight with an increase in the heat treatment temperature, the furcellaran showed a sharp decrease in molecular weight particularly at treatment temperatures above 100 °C.

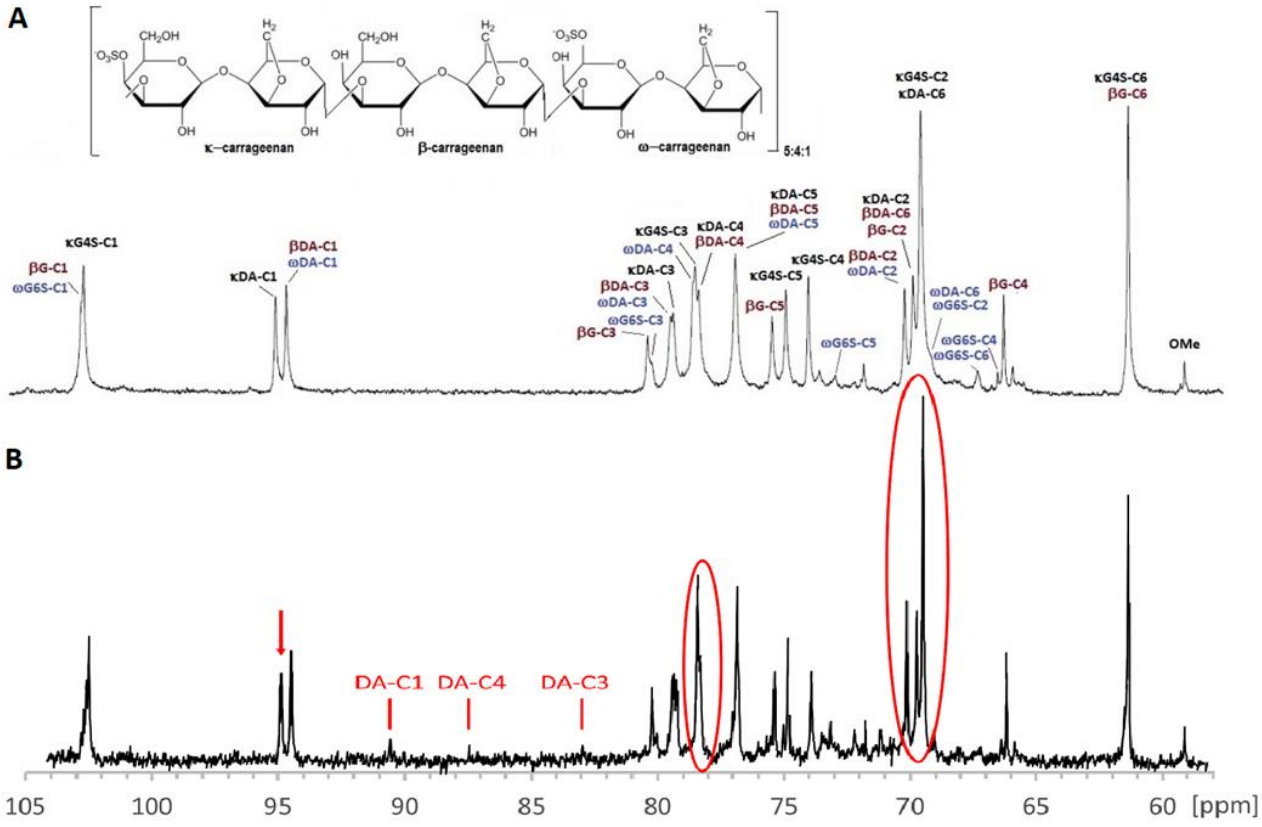

Figure 10. ^{13}C NMR spectra of (A) non-treated and (B) heat-treated at 115 °C for 15 minutes furcellaran (adapted from Paper III).

4.1.3 Water sorption behaviour

Knowledge of the thermodynamic properties associated with water sorption behaviour in foods is important for dehydration in several ways, especially in the design and optimisation of unit operation, as well as to ensure storage stability by calculating moisture changes. Based on the above, carrageenan sorption curves were constructed which characterised the behaviour of the samples in environments with different water activities.

Sorption isotherms

The moisture adsorption and desorption isotherms of furcellaran determined at 20, 35 and 50 °C are given in *Figure 11*. All sorption isotherms were Type II according to BET and IUPAC classifications and had sigmoidal shapes, which indicated multi-layers of sorption of water in a macroporous material and were consistent with the literature data (Inglezakis et al., 2018; Jhider & Bagané, 2019; Tavares & Noreña, 2021). In all cases, the equilibrium moisture content increased with increasing water activity at each temperature and decreased with increasing temperature at water activity < 0.76. This thermal behaviour may be explained by the fact that at higher temperatures the kinetic energy of water molecules increases, thereby increasing their distance and reducing the attractive forces between them. As a result, the sorption degree decreases with increasing water activity as the temperature increases (Bahloul et al., 2008). The same tendency was observed for the sorption isotherms of carrageenans (Torres et al., 2018) and agar-agar (Iglesias & Bueno, 1999).

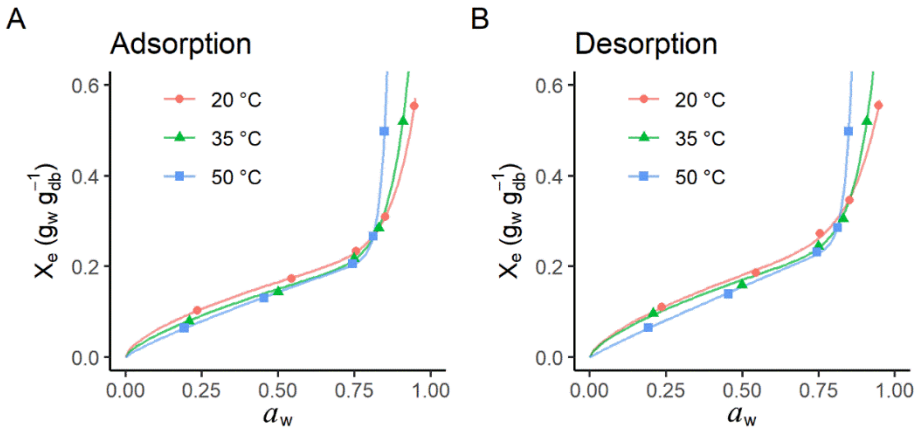

Figure 11. Water adsorption (A) and desorption (B) isotherms of furcellaran at different temperatures. X_e the equilibrium moisture content. Dots correspond to experimental data and lines to the Peleg model (adapted from Paper IV).

A clear increase in moisture content can be seen in adsorption (*Figure 11*A) and correspondingly a decrease in moisture loss for desorption (*Figure 11*B) for water activity > 0.76 at all temperatures. This phenomenon has been observed with foods rich in soluble solids (Seth, 2018; Sormoli & Langrish, 2015) as soluble solids can bind water resulting the decrease of water activity. The intersecting of different isotherms can be observed. This is probably due to faster dissolution of soluble solids at higher

temperatures. Also, more water binding sites in the furcellaran may be exposed due to the thermal effect.

Small differences in hysteresis can be seen in comparing moisture adsorption and desorption isotherms at each temperature *(Paper IV*, *Figure 2*). In all cases, the largest differences between the adsorption and desorption processes were observed at 20 °C, decreasing with increasing temperatures. These results may be related to the process of the solubility of some compounds in water, promoted by temperature and structural changes (Bell & Labuza, 2000). This thermal impact on hysteresis cycles has been previously found for agar (Iglesias & Bueno, 1999), high polysaccharide content red seaweeds (Lemus et al., 2008), and starchy flours (Moreira et al., 2010).

GAB, Caurie, Henderson Thompson, modified Oswin, Peleg and Smith models have been tested to fit the equilibrium moisture content with relative humidity. The parameter values and statistical results at 20, 35 and 50 °C for all models used for adsorption and desorption isotherms are shown in *Paper IV, Table 3* and *Table 4*, respectively. The Peleg model was found to be the most appropriate model to describe sorption curves (*Figure 11*) for different water activities.

Net isosteric heat of sorption

The isosteric heat of sorption is a measure of energy or the energy of a bond between water molecules and an absorbable surface (Mulet et al., 1999). It characterises various processes, such as the drying of food samples and the state of water molecules in foods (Tadapaneni et al., 2017).

The experimental data of sorption isotherms were used to determine the net isosteric heat of the sorption of furcellaran. The Peleg model with equation 4 was used to obtain the values of the water activity at constant moisture content at each temperature (*Figure 12*).

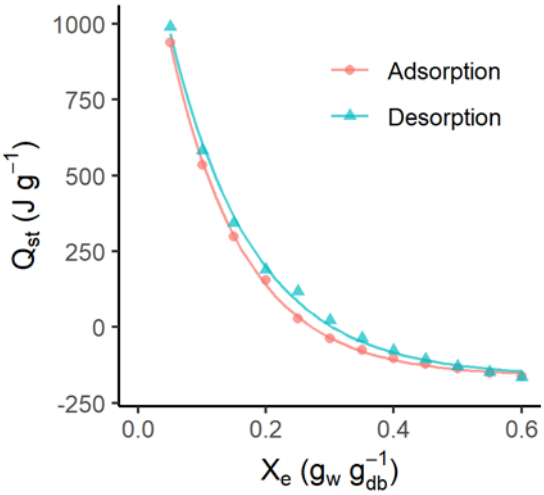

Figure 12. Net isosteric heat of furcellaran for adsorption and desorption for different moisture contents (adapted from Paper IV).

The net isosteric heat decreases as the moisture content increases, showing that the energy needed to adsorb or remove the water in the furcellaran decreases with moisture content. It is known that the sorption occurs at first on the most active polar sites having the greatest interaction energy (Tsami, 1991). When increasing the water content, the sites will occupied and sorption occurs on the less active sites and the net isosteric heat is decreasing. The net isosteric heat of desorption was slightly higher than that of adsorption, indicating that the energy required for the water desorption process was higher than that needed for the adsorption process.

The net isosteric heat of adsorption and desorption approached zero at 0.26 $g_w\ g_{db}^{-1}$ and 0.32 $g_w\ g_{db}^{-1}$, respectively. It shows that there is a critical moisture content where the net isosteric heat of adsorption and desorption is equal to the vaporisation of pure water, and the water molecules act as in the liquid state. So, at higher moisture content less energy is needed during material drying.

However, after a certain moisture content, the heat of sorption values became negative. This may be due to an increase in the moisture content by the dissolution of sugars and possibly of biopolymer at higher temperatures. Negative heat of sorption values has no physical meaning and is due to mathematical calculations.

The net isosteric heat of the sorption of water in furcellaran can be approximated mathematically by equation 7:

$$Q_{st} = a * \exp(k * X_e) + b \quad (7)$$

For adsorption: P = 5.5; RMSE = 8.95; R^2 = 0.999.

$$Q_{st} = 1682.70 \exp\ (-8.616 * X_e) - 161.40$$

For desorption: P = 15.65; RMSE = 20.74; R^2 = 0.997.

$$Q_{st} = 1655.41 \exp(-7.632 * X_e) - 162.22$$

These mathematical equations can applied to predict the heat of the sorption of furcellaran for different moisture contents.

Calorimetry

The curves that are the output of a microcalorimeter are called calorimetric thermograms or power-time curves. The typical calorimetric power-time curve for furcellaran at different water activities is shown in*, Figure 13*. The absorption of the samples at water activity of 0.51, 0.76, and 1.0 produced exothermic responses and desorption at water activity 0.26 produced a slight endothermic response. All curves showed similar shapes. The released heat Q (J g^{-1}) was expressed as equation 8 (Suurkuusk & Wadsö, 1982):

$$Q = \int \Phi d\tau \quad (8)$$

where Φ – heat flow rate (μW) and τ – reaction time (h) are equal to the net area under the curve.

There turned out to be a direct correlation between released heat and ambient water activity for all samples. The example of corrected calorimetric output (the thermal power within the first 0.52 h was not registered) is shown in *Paper II, Figure 2*, in which the experimental data for all samples is clearly described by equation 9:

$$dQ/d\tau = a \cdot e^{b\tau} \quad (9)$$

where $dQ/d\tau$ – heat flow rate (μW), τ – reaction time (h),
a and *b* are the coefficients (*Paper II, Table 2*) for each sample found by the least-squares method.

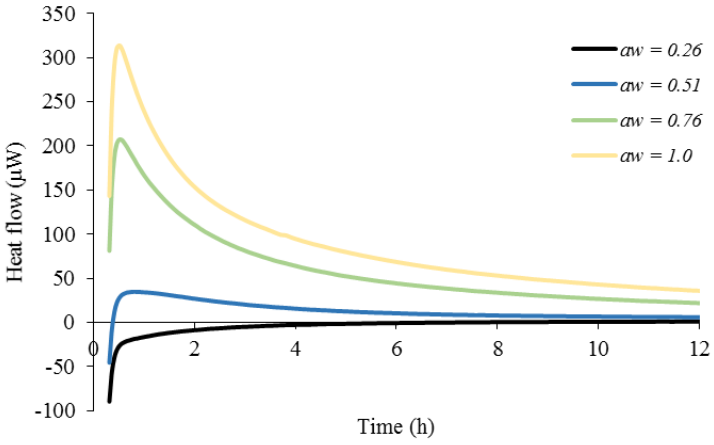

Figure 13. Typical calorimetric curves on TAM for furcellaran at four diferent water activities. Experiment conditions: temp. 35 °C, m=0.089 g (adapted from Paper II).

The total heat evolved during an experiment depends on the furcellaran's and carrageenans' specific compositions and shows variation between all samples at different water activities. No heat release was observed at low water activity (a_w = 0.26), except for ι-carrageenan. The thermal outputs of all samples (*Table 4*) at water activities of 0.51, 0.76, and 1.0 increased; the highest values were obtained for ι-carrageenan, followed by furcellaran, κ-carrageenan and the mixture of κ/λ-carrageenan.

Table 4. Water content of furcellaran and carrageenans for isothermal calorimetry measurement (12 h at 35 °C) at different water activities and the net heat evolved (adapted from Paper II).

Sample	Water content	Experimental net heat evolved Q (J g^{-1})			
	L_D, % (w/w)	a_w = 0.26	a_w = 0.51	a_w = 0.76	a_w = 1.0
furcellaran	11.2	0	6.3	29.3	43.5
ι-carrageenan	9.4	0.41	15.1	37.5	45.9
κ-carrageenan	6.7	0	10.6	28.6	31.6
κ/λ-carrageenan	9.9	0	4.7	22.6	28.1

The calorimetric responses of heat-treated samples (55, 85 and 105 °C for 15 min) show the dependence on the depth of the applied optional heat treatment (*Figure 14*). The mathematical evaluation of these results showed that the dependence of the total heat Q (J g^{-1}) on water activity a_w was satisfactorily approximated by equation 10:

$$Q = a\,[1 - (1 - a_w)^b] \quad (10)$$

where *a* and *b* are the coefficients found by the nonlinear least squares method.

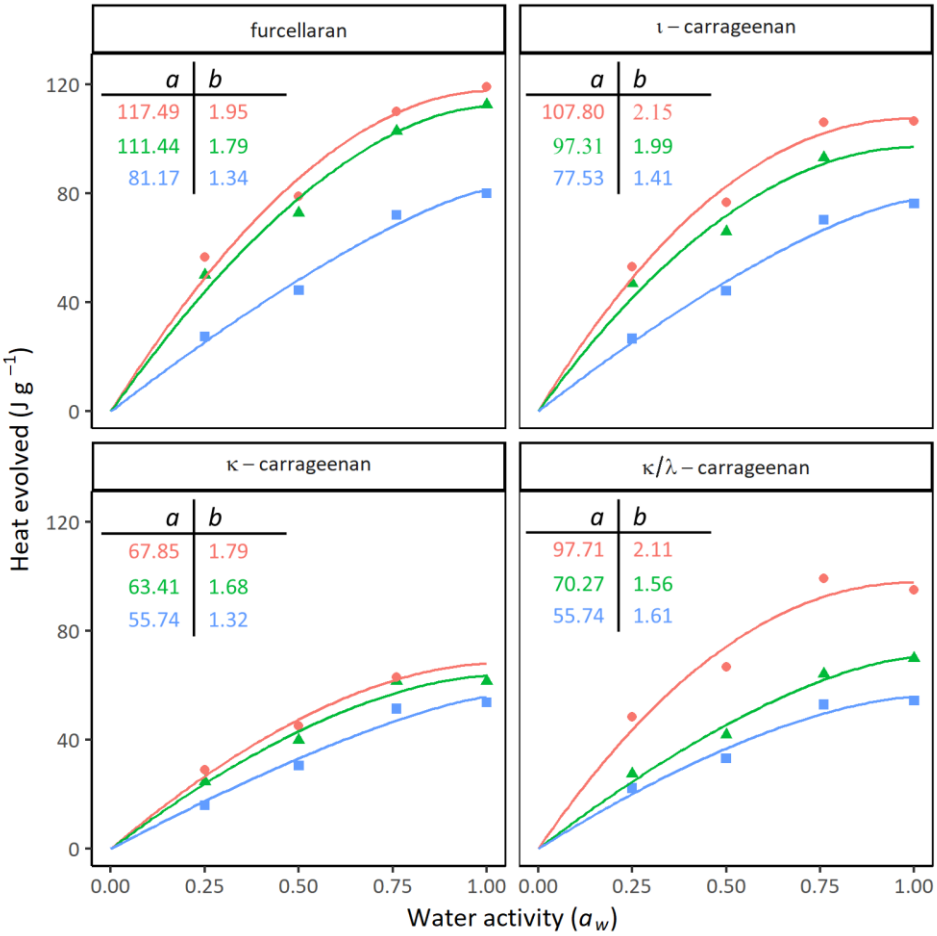

Figure 14. Heat evolved as a function of water activity for furcellaran and different carrageenans at optional heat treatment temperatures for 15 min. "dashed lines" denote fitted curves; symbols "blue square," "green triangle," "red circle" denote experimental data: "blue square" 55 °C, "green triangle" 85 °C, "red circle" 105 °C. Values of the coefcients a and b from equation 11 obtained by curve ftting are shown with the corresponding colors. $Q_{55\,°C} = 81.17[1-(1-a_w)^{1.34}]$, $Q_{85\,°C} = 111.44[1-(1-a_w)^{1.79}]$, $Q_{105\,°C} = 117.49[1-(1-a_w)^{1.95}]$ *(adapted from Paper II).*

Water activity aw was closely related to the chemical potential μ determining system at equilibrium, equation 11 (Reid, 2007):

$$\mu = \mu_0 + RT \ln (a_w) = \mu_0 + RT \ln (p/p_0) \quad (11)$$

where μ is the actual chemical potential (kJ mol^{-1}), μ_0 the reference state chemical potential (kJ mol^{-1}), R the universal gas constant (8.314 J mol^{-1} K^{-1}), T the temperature (K), p the vapour pressure of water in the headspace above the sample at T (Pa), and p_0 the vapour pressure of water in the headspace above the water at T (Pa).

Based on the obtained results, it can be assumed that additional heat treatment of carrageenans would destabilise the galactans, thus opening more binding sites for water (Fellows, 2017; Yousef & Balasubramaniam, 2013). Consequently, at higher a_w, the increase in actual chemical potential μ would lead to increased absorption energy. Thus,

the high-temperature heat treatment of low-moisture carrageenans leads to product instability, increasing water vapour absorption and causing a significant increase in heat release even under moderate storage conditions.

Temperature change due to heat release

Using equation 5, the temperature change of carrageenans due to heat release during moisture adsorption at different water activities was calculated (*Table 5*) using data from *Table 4* and *Figure 14*. From the obtained data, it can be concluded that the temperature of the product increased as a the heat treatment temperature and the water activity of the respective samples were increased. The maximum product temperature rise of 29.8 °C g^{-1} was obtained for furcellaran that was heat treated at temperature 105 °C and measured at a_w = 1.0. However, this temperature change is not sufficient to cause heating and spontaneous combustion.

Table 5. The change of product temperature depending on heat treatment temperature and ambient water activity.

Sample	Drying temperature (°C)	Change of product temperature ΔT (°C g^{-1})			
		a_w = 0.26	a_w = 0.51	a_w = 0.76	a_w = 1.0
furcellaran	non-treated	0	1.6	7.3	10.9
ι-carrageenan		0.1	3.8	9.4	11.5
κ-carrageenan		0	2.7	7.2	7.9
κ/λ-carrageenan		0	1.2	5.7	7.0
furcellaran	55	6.9	11.1	18.0	20.0
ι-carrageenan		6.7	11.1	17.6	19.1
κ-carrageenan		4.0	7.6	12.9	13.4
κ/λ-carrageenan		5.6	8.3	13.3	13.6
furcellaran	85	12.5	18.2	25.7	28.2
ι-carrageenan		11.7	16.5	23.3	-
κ-carrageenan		6.2	10.0	15.4	15.4
κ/λ-carrageenan		6.9	10.5	16.1	17.5
furcellaran	105	14.2	19.7	27.5	29.8
ι-carrageenan		13.3	19.2	26.5	26.6
κ-carrageenan		7.3	11.3	15.8	-
κ/λ-carrageenan		12.1	16.7	24.8	23.8

Rheological properties

The viscosities of non-treated and heat-treated (115 °C) carrageenan sols at 75 °C are shown in *Paper III, Table 5*. The viscosities decrease in the order of κ-carrageenan > ι- carrageenan > κ/λ-carrageenan mixture > furcellaran and correlate well with the molecular weight of the polysaccharides (R^2 = 0.945). A short-length polymer forms smaller coils with less drag and intermolecular attraction, and so lowers the viscosity of the solution, which was reflected in the low molecular weight values of furcellaran.

In order to investigate the microstructure of the gels without altering the internal network structure, dynamic rheology tests were carried out using low heat treatment temperatures (65–95 °C). First, an amplitude sweep test was performed to define the linear viscoelastic (LVE) region (*Figure 15*). The storage modulus (G') was greater than the loss modulus (G'') in all carrageenan gels in the LVE region, indicating dominant

elastic behaviour, and both moduli were raised in the order ι-carrageenan < furcellaran < κ-carrageenan < κ/λ-carrageenan due to the differences in the network strength. The G' and G'' values were stable up to 4% for κ-carrageenan and κ/λ-carrageenan gels and 10% for furcellaran gels, then tended to collapse. For ι-carrageenan the LVE region occurred up to 90%. The crossover points at 1190 Pa for κ-carrageenan, 1250 Pa for furcellaran and 4540 Pa for κ/λ-carrageenan are good indicators of yield stress, in which the structure ruptured and material began to flow. The constant strain value 0.1% was selected from the LVE range for frequency, time and temperature sweep tests.

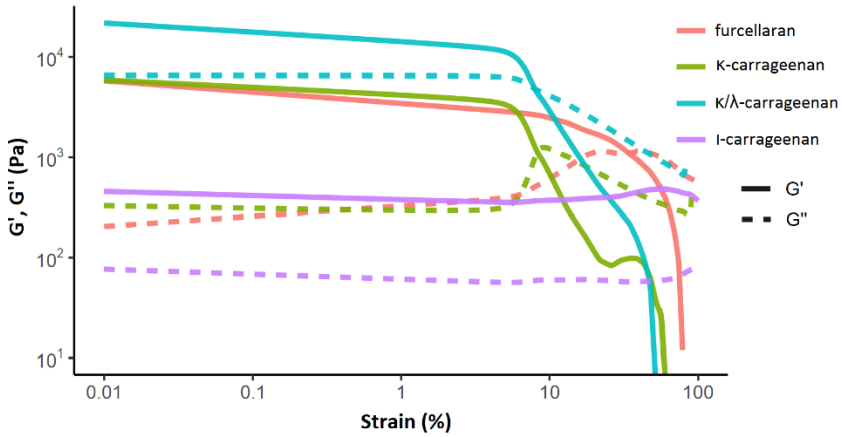

Figure 15. Storage modulus (G') and loss modulus (G") as a function of strain for 2.5% carrageenan gels (adapted from Paper III).

An example of the measurement run for non-treated 2.5% (w/w) furcellaran and carrageenan gels are shown in *Figure 16*.

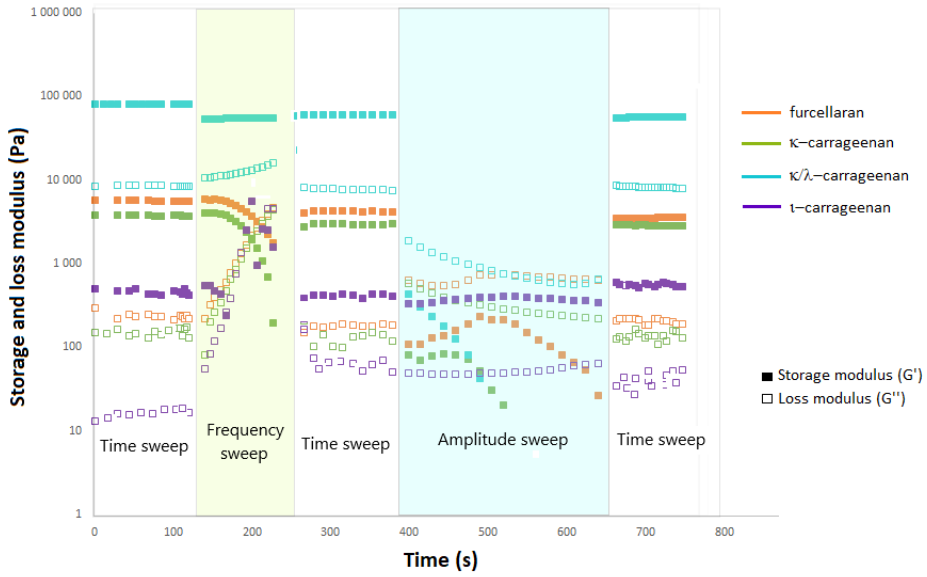

Figure 16. Measurement run for non-treated 2.5% (w/w) furcellaran and carrageenan gels.

Frequency sweep experiments were carried out to investigate the stability of the gel networks. The data obtained reveal that for all of the carrageenans, G' exhibits a plateau at low frequencies of 0.01–10 Hz, which is indicative of a stable cross-linked network *(Paper III, Figure 6)*. At higher frequencies, the G' decreased for κ-carrageenan, furcellaran and ι-carrageenan and crossover points at 33 Hz, 30 Hz and 13 Hz, respectively, were observed. This means that these systems behave as reversible networks and are able to pass from a gel state at low frequencies to a very viscous liquid state at high frequencies. For κ/λ-carrageenan gels, the slightly increasing G' curves were all above the G" curves, which is indicative of the gel character over the entire frequency domain applied and the frequency of oscillations has a low influence on the viscoelastic properties.

The G' values of all studied carrageenan gels slightly decreased with an increase in the heat treatment temperature (65–95 °C) (*Paper III, Figure 7*). The greatest decrease in G' values occurred with κ/λ-carrageenan gels, followed by ι-carrageenan > furcellaran > κ- carrageenan. Also the stability against the frequency oscillations decreased after heat treatment (*Figure 17*A). After drying at 115 degrees, the stability from the original value decreased by 9%, 8% and 7% for furcellaran, κ-carrageenan and ι-carrageenan, respectively. κ/λ-carrageenan gels showed no crossover between G' and G'' values until 85 °C, after which the crossover point occurred at 67 Hz.

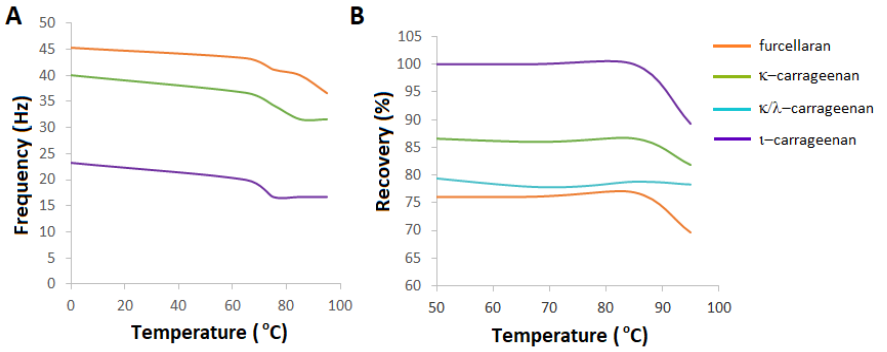

Figure 17. The influence of heat treatment temperature on the (A) frequency where the gel network rupture and (B) recovery of the gels after amplitude sweep test.

The cyclic time sweeps (*Figure 16*) show that all carrageenan gels have the ability to instantly recover after frequency and amplitude sweep tests. 100% recovery was observed for ι-carrageenan, followed by κ-carrageenan (89%), κ/λ-carrageenan (85%) and furcellaran (76%) after the amplitude sweep (*Figure 17*B). A decrease in recovery was observed after heat treatment at 85 °C for ι-carrageenan, κ-carrageenan and furcellaran and at 115 °C the highest decrease (11%) occurred for ι-carrageenan, while for all other carrageenans a 7% drop was recorded.

Gelling and melting temperatures

The temperature sweep test was applied to analyse the temperature dependent rheological behaviour of the carrageenans. A representative temperature sweep curve for non-treated 1.5% (w/w) furcellaran gel is provided in *Figure 18*. Two distinct crossover points are seen in the graph corresponding to the sol-gel transition temperature (T_{sg}) at 32 °C during the cooling cycle and gel-sol transition temperature (T_{gs}) at 53 °C during heating.

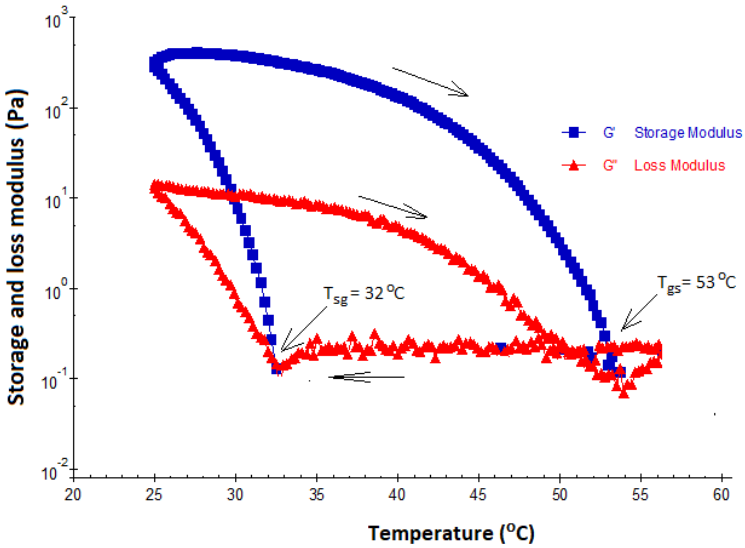

Figure 18. The gelling and melting temperatures of non-treated furcellaran sols and gels.

Similar curves were obtained with other carrageenan samples, differences occurring only in T_{sg} and T_{gs} values (*Paper III, Table 5*), confirming that all carrageenan gels had the thermo-reversible property. The transition temperatures were much higher for ι- carrageenan and κ/λ-carrageenan. As κ/λ-carrageenan contains the ι-type instead of λ- carrageenan, the higher transition temperatures were due to the content of ι- carrageenan. An increase in the gelling and melting temperatures with an increase in the ι-carrageenan proportion has been reported before (Cosenza et al., 2014; Souza et al., 2011). It is apparent that both T_{sg} and T_{gs} shift to lower temperatures with an increase in the heat treatment temperature, indicating that heat treatment impedes coil-helix transition. Also the thermal hysteresis between T_{sg} and T_{gs} decreased with heat treatment for all of the samples except for κ/λ-carrageenan. Furcellaran showed the greatest shift in transition temperatures and in thermal hysteresis, probably due to greater degradation of the polysaccharide.

Microstructure of the gels

The microstructural changes in carrageenan gels (*Figure 19*) that may be caused by heat treatment were studied by Scanning Electron Microscopy (SEM). It was observed that the gel skeleton structures of the hydrogel samples formed porous networks; furcellaran was similar to ι-carrageenan and κ-carrageenan was similar to κ/λ-carrageenan. The first mentioned samples had characteristic honeycomb structures, whereas κ-carrageenan and κ/λ-carrageenan exhibited long cross-linked tubular structures with rectangular pores. The results indicate that the heat treatment was reflected only in the morphology of the furcellaran, whose honeycomb structure thickened visually and due to the decrease in the proportion of the thin polysaccharide network, leading to a decrease in gel strength.

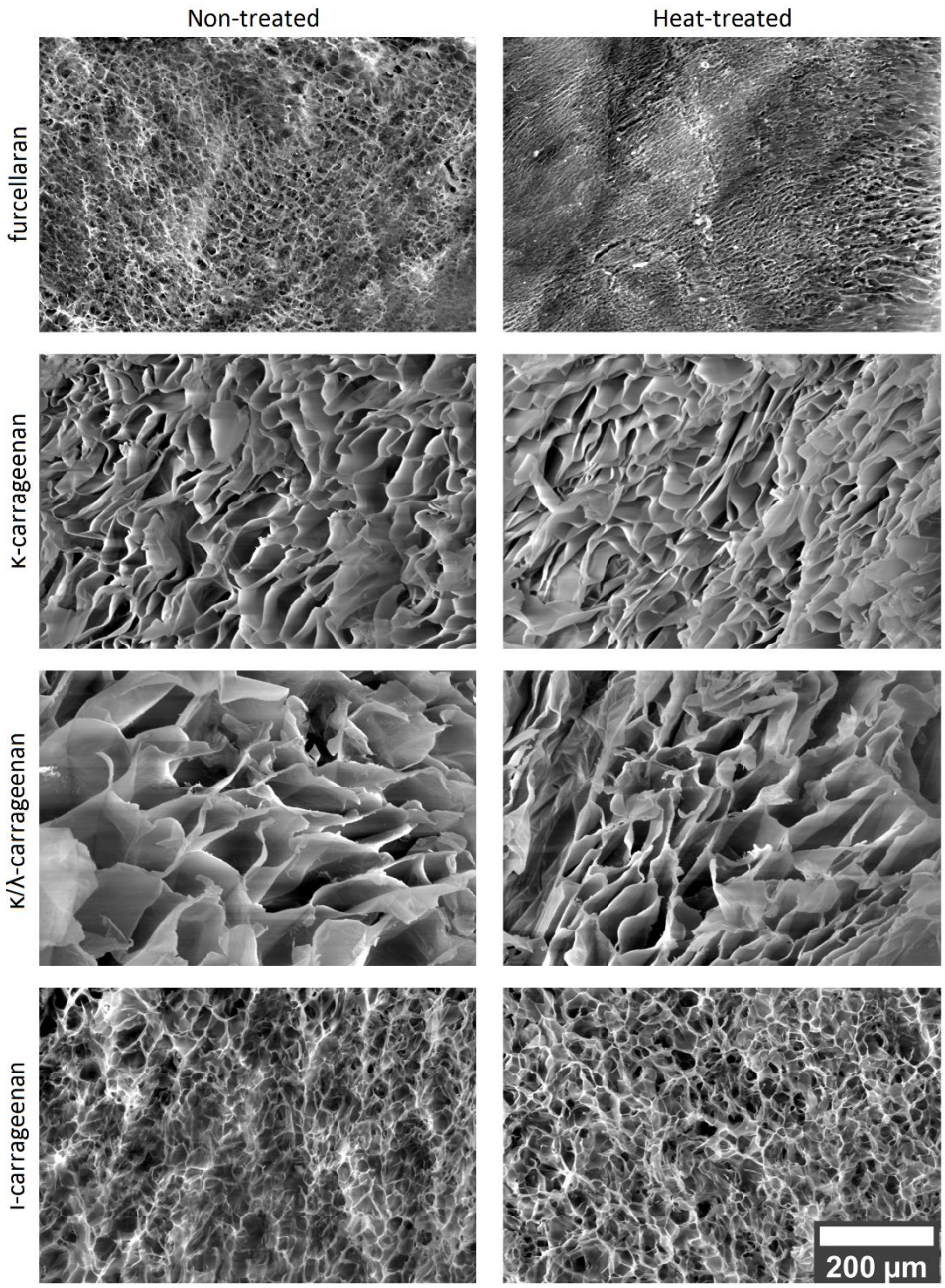

Figure 19. SEM images of 2.5% (w/w) gel network structures of non-treated and heat-treated (115 °C 15 min) carrageenans (adapted from Paper III).

Temperature degradation of furcellaran

The changes in structure, decrease in molecular weight and gel strength lead to the conclusion that commercial furcellaran degrades at temperatures above 115 °C. The degradation is likely the result of acid hydrolysis as the pH of the heat-treated samples decreases from 7.4 to 2.4 (*Paper III, Figure 4*). During short-term drying at high

temperature when there is a sufficient amount of moisture present, the sulphate groups from galactose-4-sulphate (*Figure 20*A) or galactose-6-sulphate *(Figure 20*B) are presumably released and sulphuric acid is formed in the presence of water (residual moisture). Glycosidic linkages between constituent units may be broken by acid hydrolysis, resulting in the shortening of polysaccharide chains and a continual decrease in molecular weight. Also, in the presence of water, strong acid will dissociate and release heat that could potentially lead to a "spontaneous ignition".

It was expected that the degradation of carrageenans would depend on the thermal history relevant to the process of the studied material. One possible explanation is that the furcellaran used in this study had more pronounced water uptake repeated heat treatment in drum-drying, causing more harsh degradation conditions.

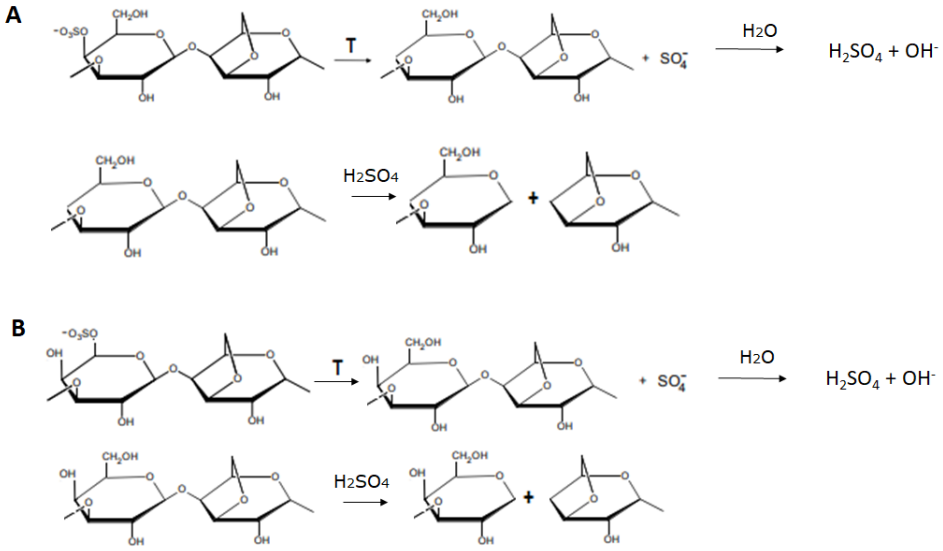

Figure 20. Degradation scheme of furcellaran. Cleavage of the sulphate groups from galactose-4-sulphate (A) and from galactose-6-sulphate (B).

The thermal treatment also affected the counterions of the sample. There was an increase in the conductance of sample filtrate by increasing the drying temperature and time *(Paper I, Figure 3*). From the atomic absorption measurements bound Ca^{2+}, K^{+} and Na^{+}-ions were determined to have been released from the polysaccharide, whereas Mg^{2+}-ions remained in the matrix (*Paper I, Table 1*). Robal et al. (2017) also found that the degradation process caused by the temperature effect on the stability of dry samples is particularly low when the samples consist only of divalent cations, whereas Mg^{2+} is less durable under thermal treatment. It is assumed that the decrease in positively charged counterions is related to the weakening of intramolecular bonds. K^{+} is known to be a structure-disordering ion (Watase & Nishinari, 1981). On the other hand, it has been proven that the thermal stability of guar gum can be substantially extended by cross-linking with transitional metal ions (Maier et al., 1993). Consequently, the ion binding to the polysaccharide matrix is liable to affect the structural features and processing conditions.

5 CONCLUSIONS

Based on the current study, the following conclusions can be drawn.

- Drying at 115 °C for 15 minutes had no influence on the structure of the carrageenans, except for furcellaran, where desulphation of the galactans occurred.
- Released sulphate groups can react with water, forming sulphuric acid, which causes the degradation of the polysaccharide.
- The degradation process caused the loss of viscosity and gelling ability as the average molecular weight fell below the minimum value required for gelation.
- The gelling and melting temperatures decreased with an increase in the heat treatment temperature, indicating that heat treatment impedes coil-helix transition.
- High temperatures destabilised the galactans, thus opening more binding sites for water.
- Stronger water binding together with the formation of sulphuric acid may significantly raise the product temperature leading to the discolorisation and spontaneous ignition of the product.

References

Abad, L.V., Kudo, H., Saiki, S., Nagasawa, N., Tamada, M., Katsumura, Y., Aranilla, C.T., Relleve, L.S., & De La Rosa, A.M. (2009). Radiation degradation studies of carrageenans. Carbohydrate Polymers 78(1), 100–106.

Abad, L.V. (2010). Radiolysis studies of kappa carrageenan for biobased materials development: doctoral thesis. Department of Nuclear Engineering and Management, University of Tokyo.

Abad, L.V., Aurigue, F.B., Relleve, L.S., Montefalcon, D.R.V., & Lopez, G.E.P. (2016). Characterization of low molecular weight fragments from gamma irradiated κ- carrageenan used as plant growth promoter. Radiation Physics and Chemistry 118, 75– 80.

Abdul Khalil, H.P.S., Lai, T.K., Tye, Y.Y., Rizal, S., Chong, E.W.N., Yap, S.W., Hamzah, A.A., Nurul Fazita, M.R., & Paridah, M.T. (2018). A review of extractions of seaweed hydrocolloids: Properties and applications. Express Polymer Letters 12, 296–317.

Abu-Galiyun, E., Huleihel, M., & Levy-Ontman, O. (2019). Antiviral bioactivity of renewable polysaccharides against Varicella Zoster. Cell Cycle 18(24), 3540–3549.

Aguirre, J.C.L., Giraldo, G.A.G., & Rodriguez, M.C. (2021). Modeling of moisture sorption isotherm and evaluation of net isosteric heat for spray-dried fortified coconut (Cocos nucifera L.) powder. British Food Journal 123(7), 2349-2364.

Aguirre-Loredo, R.Y., Rodríguez-Hernández, A.I., Morales-Sánchez, E., Gómez-Aldapa, C.A., & Velazquez, G. (2016). Effect of equilibrium moisture content on barrier, mechanical and thermal properties of chitosan films. Food Chemistry 196, 560–566.

Aguerre, R.J., Suarez, C., & Viollaz, P.Z. (1989). New BET type multi-layer sorption isotherms. – Part II: Modelling water sorption in foods. Lebensmittel-Wissenschaft und Technologie 22(4), 192–195.

Ajibola, O.O., Aviara, N.A., & Ajetumobi, O.E. (2003). Sorption equilibrium and thermodynamic properties of cowpea (Vigna unguiculata). Journal of Food Engineering 58(4), 317–324.

Alhamdan, A.M., & Hassan, B.H. (1999). Water sorption isotherms of date pastes as influenced by date cultivar and storage temperature. Journal of Food Engineering 39(3), 301–306.

Al-Muhtaseb, A.H., McMinn, W.A.M., & Magee, T.R.A. (2004). Water sorption isotherms of starch powders. Part 1: mathematical description of experimental data. Journal of Food Engineering 61, 297–307.

Al-Nahdi, Z.M., Al-Alawi, A., & Al-Marhobi, I. (2019). The effect of extraction conditions on chemical and thermal characteristics of kappa-carrageenan extracted from *Hypnea bryoides*. Journal of Marine Sciences, 2019, Article ID 5183261.

Anastyuk, S.D., Barabanova, A.O., Correc, G., Nazarenko, E.L., Davydova, V.N., Helbert, W., Dmitrenok, P.S., & Yermak, I.M. (2011). Analysis of structural heterogeneity of κ/β- carrageenan oligosaccharides from *Tichocarpus crinitus* by negative-ion ESI and tandem MALDI mass spectrometry. Carbohydrate Polymers 86(2), 546–554.

Andrade, P.R.D., Lemus, M.R., & Perez, C.C.E. (2011). Models of sorption isotherms for food: Uses and limitations. Vitae-Revista De La Facultad De Quimica Farmaceutica 18(3), 325–334.

Arltoft, D., Ipsen, R., Madsen, F., & de Vries, J. (2007). Interactions between carrageenans and milk proteins: A microstructural and rheological study. Biomacromolecules 8(2), 729-736.

Arslan-Tontul, S. (2021). Moisture sorption isotherm and thermodynamic analysis of quinoa grains. Heat and Mass Transfer 57(3), 543–550.

Aviara, N.A. (2020). Moisture sorption isotherms and isotherm model performance evaluation for food and agricultural products. In G. Kyzas, & N. Lazaridis (Eds.), Sorption in 2020s. IntechOpen.

Azevedo, G., Torres, M.D., Sousa-Pinto, I., & Hillio, L. (2015). Effect of pre-extraction alkali treatment on the chemical structure and gelling properties of extracted hybrid carrageenan from *Chondrus crispus* and *Ahnfeltiopsis devoniensis.* Food Hydrocolloids 50, 150–158.

Bahloul, N., Boudhrioua, N., & Kechaou, N. (2008). Moisture desorption-adsorption isotherms and isosteric heats of sorption of Tunisian olive leaves (Olea europaea L.). Industrial Crops and Produtcs 28(2), 162–176.

Barahona, T., Chandía, N.P., Encinas, M.V., Matsuhiro, B., & Zúñiga, E.A. (2011). Antioxidant capacity of sulfated polysaccharides from seaweeds. A kinetic approach. Food Hydrocolloids 25(3), 529–535.

Barbeyron, T., Michel, G., Potin, P., Henrissat, B., & Kloareg, B. (2000). iota-Carrageenases constitute a novel family of glycoside hydrolases, unrelated to that of κappa-carrageenases. Journal of Biological Chemistry 275(45), 35499–35505.

Barbosa-Cánovas, G.V., & Vega-Mercado, H. (1996). Physical, chemical, and microbiological characteristics of dehydrated foods. In Dehydration of Foods (pp. 29–99). Springer, Boston.

Bazardeh, M.E., & Esmaiili, M. (2014). Sorption isotherm and state diagram in evaluating storage stability forsultana raisins. Journal of Stored Products Research 59, 140–145.

Bell, L.N., & Labuza, T.P. (2000). L.N. Bell (Ed.), Moisture Sorption: Practical Aspects of Isotherm Measurement and Use (2nd ed.) American Association Cereal Chemistry, St. Paul, Minnesota.

Bernal, V., Giraldo, L., & Moreno-Piraján, J.C. (2021). Physicochemical parameters of the methylparaben adsorption from aqueous solution onto activated carbon and their relationship with the surface chemistry. ACS Omega 6(13), 8797–8807.

BeMiller, J.N. (2018). Carrageenan. In J.N. BeMiller (Ed.), Carbohydrate Chemistry for Food Scientists (3rd ed., pp. 279–291). Woodhead Publishing and AACC International Press.

Bispo, J.A.C., Bonafe, C.F.S., Santana, K.M.O.V., & Santos, E.C.A. (2015). A comparison of drying kinetics based on the degree of hydration and moisture ratio. LWT – Food Science and Technology 60(1), 192–198.

Blakemore, W.R., & Harpell, A.R. (2010). Carrageenan. In A. Imeson (Ed.), Food Stabilisers, Thickeners and Gelling Agent (pp. 73–94). Blackwell Publishing.

Blanco-Pascual, N., Alemán, A., Gómez-Guillén, M.C., & Montero, M.P. (2014). Enzyme-assisted extraction of κ/ι-hybrid carrageenan from *Mastocarpus stellatus* for obtaining bioactive ingredients and their application for edible active film development. Food & Function 5(2), 319–329.

Boulho, R., Marty, C., Freile-Pelegrín, Y., Robledo, D., Bourgougnon, N., & Bedoux, G. (2017). Antiherpetic (HSV-1) activity of carrageenans from the red seaweed *Solieria chordalis* (Rhodophyta, Gigartinales) extracted by microwave-assisted extraction (MAE). Journal of Applied Phycology 29(5), 2219–2228.

Bradley, T., & Mitchell, J.R. (1988). The determination of the kinetics of polysaccharide thermal-degradation using high-temperature viscosity measurements. Carbohydrate Polymers 9(4), 257–267.

Bui, T.N.T.V. (2019). Structure, rheological properties and connectivity of gels formed by carrageenan extracted from different red algae species: doctoral thesis. Organic chemistry, Université du Maine.

Caballero-Cerón, C., Guerrero-Beltrán, J.A., Mújica-Paz, H., Torres, J.A., & Welti-Chanes, J. (2015). Moisture sorption isotherms of foods: Experimental methodology, mathematical analysis, and practical applications. In G. Gutiérrez-López, L. Alamilla-Beltrán, M. del Pilar Buera, J. Welti-Chanes, E. Parada-Arias, & G. Barbosa-Cánovas (Eds.), Water Stress in Biological, Chemical, Pharmaceutical and Food Systems. (pp. 187–214). Springer, New York.

Campo, V.L., Kawano, D.F., da Silva, D.B., Jr & Carvalho, I. (2009). Carrageenans: biological properties, chemical modifications and structural analysis – A review. Carbohydrate Polymers 77(2), 167–180.

Castillo, M.D., Martinez, E.J., Gonzalez, H.H.L., Pacin, A.M., & Resnik, S.L. (2003). Study of mathematical models applied to sorption isothermsof Argentinean black bean varieties. Journal of Food Engineering 60, 343–348.

Cervenka, L., Rezkova, S., Hejdrychova, J., Kralovsky, J., Brozkova, I., Pejchalova, M., & Vytrasova, J. (2007). Study of moisture adsorption characteristics of gingerbreads and biscuits using a hygrometric method. Acta Alimentaria 36(3), 311–320.

Chan, W.I., Zhang, G.P., Li, X., Leung, C.H., Ma, D.L., Dong, L., & Wang, C.M. (2017). Carrageenan activates monocytes via type-specific binding with interleukin-8: an implication for design of immuno-active biomaterials. Biomaterials Science 5(3), 403–407.

Chen, C.-C. (1990). Modification of Oswin EMC/ERH equation. Journal of Agricultural Research of China 39(4), 367–376.

Chen, C. (2000). Rapid model to determinate the sorption isotherms of peanuts. Journal of Agricultural Engineering Research 75, 401–408.

Chen, Y., Liao, M.-L., & Dunstan, D.E. (2002). The rheology of K^+-κ-carrageenan as a weak gel. Carbohydrate Polymers 50(2), 109–116.

Chen, H.M., Yan, X.J., Wang, F., Xu, W.F., Zhang, L. et al. (2010). Assessment of the oxidative cellular toxicity of a kappa-carrageenan oxidative degradation product towards Caco-2 cells. Food Research International 43(10), 2390–2401.

Chen, M.H., Wang, L.L., Chung, J.J., Kim, Y.-H., Atluri, P., & Burdick, J.A. (2017). Methods to assess shear-thinning hydrogels for application as injectable biomaterials. ACS Biomaterials Science & Engineering 3, 3146−3160.

Chen, X.Y., Han, W.W., Wang, G.X., & Zhao, X. (2020). Application prospect of polysaccharides in the development of anti-novel coronavirus drugs and vaccines. International Journal of Biological Macromolecules 164, 331–343.

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(2), 95–98.

Cohen, S.M. & Ito N. (2002). A critical review of the toxicological effects of carrageenan and processed eucheuma seaweed on the gastrointestinal tract. Critical Reviews in Toxicology 32(5), 413–44.

Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council Text with EEA relevance, OJ L 83, 22.3.2012, p. 1–295.

Correc, G., Barabanova, A., Tuvikene, R., Truus, K., Yermak, I., & Helbert, W. (2012). Comparison of the structures of hybrid κ-/β-carrageenans extracted from *Furcellaria lumbricalis* and *Tichocarpus crinitus*. Carbohydrate Polymers 88(1), 31–36.

Cosenza, V.A., Navarro, D.A., Fissore, E.N., Rojas, A.M., & Stortz, C.A. (2014). Chemical and rheological characterization of the carrageenans from Hypnea musciformis (Wulfen) Lamoroux. Carbohydrate Polymers 102, 780–789.

CP Kelco. (2002). GENU® Carrageenan Book, 1–28.

Craigie, J.S. (1990). Cell walls. In K.M. Cole, & R.G. Sheath (Eds.), The Biology of Red Algae (pp. 221–257). Cambridge University Press, Cambridge.

Crapiste, G.H., & Rotstein, E. (1982). Prediction of sorptional equilibrium data for starch-containing foodstuffs. Journal of Food Science 47(5), 1501–1507.

Cunha, L., & Grenha, A. (2016). Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. Marine Drugs 14(3), 42.

Das, A.K., Sharma, M., Mondal, D., & Prasad, K. (2016). Deep eutectic solvents as efficient solvent system for the extraction of κ-carrageenan from *Kappaphycus alvarezii*. Carbohydrate Polymers 136, 930–935.

Dea, I.C.M. (1993). Conformational origins of polysaccharide solution and gel properties. In R.L. Whistler, & J.N. BeMiller (Eds.), Industrial Gums (3rd ed., pp. 21–52). Academic Press, California.

De Faria, G.S.M., Hayashi, L., & Monteiro, A.R. (2014). Effect of drying temperature on carrageenan yield and quality of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) cultivated in Brazil. Journal of Applied Phycology 26(2), 917–922.

Demarchi, S.M., Ruiz, N.A.Q., De Michelis, A., & Giner, S.A. (2013). Sorption characteristics of rosehip, apple and tomato pulp formulations as determined by gravimetric and hygrometric methods. LWT – Food Science and Technology 52(1), 21–26.

Diogo, J.V., Novo, S.G., Gonzalez, M.J., Ciancia, M., & Bratanich, A.C. (2015). Antiviral activity of lambda-carrageenan prepared from red seaweed (*Gigartina skottsbergii*) against BoHV-1 and SuHV-1. Res. Research in Veterinary Science 98, 142–144.

Djaeni, M., Sasongko, S.B., Prasetyaningrum, A.A., Jin, X., & van Boxtel, A.J. (2012). Carrageenan drying with dehumidified air: drying characteristics and product quality. International Journal of Food Engineering 8(3), Article 32.

Dodgson, K.S., & Price, R.G. (1962). A note on the determination of the ester sulphate content of sulphated polysaccharides. The Biochemical Journal 84, 106–110.

Dos Santos-Fidencio, G.C., Gonçalves, A.G., Noseda, M.D., Duarte, M.E.R., & Ducatti, D.R.B. (2019). Effects of carboxyl group on the anticoagulant activity of oxidized carrageenans. Carbohydrate Polymers 214, 286–293.

Du, L., Brenner, T., Xie, J.L., & Matsukawa, S. (2016). A study on phase separation behavior in kappa/iota carrageenan mixtures by micro DSC, rheological measurements and simulating water and cations migration between phases. Food Hydrocolloids 55, 81–88.

Eccles, R., Winther, B., Johnston, S.L., Robinson, P., Trampisch, M., & Koelsch, S. (2015). Efficacy and safety of iota-carrageenan nasal spray versus placebo in early treatment of the common cold in adults: The ICICC trial. Respiratory Research 16, Article ID 121.

Eha, K. (2001). Interactions between drying and storage of furcellaran preparations: master's thesis. Tallinn University of Technology, Tallinn.

Est-Agar AS. (n.d.). Furcellaran manufacturing. http://estagar.ee/manufacturing/

Farahnaky, A., Azizi, R., Majzoobi, M., Mesbahi, Gh., & Maftoonazad, N. (2013). Using power ultrasound for cold gelation of kappa-carrageenan in presence of sodium ions. Innovative Food Science and Emerging Technologies 20, 173–181.

Fellows, P.J. (2017). Food Processing Technology – Principles and Practice (4th ed.). Elsevier, Kent.

Feng, W., Feng, S., Tang, K., He, X., Jing, A., & Liang, G. (2017). A novel composite of collagen-hydroxyapatite/kappa-carrageenan. Journal of Alloys and Compounds 693, 482–489.

Fennema, O. (1981). Water activity at subfreezing temperatures. In L.B. Rockland & G.F. Stewart (Eds.), Water Activity: Influences on Food Quality (pp. 713–732). Academic Press, New York.

Frediansyah, A. (2021). The antiviral activity of iota-, kappa-, and lambda-carrageenan against COVID-19: A critical review. Clinical Epidemiology and Global Health 12, Article ID 100826.

Getahun, E., Gabbiye, N., Delele, M.A., Fanta, S.W., Gebrehiwot, M.G., & Vanierschot, M. (2020). Effect of maturity on the moisture sorption isotherm of chili pepper (Mareko Fana variety). Heliyon 6(8), Article Number: e04608.

Glicksman, M. (1983). Red seaweed extracts (agar, carrageenan, furcellaran). In M. Glicksman (Ed.), Food Hydrocolloids (pp. 73–113). CRC Press, New York.

Goff H.D., & Guo, Q. (2019). The role of hydrocolloids in the development of food structure. In F. Spyropoulos, A. Lazidis, & I. Norton (Eds.), Handbook of Food Structure Development (1st ed., pp. 1–28). Royal Society of Chemistry.

Goneli, A.L.D., Correa, P.C., Oliveira, G.H.H., & Afonso, P.C. (2013). Water sorption properties of coffee fruits, pulped and green coffee. LWT – Food Science and Technology 50(2), 386-391.

Goula, A.M., Karapantsios, T.D., Achilias, D.S., & Adamopoulos, K.G. (2008). Water sorption isotherms and glass transition temperatureof spray dried tomato pulp. Journal of Food Engineering 85(1), 73–83.

Grenha, A., Gomes, M.E., Rodrigues, M., Santo, V.E., Mano, J.F., Neves, N.M., & Reis, R.L. (2010). Development of new chitosan/carrageenan nanoparticles for drug delivery applications. Journal of Biomedical Materials Research 92A(4), 1265–1272.

Guan, J., Li, L., & Mao, S. (2017). Applications of carrageenan in advanced drug delivery. In J. Venkatesan, S. Anil, & S.-K. Kim (Eds.), Seaweed Polysaccharides (pp. 283–303). Elsevier, Amsterdam.

Hawa, L.C., Ubaidillah, U., Damayanti, R., & Hendrawan, Y. (2020). Moisture sorption isotherms of modified cassava flour during drying and storage. Heat and Mass Transfer 56(8), 2389–2396.

Hebar, A., Koller, C., Seifert, J.-M., Chabicovsky, M., Bodenteich, A., Bernkop-Schnürch, A., Grassauer, A., & Prieschl-Grassauer, E. (2015). Non-clinical safety evaluation of intranasal iota-carrageenan. Plos One 10(4), e0122911.

Hernández-Carmona, G. (2013). Conventional and alternative technologies for the extraction of algal polysaccharides. In H. Dominguez (Ed.), Functional Ingredients from Algae for Foods and Nutraceuticals (pp. 495–503). Woodhead Publishing.

Hilliou, L., Larotonda, F.D.S., Abreu, P., Ramos, A.M., Sereno, A.M., & Gonçalves, M.P. (2006). Effect of extraction parameters on the chemical structure and gel properties of κ/ι-hybrid carrageenans obtained from *Mastocarpus stellatus*. Biomolecular Engineering 23(4), 201–208.

Hoffmann, R.A., Gidley, M.J., Cooke, D., & Frith, W.J. (1995). Effect of isolation procedures on the molecular composition and physical properties of *Eucheuma cottonii* carrageenan. Food Hydrocolloids 9(4), 281–289.

Hotchkiss, S., Brooks, M., Campbell, R., Philp, K., & Trius, A. (2016). The use of carrageenan in food. In L. Pereira (Ed.), Carrageenans: Sources and Extraction Methods, Molecular Structure, Bioactive Properties and Health Effects. Nova Science Publications, New York.

Iglesias, H.A., & Chirife, J. (1978). Delayed crystallization of amorphous sucrose in humidified freeze dried model systems. International Journal of Food Science & Technology 13(2), 137–144.

Iglesias, O., & Bueno, J.L. (1999). Water agar-agar equilibrium: determination and correlation of sorption isotherms. International Journal of Food Science and Technology 34(3), 209–216.

Iijima, M., Hatakeyama, T., Takahashi, M., & Hatakeyama, H. (2007). Effect of thermal history on kappa-carrageenan hydrogelation by differential scanning calorimetry. Thermochimica Acta 452(1), 53–58.

Imeson, A.P. (2009). Carrageenan and furcellaran. In G.O. Phillips, & P.A. Williams (Eds.), Handbook of Hydrocolloids (2nd ed., pp. 164–185). Woodhead Publishing.

Inglezakis, V.J., Poulopoulos, S.G., & Kazemian, H. (2018). Insights into the S-shaped sorption isotherms and their dimensionless forms. Microporous and Mesoporous Materials 272, 166–176.

Ito, K., & Hori, K. (1989). Seaweed: Chemical composition and potential food uses. Food Reviews International 5(1), 101–144.

Jamali, A., Kouhila, M., Mohamed, L.A., Jaouhari, J.T., Idlimam, A., & Abdenouri, N. (2006). Sorption isotherms of *Chenopodium ambrosioides* leaves at three temperatures. Journal of Food Engineering 72, 77–84.

Jhider, N., & Bagané, M. (2019). Moisture sorption isotherms and isosteric heat of sorption of Tunisian clay product. Heat and Mass Transfer 55, 571–579.

Jouppila, K. & Roos, Y.H. (1994). Glass transitions and crystallization in milk powders. Journal of Dairy Science 77(10), 2907–2915.

Kaleemullah, S., & Kailappan, R. (2004). Moisture sorption isotherms of red chillies. Biosystems Engineering 88(1), 95–104.

Kaminska-Dworznicka, A., Janczewska-Dupczyk, A., Kot, A., Laba, S., & Samborska, K. (2020). The impact of iota- and kappa-carrageenan addition on freezing process and ice crystals structure of strawberry sorbet frozen by various methods. Journal of Food Science 85(1), 50–56.

Kapsalis, J.G. (1987). Influences of hysteresis and temperature on moisture sorption isotherms. In L.B. Rockland, & L.R. Beuchat (Eds.), Water Activity: Theory and Applications to Food (pp. 173–213). Marcel Dekker, New York.

Karlsson, A., & Singh, S.K. (1999). Acid hydrolysis of sulphated polysaccharides. Desulphation and the effect on molecular mass. Carbohydrate Polymers 38(1), 7–15.

Kent, M., Christiansen, K., van Haneghem, I.A., Holtz, E., Morley, M.J., Nesvadba, P., & Poulsen, K.P. (1984). Cost 90 collaborative measurements of thermal properties of foods. Journal of Food Engineering 3(2), 117–150.

Kersen, P., Paalme, T., Pajusalu, L., & Martin, G. (2017). Biotechnological applications of the red alga *Furcellaria lumbricalis* and its cultivation potential in the Baltic Sea. Botanica Marina 60(2), 207–218.

Kilinc, B., Cirik, S., Turan, G., Tekogul, H., & Koru, E. (2013). Seaweeds for food and industrial applications. In I. Muzzalupo (Ed.), Food Industry (pp. 735–748). InTech, Rijeka.

Klojdová, I., Troshchynska, Y., & Štětina, J. (2018). Influence of carrageenan on the preparation and stability of w/o/w double milk emulsions. International Dairy Journal 87, 54–59.

Knutsen, S.H., Myslabodski D.E., & Grasdalen, H. (1990). Characterization of carrageenan fractions from Norwegian *Furcellaria lumbricalis* (Huds.) Lamour. by ^{1}H-n.m.r. spectroscopy. Carbohydrate Research 206(2), 367–372.

Knutsen, S.H., Myslabodski, D.E., Larsen, B., & Usov, A.I. (1994). A modified system of nomenclature for red algal galactans. Botanica Marina 37(2), 163–169.

Kuhn, K.R., Picone, C.S.F., & Cunha, R.L. (2012). Food gels. In V.R.N. Telis (Ed.), Biopolymer Engineering in Food Processing (pp. 111–144). CRC Press, Florida.

Labuza, T.P., & Altunakar, L. (2007). Water activity prediction and moisture sorption isotherms. In G.V. Barbosa-Cánovas, A.J. Fontana Jr., S.J. Schmidt, & T.P. Labuza (Eds.), Water Activity in Foods: Fundamentals and Applications (pp. 109¬154). Blackwell Publishing.

Labuza, T.P., Tannenbaum, S.R., & Karel, M. (1970). Water content and stability of low-moisture and intermediate-moisture foods. Journal of Food Technology 24, 543–550.

Labuza, T.P., Acott, K., Tatini, S.R., Lee, R.Y., Flink, J., & McCall, W. (1976). Water activity determination: A collaborative study of different methods. Journal of Food Science 41, 910–917.

Labuza, T.P., Kaanane, A., & Chen, J.Y. (1985). Effect of temperature on the moisture sorption isotherms and water activity shift of two dehydrated foods. Food Science and Nutrition 50(2), 385–392.

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(3), 319–325.

Lai, V.M.F., Wong, P.A.L., & Lii, C.Y. (2000). Effects of cation properties on sol-gel transition and gel properties of κappa-carrageenan. Journal of Food Science 65(8), 1332–1337.

Lang, K.W., McCune, T.D., & Steinberg, M.P. (1981). A proximity equilibration cell for rapid determination of sorption isotherms. Journal of Food Science 46, 936-938.

Lasekan, O.O., & Lasekan, W.O. (2000). Moisture sorption and the degree of starch polymer degradation on flours of popped and malted sorghum (Sorghum bicolor). Journal of Cereal Science 31(1), 55–61.

Lemus, R.A., Pérez, M., Andrés, A., Roco, T., Tello, C.M., & Vega, A. (2008). Kinetic study of dehydration and desorption isotherms of red alga Gracilaria. LWT - Food Science and Technology 41(9), 1592-1599.

Liu, Z.X., Hiwatashi, N., Noguchi, M., & Toyota, T. (1997). Increased expression of costimulatory molecules on peripheral blood monocytes in patients with Crohn's disease. Scandinavian Journal of Gastroenterology 32(12), 1241–1246.

Liu, J.J., Zhan, X.D., Wan, J.B., Wang, Y.T., & Wang, C.M. (2015). Review for carrageenan-based pharmaceutical biomaterials: Favourable physical features versus adverse biological effects. Carbohydrate Polymers 121, 27–36.

Liu, Z., Gao, T., Yang, Y., Meng, F., Zhan, F., Jiang, Q., & Sun, X. (2019). Anti-cancer activity of porphyran and carrageenan from red seaweeds. Molecules 24(23), Article ID 4286.

MacArtain, P., Jacquier, J.C., & Dawson, K.A. (2003). Physical characteristics of calcium induced kappa-carrageenan networks. Carbohydrate Polymers 53(4), 395–400.

Madruga, L.Y.C., Sabino, R.M., Santos, E.C.G., Popat, K.C., Balaban, R.D., Kipper, M.J. (2020). Carboxymethyl-kappa-carrageenan: A study of biocompatibility, antioxidant and antibacterial activities. International Journal of Biological Macromolecules 152, 483–491.

Maier, H., Anderson, M., Karl, C., Magnuson, K., & Whistler, R.L. (1993). Guar, locust bean, tara, and fenugreek gums. In: R.L. Whistler, & J.N. BeMiller (Eds.), Industrial Gums (3rd ed., pp. 181–226). Academic Press, California.

Makshakova, O.N., Faizullin, D.A., & Zuev, Y.F. (2020). Interplay between secondary structure and ion binding upon thermoreversible gelation of kappa-carrageenan. Carbohydrate Polymers 227, Article ID 115342.

Mangin, C.M. (2000). Analysis of carrageenans using capillary electrophoresis: doctoral thesis. Department of Chemistry, University of York.

Mangione, M.R., Giacomazza, D., Bulone, D., Martorana, V., Cavallaro, G., & San Biagio, P.L. (2005). $K^+$and Na^+ effects on the gelation properties of κ-Carrageenan. Biophysical Chemistry 113(2), 129–135.

Marchetti, L., Andrés, S.C., & Califano, A.N. (2014). Low-fat meat sausages with fish oil: Optimization of milk proteins and carrageenan contents using response surface methodology. Meat Science 96(3), 1297–1303.

Masson, C.R. (1954). The degradation of carrageenin. I. Kinetics in aqueous solution at pH 7. Canadian Journal of Chemistry 3(4), 597-603.

McHugh, D.J. (2003). Carrageenan. In: A Guide to the Seaweed Industry. FAO Fisheries Technical Paper 441 (pp. 61–72). Food and Agriculture Organization of the United Nations: Rome, Italy.

McHugh, D.J. (1987). Production and utilization of products from commercial seaweeds. FAO Fisheries Technical Paper 288, 189 p.

McKim, J.M., Willoughby, J.A., Sr., Blakemore, W.R., & Weiner, M.L. (2018). Clarifying the confusion between poligeenan, degraded carrageenan, and carrageenan: A review of the chemistry, nomenclature, and invivo toxicology by the oral route. Critical Reviews in Food Science and Nutrition 59(19), 3054–3073.

Mehta, A.S., Mody, K.H., Iyer, A., & Ghosh, P.K. (2008). Preparation of semi-refined kappa-carrageenan: Recycling of alkali solution and recovery of alkali from spent liquor. Indian Journal of Chemical Technology 15(1), 45–52.

Miura, M., & Hiwatashi, N. (1987). Impaired monocyte macrophages function in patients with Crohn's disease. Journal of Clinical & Laboratory Immunology 24(4), 167–170.

Mokhtari, H., Kharaziha, M., Karimzadeh, F., & Tavakoli, S. (2019). An injectable mechanically robust hydrogel of kappa-carrageenan-dopamine functionalized graphene oxide for promoting cell growth. Carbohydrate Polymers 214, 234–249.

Montero, P., & Pérez-Mateos, M. (2002). Effects of Na^+, K^+ and Ca^{2+} on gels formed from fish mince containing a carrageenan or alginate. Food Hydrocolloids 16(4), 375–385.

Moreira, R., Chenlo, F., & Torres, M.D. (2009). Simplified algorithm for the prediction of water sorption isotherms of fruits, vegetables and legumes based upon chemical composition. Journal of Food Engineering 94(3–4), 334–343.

Moreira, R., Chenlo, F., Torres, M.D., & Prieto, D.M. (2010). Water adsorption and desorption isotherms of chestnut and wheat flours. Industrial Crops and Products 32(3), 252–257.

Mulet, A., Garcia-Reverter, J., Sanjuan, N. & Bon, J. (1999). Sorption isosteric heat determination by thermal analysis and sorption isotherms. Journal of Food Science 64(1), 64–68.

Mustapha, S., Chandar, H., Abidin, Z.Z., Saghravani, R., & Harun, M.Y. (2011). Production of semi-refined carrageenan from *Eucheuma cotonii*. Journal of Scientific & Industrial Research 70(10), 865–870.

Naeem, M., Idrees, M., Aftab, T., Alam, M.M., Khan, M.M.A., Uddin, M., & Varshney, L. (2015). Radiation processed carrageenan improves plant growth, physiological activities, and alkaloids production in *Catharanthus roseus* L. Advances in Botany 2015, Article ID 150474.

Naseri, A., Holdt, S.L., & Jacobsen, C. (2019). Biochemical and nutritional composition of industrial red seaweed used in carrageenan production. Journal of Aquatic Food Product Technology 28(9), 967–973.

Necas, J., & Bartosikova, L. (2013). Carrageenan: a review. Veterinarni Medicina 58(4), 187–205.

Nieto, T.V.N., Wang, Y., Ozimek, L., & Chen, L. (2016). Improved thermal gelation of oat protein with the formation of controlled phase-separated networks using dextrin and carrageenan polysaccharides. Food Research International 82, 95–103.

Noriega, M.D., Estrada, O., & López, I. (2014). Computational model to design plastic multi-layer films for food packaging to assure a shelf life at the best cost. Journal of Plastic Film & Sheeting 30(1), 48–76.

Ocieczek, A., & Ruszkowska, M. (2011). Kinetics and statics of water vapour of pudding powders as their storage stability factors. Milchwissenschaft-Milk Science International 66(4), 401–405.

Okos, M.R., Narsimhan, G., Singh, R.K., & Weitnauer, A.C. (1992). Food dehydration. In D.R. Heldman, & D.B. Lund (Eds.), Handbook of Food Engineering (pp. 437–562). Marcel Dekker, New York.

Ouyang, Q.-Q., Hu, Z., Li, S.-D., Quan, W.-Y., Wen, L.-L., Yang, Z.-M., & Li, P.-W. (2018). Thermal degradation of agar: Mechanism and toxicity of products. Food Chemistry 264, 277–283.

Pacheco-Quito, E.-M., Ruiz-Caro, R., & Veiga, M.-D. (2020). Carrageenan: Drug delivery systems and other biomedical applications. Marine Drugs 18(11), 583.

Parvar, M.B., Razavi, S.M.A., Tehrani, M.M., & Alipour, A. (2013). Optimization of functional properties of three stabilizers and kappa-carrageenan in ice cream and study of their synergism. Journal of Agricultural Science and Technology 15(4), 757–769.

Peleg, M. (1993). Assessment of a semi-empirical four parameter general model for sigmoid moisture sorption isotherms. Journal of Food Process Engineering 16, 21–37.

Peng, Q.J., & Perlin, A.S. (1987). Observations on N.M.R. spectra of starches in dimethyl sulfoxide, iodine-complexing, and solvation in water-di-methyl sulfoxide. Carbohydrate Research 160, 57–72.

Pereira, M.G., Benevides, N.M.B., Melo, M.R.S., Valente, A.P., Melo, F.R., & Mourão, P.A.S. (2005). Structure and anticoagulant activity of a sulfated galactan from the red alga, *Gelidium crinale*. Is there a specific structural requirement for the anticoagulant action? Carbohydrate Research 340(12), 2015–2023.

Pereira, L., Sousa, A., Coelho, H., Amado, A.M., & Ribeiro-Claro, P.J.A. (2003). Use of FTIR, FT-Raman and (13)GNMR spectroscopy for identification of some seaweed phycocolloids. Biomolecular Engineering 20(4–6), 223–228.

Philips Scientific Atomic Absorption Data Book (1988). Black Bear Press, Cambridge.

Pollatos, E.P., Riganakos, K.A., & Demertzis, P.G. (2013). Moisture sorption characteristics of Greek durum wheat semolina. Starch-Starke 65 (11–12), 1051–1060.

Popa, E.G., Carvalho, P.P., Dias, A.F., Santos, T.C., Santo, V.E., Marques, A.P., Viegas, C.A., Dias, I.R., Gomes, M.E., & Reis, R.L. (2014). Evaluation of the *in vitro* and *in vivo* biocompatibility of carrageenan-based hydrogels. Journal of Biomedical Materials Research. Part A 102(11), 4087–4097.

Prado-Fernandez, J., Rodriguez-Vazquez, J.A., Tojo, E., & Andrade, J.M. (2003). Quantitation of kappa-, iota- and lambda-carrageenans by mid-infrared spectroscopy and PLS regression. Analytica Chimica Acta 480(1), 23–37.

Prasetyaningrum, A., Ratnawati, R., & Jos, B. (2017). Kinetics of oxidative depolymerization of κ-carrageenan by ozone. Bulletin of Chemical Reaction Engineering & Catalysis 12(2), 235–242.

Prasetyaningrum, A., Utomo, D.P., Raemas, A.A., Kusworo, T.D., Jos, B., & Djaeni, M. (2021). Alginate/κappa-carrageenan-based edible films incorporated with clove essential oil: physico-chemical characterization and antioxidant-antimicrobial activity. Polymers 13(3), Article Number 354.

Price, W.J. (1979). Spectrochemical Analysis by Atomic Absorption. (pp. 287–357). Heyden & Son, London, Philadelphia, Rheine.

Quemener, B., & Lahaye, M. (1998). Comparative analysis of sulfated galactans from red algae by reductive hydrolysis and mild methanolysis coupled to two different HPLC techniques. Journal of Applied Phycology 10(1), 75–81.

Quemener, B., Marot, C., Mouillet, L., Da Riz, V., & Diris, J. (2000). Quantitative analysis of hydrocolloids in food systems by methanolysis coupled to reverse HPLC. Part 1. Gelling carrageenans. Food Hydrocolloids 14, 9–17.

Rahman, M.S. & Labuza, T.P. (2007). Water activity and food preservation. In M.S. Rahman (Ed.), Handbook of Food Preservation (2nd ed., pp. 447–476). CRC Press.

Rahman, M.S. (2009). Food stability beyond water activity and glass transtion: macro-micro region concept in the state diagram. International Journal of Food Properties 12(4), 726–740.

Rao, C.G. (2015). Water activity and shelf life. In Engineering for Storage of Fruits and Vegetables. Cold Storage, Controlled Atmosphere Storage, Modified Atmosphere Storage (pp. 699–728). Elsevier.

Reid, D.S. (2007). Water activity: fundamentals and relationships. In G.V. Barbosa-Cánovas, A.J. Fontana, Jr., S.J. Schmidt, & T.P. Labuza (Eds.), Water Activity in Food: Fundamentals and Applications (1st ed., pp. 15–28). Blackwell Publishing and IFT Press.

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(3), 403–410.

Rhein-Knudsen, N., Ale, M.T., & Meyer, A.S. (2015). Seaweed hydrocolloid production: An update on enzyme assisted extraction and modification technologies. Marine Drugs 13(6), 3340–3359.

Rhein-Knudsen, N., & Meyer, A.S. (2021). Chemistry, gelation, and enzymatic modification of seaweed food hydrocolloids. Trends in Food Science & Technology 109, 608–621.

Robal, M., Truus, K., Volobujeva, O., Mellikov, E., & Tuvikene R. (2017). Thermal stability of red algal galactans: Effect of molecular structure and counterions. International Journal of Biological Macromolecules 104, 213–223.

Rochas, C., & Rinaudo, M. (1980). Activity coefficients of counterions and conformation in kappa-carrageenan systems. Biopolymers 19(9), 1675–1687.

Saareleht, M. (2010). The introduction of producing method of furcellaran from Baltic Sea algae *Furcellaria lumbricalis* and comparision of product samples with flaky furcellaran: master's thesis. Department of Food Processing, Tallinn University of Technology.

Saha, D., & Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: a critical review. Journal of Food Science and Technology-Mysore 47(6), 587–597.

Sahin, S., & Sumnu, S.G. (2006). Physical properties of foods. New York: Springer.

Saluri, M., Robal, M., & Tuvikene, R. (2019). Hybrid carrageenans as beer wort fining agents. Food Hydrocolloids 86, 26–33.

Saluri, M., Kaljuvee, K.-L., Paalme, T., Reile, I., & Tuvikene, R. (2021). Structural variability and rheological properties of furcellaran. Food Hydrocolloids 111, 106227.

Sánchez-Sánchez, M.-P., Martín-Illana, A., Ruiz-Caro, R., Bermejo, P., Abad, M.-J., Carro, R., Bedoya, L.-M., Tamayo, A., & Rubio, J., Fernández-Ferreiro, A., et al. (2015). Chitosan and kappa-carrageenan vaginal acyclovir formulations for prevention of genital herpes. In vitro and ex vivo evaluation. Marine Drugs 13(9), 5976–5992.

Santalla, E.M., & Mascheroni, R.H. (2003). Equilibrium moisture characteristics of high oleic sunflower seeds and kernels. Drying Technology 21(1), 147–163.

Saravacos, G.D., Tsiourvas, D.A., & Tsami, E. (1986). Effect of temperature on the water adsorption isotherms of sultana raisins. Journal of Food Science 51(2), 381–383.

Seth, D., Dash, K.K., Mishra, H.N., & Deka, S.C. (2018). Thermodynamics of sorption isotherms and storage stability of spray dried sweetened yoghurt powder. Journal of Food Science and Technology 55(10), 4139–4147.

Shanthilal, J., & Bhattacharya, S. (2015). The process of gelling. In S. Bhattacharya (Ed.), Conventional and Advanced Food Processing Technologies (pp. 99–114). Wiley Blackwell.

Singh, S.K., & Jacobsson, S.P. (1994). Kinetics of acid hydrolysis of κ -carrageenan as determined by molecular weight (SEC-MALLSRI), gel breaking strength, and viscosity measurements. Carbohydrate Polymers 23, 89–103.

Smith, S.E. (1947). Sorption of wheat vapour by high polymers. Journal of the American Chemical Society 69, 646–651.

Sormoli, M.E., & Langrish, T.A.G. (2015). Moisture sorption isotherms and net isosteric heat of sorption for spray-dried pure orange juice powder. LWT – Food Science and Technology 62(1), 875–882.

Souza, H.K.S., Hilliou, L., Bastos, M., & Goncalves, M.P. (2011). Effect of molecular weight and chemical structure on thermal and rheological properties of gelling kappa/iota-hybrid carrageenan solutions. Carbohydrate Polymers 85(2), 429–438.

Souza, M.P., Vaz, A.F.M., Costa, T.B., Cerqueira, M.A., De Castro, C.M.M.B., Vicente, A.A., & Carneiro-da-Cunha, M.G. (2018). Construction of a biocompatible and antioxidant multilayer coating by layer-by-layer assembly of κappa-carrageenan and quercetin nanoparticles. Food and Bioprocess Technology 11(5), 1050–1060.

Staudt, P.B., Kechinski, C.F., Tessaro, I.C., Marczak, L.D.F., Soares, R.D., & Cardozo, N.S.M. (2013). A new method for predicting sorption isotherms at different temperatures using the BET model. Journal of Food Engineering 114(1), 139–145.

Stepien, A., Witczak, M., & Witczak, T. (2020). Moisture sorption characteristics of food powders containing freeze dried avocado, maltodextrin and inulin. International Journal of Biological Macromolecules 149, 256–261.

Sun, T., Tao, H.N., Xie, J., Zhang, S.O., & Xu, X. (2010). Degradation and antioxidant activity of κappa-carrageenans. Journal of Applied Polymer Science 117(1), 194–199.

Suurkuusk, J., & Wadsö, I. (1982). A multichannel microcalorimetry system. Chemica Scripta 20(4), 155–163.

Swami, S.B., Das, S.K., & Maiti, B. (2005). Moisture sorption isotherms of black gram nuggets (bori) at varied temperatures. Journal of Food Engineering 67(4), 477–482.

Tadapaneni, R.K., Yang, R., Carter, B., & Tang, J.M. (2017). A new method to determine the water activity and the net isosteric heats of sorption for low moisture foods at elevated temperatures. Food Research International 102, 203–212.

Tavares, L., & Noreña, C.P.Z. (2021). Characterization of the physicochemical, structural and thermodynamic properties of encapsulated garlic extract in multilayer wall materials. Powder Technology 378(A), 388–399.

Thanh, T.T.T., Yuguchi, Y., Mimura, M., Yasunaga, H., Takano, R., Urakawa, H., & Kajiwara, K. (2010). Structure and gelling properties of carrageenan family studied by scattering techniques. Asian Journal of Chemistry 22(5), 3989–4002.

Thompson, T.L., Peart, R.M., & Foster, G.H. (1968). Mathematical simulation of corn drying – a new model. Transactions of the American Society of Agricultural Engineers 24, 582–586.

Tobacman, J.K. (2001). Review of harmful gastrointestinal effects of carrageenan in animal experiments. Environmental Health Perspectives 109(10), 983–994.

Torres, M.D., Chenlo, F., & Moreira, R. (2018). Structural features and water sorption isotherms of carrageenans: A prediction model for hybrid carrageenans. Carbohydrate Polymers 180, 72–80.

Truus, K., Tuvikene, R., Vaher, M., Kailas, T., Toomik, P., & Pehk, T. (2006). Structural and compositional characteristics of gelling galactan from the red alga *Ahnfeltia tobuchiensis* (Ahnfeltiales, the Sea of Japan). Carbohydrate Polymers 63(1), 130–135.

Tsami, E. (1991). Net isosteric heat of sorption in dried fruits. Journal of Food Engineering 14, 327–335.

Tuvikene, R., Truus, K., Vaher, M., Kailas, T., Martin, G., & Kersen, P. (2006). Extraction and quantification of hybrid carrageenans from the biomass of the red algae Furcellaria lumbricalis and Coccotylus truncatus. Proceedings of the Estonian Academy of Sciences. Chemistry 55(1), 40–53.

Tuvikene, R., Truus, K., Robal, M., Pehk, T., Kailas, T., Vaher, M., & Paalme, T. (2009). Structure and thermal stability of pyruvated carrageenans from the red alga Coccotylus truncatus. Carbohydrate Research 344(6), 788–794.

Tuvikene, R., Truus, K., Robal, M., Volobujeva, O., Mellikov, E., Pehk, T., Kollist, A., Kailas, T., & Vaher, M. (2010). The extraction, structure and gelling properties of hybrid galactan from the red alga *Furcellaria lumbricalis* (Baltic Sea, Estonia). Journal of Applied Phycology 22(1), 51−63.

Uno, Y., Omoto, T., Goto, Y., Asai, I., Nakamura, M., & Maitani, T. (2001). Molecular weight distribution of carrageenans studied by a combined gel permeation/inductively coupled plasma (GPC/ICP) method. Food Additives and Contaminants 18(9), 763–772.

Usov, A.I., & Shashkov, A.S. (1985). Polysaccharides of algae. XXXIV: detection of iota-carrageenan in *Phyllophora-brodiaei* (turn.) J. Ag. (Rhodophyta) using 13C-NMR spectroscopy. Botanica Marina 28, 367–373.

Usov, A.I. (1992). Sulfated polysaccharides of the red seaweeds. Food Hydrocolloids 6(1), 9–23.

Valderrama, D., Cai, J., Hishamunda, N., & Ridler, N. (2013). Social and economic dimensions of carrageenan seaweed farming. FAO Fisheries and Aquaculture Technical Paper No. 580. Food and Agriculture Organization of the United Nations: Rome, Italy.

van der Berg, C., & Bruin, S. (1981). Water activity and its estimation in food systems; theoretical aspects. In L.B. Rockland, & G.F. Stewart (Eds.), Water Activity: Influences on Food Quality (pp. 1–61). Academic Press, New York.

van de Velde, F., Knutsen, S.H., Usov, A.I., Rollema, H.S., & Cerezo, A.S. (2002). 1H and 13C high resolution NMR spectroscopy of carrageenans: Application in research and industry. Trends in Food Science and Technology 13(3), 73–92.

van de Velde, F. (2008). Structure and function of hybrid carrageenans. Food Hydrocolloids 22(5), 727–734.

Varadarajan, S.A., Ramli, N., Ariff, A., Said, M., & Yasir, S.M. (2009) Development of high yielding carragenan extraction method from *Eucheuma cotonii* using cellulase and Aspergillus niger. Presiding Seminar Kimia Bersama UKM-ITB VIII, 461–469.

Vázquez-Delfín, E.; Robledo, D.; Freile-Pelegrín, Y. (2014). Microwave-assisted extraction of the carrageenan from Hypnea musciformis (Cystocloniaceae, Rhodophyta). Journal of Applied Phycology 26(2), 901–907.

Viitak, A., & Treumann, M. (1996). Electrochemical AAS for the determination of arsenic and selenium in hair. Proceedings of the Estonian Academy of Sciences. Chemistry 45 (3/4), 91–96.

Wang, F.F., Yao, Z., Wu, H.G., Zhang, S.X., Zhu, N.N., & Gai, X. (2011). Antibacterial activities of kappa-carrageenan oligosaccharides. Applied Mechanics and Materials 108, 194–199.

Watase, M., & Nishinari, K. (1981). Effect of alkali metal ions on the rheological properties of κ-carrageenan and agarose gels. Journal of Texture Studies 12(4), 427–445.

Watt, J., & Marcus, R. (1969). Ulcerative colitis in the guinea-pig caused by seaweed extract. Journal of Pharmacy and Pharmacology 21, 187S–188S.

Weisser, H. (1985). Influence of temperature on sorption equilibria. In D. Simatos, & J.L. Multon (Eds.), Properties of Water in Foods in Relation to Quality and Stability (pp. 95–118). Martinus Nijhoff Publishers, Dordrecht.

Wembabazi, E., Mugisha, P.J., Ratibu, A., Wendiro, D., Kyambadde, J., & Vuzi, P.C. (2015). Spectroscopic analysis of heterogeneous biocatalysts for biodiesel production from expired sunflower cooking oil. Journal of Spectroscopy 2015, Article ID 714396.

Widjanarko, S.B., Amalia, Q., Hermanto, M. B., et al. (2018). Evaluation of the effect of yellow konjac flour-kappa-carrageenan mixed gels and red koji rice extracts on the properties of restructured meat using response surface methodology. Journal of Food Science and Technology-Mysore 55(5), 1781–1788.

Wu, P., & Imai, M. (2012). Novel biopolymer composite membrane involved with selective mass transfer and excellent water permeability. In R.Y. Ning (Ed.), Advancing Desalination. IntechOpen.

Yang, B., Yu, G.L., Zhao, X., Jiao, G.L., Ren, S.M., & Chai, W.G. (2009). Mechanism of mild acid hydrolysis of galactan polysaccharides with highly ordered disaccharide repeats leading to a complete series of exclusively odd-numbered oligosaccharides. FEBS Journal 276(7), 2125–2137.

Yang, B., Yu, G.L., Zhao, X., Ren, W.N., Jiao, G.L., Fang, L.H., Wang, Y.H., Du, G.H., Tiller, C., & Girouard, G. (2011). Structural characterisation and bioactivities of hybrid carrageenan – like sulphated galactan from red alga *Furcelaria lumbricalis*. Food Chemistry 124(1), 50–57.

Yang, Z., Zhu, E., & Zhu, Z. (2015). Water desorption isotherm and drying characteristics of green soybean. Journal of Stored Products Research 60, 25–30.

You, S.G., Yang, C., Lee, H.Y., & Lee, B.-Y. (2010). Molecular characteristics of partially hydrolyzed fucoidans from sporophyll of Undaria Pinnatifida and their in vitro anticancer activity. Food Chemistry 119(2), 554–559.

Yousef, A.Y., & Balasubramaniam, V.M. (2013). Physical methods of food preservation. In M.P. Doyle, & R.L. Buchanan (Eds.), Food Microbiology – Fundamentals and Frontiers (4th ed., pp. 737–763). American Society for Microbiology Press, Washington.

Yousefi, M., & Jafari, S.M. (2019). Recent advances in application of different hydrocolloids in dairy products to improve their techno-functional properties. Trends in Food Science & Technology 88, 468–483.

Youssouf, L., Lallemand, L., Giraud, P., Soulé, F., Bhaw-Luximon, A., Meilhac, O., D'Hellencourt, C.L., Jhurry, D., & Couprie, J. (2017). Ultrasound-assisted extraction and structural characterization by NMR of alginates and carrageenans from seaweeds. Carbohydrate Polymers 166, 55–63.

Yu, H.C., Zhang, He., Ren, K.F, Ying, Z.M., Zhu, F.B., Qian, J., Ji, J., Wu, Z.L., Zheng, Q. (2018). Ultrathin κappa-carrageenan/chitosan hydrogel films with high toughness and antiadhesion property. ACS Applied Materials & Interfaces 10(10), 9002–9009.

Yuan, H.M., Song, J.M., Li, X.G., Li, N., & Liu, S. (2011). Enhanced immunostimulatory and antitumor activity of different derivatives of kappa-carrageenan oligosaccharides from Kappaphycus striatum. Journal of Applied Phycology 23(1), 59–65.

Yuguchi, Y., Thuy, T.T.T., Urakawa, H., & Kajiwara, K. (2002). Structural characteristics of carrageenan gels: temperature and concentration dependence. Food Hydrocolloids 16(6), 515–522.

Zainal-Abidin, M.H., Hayyan, M., Hayyan, A., & Jayakumar, N.S. (2017). New horizons in the extraction of bioactive compounds using deep eutectic solvents: A review. Analytica Chimica Acta 979, 1–23.

Zamora, R., Gómez, G., & Hidalgo, F.J. (2002). Classification of vegetable oils by high-resolution 13C NMR spectroscopy using chromatographically obtained oil fractions. Journal of the American Oil Chemists Society 79(3), 267–272.

Zapata, M.J.E., Quintero, C.O.A., & Porras, B.L.D. (2014). Sorption isotherms for oat flakes (Avena sativa L.). Agronomía Colombiana 32(1), 52–58.

Zhang, W., Piculell, L., & Nilsson, S. (1991). Salt dependence and ion specificity of the coil helix transition of furcellaran. Biopolymers 31(14), 1727–1736.

Zhu, M.J., Ge, L.M., Lyu, Y.B., Zi, Y.X., Li, X.Y., Li, D.F., & Mu, C.D. (2017). Preparation, characterization and antibacterial activity of oxidized κappa-carrageenan. Carbohydrate Polymers 174, 1051–1058.

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Abstract

Impact of short-term heat treatment on the structure and functional properties of carrageenans

This PhD research involves the study of the functional properties of commercial carrageenan products, including the furcellaran produced in Estonia, and their changes during heat treatment. It was determined that some batches of the stored drum-dried furcellaran powder showed an exothermic heat flow resulting in a local self-browning and limited burning. In general, the pronounced exothermic behaviour can be attributed to the onset of pyrolytic degradation, which can affect the final product properties. So far, few studies have focused on the thermal stability of carrageenans. A better understanding of the structural and functional changes in carrageenans after thermal processing at elevated temperatures is expected to lead ultimately to better process control and higher final product quality, valorisation and acceptance.

The objective of this study was to investigate the effect of short-term heat treatment on the structure and functional properties of furcellaran compared with other carrageenans using NMR, SEC and rheology. It was found that drying at 115 °C for 15 minutes had no influence on the structures of carrageenans, except for furcellaran, where degradation of the polysaccharide and desulphation of the galactans occurred. Heat treatment decreased the molecular weight of carrageenans and the degradation accelerated at higher temperatures. Furcellaran had the lowest molecular weight compared with the other carrageenans as it had passed through a previous severe repeated heat treatment in drum-drying during processing. It showed more sensitivity to heat, as drying above 115 °C caused the loss of viscosity and gelling ability, as the average molecular weight fell below the minimum value required for gelation. The viscosity and hardness of the other studied carrageenan gels were not dependent on the drying temperature. The storage modulus of carrageenan gels, as well as gelling and melting temperatures, decreased with an increase in heat treatment temperature, preventing a coil-helix transition. The exothermic heat flow caused by the water absorption in media with different water activities was also investigated, using an isothermal microcalorimeter. Experiments revealed the structural transformation and following water interaction with samples, which must be stored with limited water access. In order to improve the quality of carrageenans, the use of high temperatures in production must be avoided in order to prevent the degradation of carrageenans and the loss of functional properties.

Lühikokkuvõte

Lühiajalise termilise töötluse mõju karragenaanide struktuurile ja funktsionaalsetele omadustele

Karragenaanid on punavetika polüsahhariidid, mida kasutatakse toiduainete ja farmaatsiatööstuses geelistavate, stabiliseerivate ja emulgeerivate ainetena. Eesti vetes asuvad looduslikud punavetika *Furcellaria lumbricalise* varud, millest Saaremaal asuv vetikatööstus toodab juba üle 60 aasta furtsellaraani – karragenaanide alaliiki kuuluvat toodet. Tootmistehnoloogias kasutatakse furtsellaraani eraldamiseks vesiekstraktsiooni ning kuivatamine toimub trummelvaltsidel. Saadav helbeline toode on unikaalsete omadustega ning eriti sobiv kasutamiseks marmelaadis, sefiiris, “linnupiimas“ ja teistes sarnastes maiustustes.

Siiski on lõpptoote kvaliteediga probleeme – täheldatud on partiidevahelisi kvaliteedi kõikumisi, säilimisel geelitugevuse langust ning mõnede partiide puhul ka tumenemist ja söestumist. Üheks probleemide hüpoteesiks on, et karragenaanid võivad tootmises kasutatavatel kõrgetel temperatuuridel laguneda, tekivad laguproduktid ning toote omadused muutuvad. Termiline lagunemine on seotud eksotermilise käitumisega, mis võib põhjustada iseeneslikku kuumenemist ja isegi süttimist. Seni on vähesed uuringud keskendunud karragenaanide termilisele stabiilsusele. Karragenaanide struktuursete ja funktsionaalsete muutuste tundmine pärast termilist töötlemist võimaldab tootmisprotsessi paremini juhtida ning tagada kvaliteetne lõpptoode.

Käesoleva doktoritöö eesmärk oli uurida furtsellaraani funktsionaalseid omadusi ja nende muutust lühiajalise kuumtöötluse järel kasutades NMR, SEC ja reoloogiat ning võrrelda saadud tulemusi kaubanduslike karragenaanidega. Kuumtöötlus 115 °C juures 15 minutit põhjustas furtsellaraani lagunemise ja desulfatatsiooni, karragenaanide struktuurides mingisugust muutust ei täheldatud. Kuumtöötlus vähendas karragenaanide molekulmassi, kusjuures lagunemine kiirenes kõrgematel temperatuuridel. Furtsellaraanil oli teiste karragenaanidega võrreldes tunduvalt madalam molekulmass ilmselt seetõttu, et toode oli juba tootmise käigus läbinud eelneva korduva kuumtöötluse trummelvaltsidel. Samuti oli furtsellaraan kõige temperatuuritundlikum, kuumtöötlus üle 115 °C põhjustas viskoossuse ja geelistumisvõime täieliku kadumise. Teiste karragenaanide geelide viskoossused ja tugevused ei sõltunud uuritud temperatuuridest. Karragenaanide geelide elastsusmoodul, samuti geelistumis- ja sulamistemperatuurid vähenesid kuumtöötlustemperatuuri tõusuga, mis osutab ka antud näitajate temperatuursõltuvusele.

Töös uuriti ka töötlemata ja termiliselt töödeldud proovide soojusvooge, kasutades isotermilist mikrokalorimeetrit. Soojusvood oli põhjustatud vee sorptsioonist tootesse erinevate vee aktiivsustega keskkondadest. Katsete tulemused näitasid proovide töötlustemperatuuri ja keskkonna vee aktiivsuse vahelist ühest seost – mida kõrgem töötlustemperatuur ja keskkonna vee aktiivsus, seda suurem on eralduv soojusvoog. Seega võib oletada, et kuumtöötlus destabiliseerib karragenaane, avades veele enam sidumiskohti. See soodustab veeauru imendumist tootesse ja põhjustab suuremat soojuse eraldumist isegi mõõdukatel säilitustingimustel. Lõpptoote kvaliteedi parandamiseks tuleb tootmises vältida kõrgete temperatuuride kasutamist, et ära hoida karragenaanide lagunemine ja funktsionaalsete omaduste kadu.

Appendix 1

Publication I

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Effects of drying on the gel strength and cation mobility of furcellaran

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Abstract

Thermal stability, by means of air drying a furcellaran powder, and its impact on gel strength and cation mobility were studied. Halogen heating in the temperature range 90–115°C for 15 min resulted in loss on drying (L_D, %). These results can be described by polynom $L_D = -9.583 + 2.989\tau - 0.249\tau^2 + 0.00729\tau^3 + 0.1034t$ ($R^2 = 0.9976$), indicating a gradual decomposition of carbohydrates. Air-drying induced a decrease in gel strength and the partial removal of potassium, calcium and sodium ions from the matrix. Air drying above 115°C yielded a remarkable destruction of polysaccharides with a total collapse in gelling power.

Keywords: Furcellaran; Drying; Gelation; Conductivity

1. Introduction

Carrageenans, particularly kappa- and ioota-carrageenans are two of the most important gel-forming polysaccharides (Painter, 1983). Despite the similarity of their molecular structures, the gels have very different properties (Smewing, 1999). On the basis of these properties, they are used extensively in the food and other industries as thickening-gelling agents or texture enhancers, stabilisers, etc. (Thomas, 1997; Nussinovitch, 1997). Furcellaran is currently considered to be a type of κ-carrageenan (Glicksman, 1983).

Gelling galactans of *Furcellaria lumbricalis* and *Coccatylus truncatus* from the red algae stratum in Kassari Bay (the Baltic Sea, Estonia) have been reviewed by Truus et al. (Truus, Vaher, Kukk, Pehk & Kollist, 1996). It was summarised that the galactans have a structure of partially sulphated κ-carrageenan mainly with a small inclusion of ι-carrageenan units. Later, from gas–liquid chromatography analyses, it was shown that the composition of polysaccharides from *F. lumbricalis* appears to be much more complicated (Truus, Vaher, Usov, Pehk & Kollist, 1997) and certainly, no iota fragments are present in this polysaccharide. Obviously, the source of ι-carrageenan patterns in mixed galactans can only be the species *C. truncatus* (Vaher, Truus, Taure, Leito, Lahe, Kallavus & Pehk, 1998). The versatility of primary structure of carrageenans has been confirmed by modern structural analysis (Usov, 1998).

A commercial gelling agent based on the sum of red algae stratum of *F. lumbricalis* and *C. truncatus* is a tasteless odourless bright yellow flaky powder with a broad application in various marmalade and pastry products. In addition, the producer reported that the baled seaweed collected from the different suppliers

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also contained minor variable amounts of contaminant species, catalogued as *Fucus vesiculosus*, *Polisiphonia nigrercens*, etc. The technological impact on physicochemical properties (particularly the influence of the drying procedure, in our case) of manufactured furcellaran will be of crucial importance (Friedenthal, Sirendi, Vokk & Köster, 1999). Any single or recurrent drying step aimed at the diminishing of residual moisture in the product needs secure process settings. Surprisingly for us, there are no published studies on thermal drying vs. gelling properties of processed furcellarans. Only available data are based on results of Research Report No 204 (1994) in which the gel strength of alcohol precipitated furcellaran was investigated in drying chamber. It was found that the product survives short time heat treatment (15 min at 130°C). Above this temperature, a remarkable decrease in gel strength was observed. The thermal degradation of food carrageenans has also been elucidated in water solutions (Bradley & Mitchell, 1988; Lai, Lii, Hung & Lu, 2000).

Regardless of the fact that drum drying is very seldom used in industrial gums production nowadays (Therkelsen, 1993), its proper operation will still have a purpose, particularly for a small producer. Accordingly, the purpose of present study was to investigate the thermal stability of a commercial furcellaran by air drying procedure in its relation to gel strength and cation mobility.

2. Materials and methods

2.1. Furcellaran

Commercial furcellaran was supplied by AS EstAgar (Kärla, Saare County, Estonia). The product has been made by hot water extraction and subsequent drum drying followed by bleaching, standardising, final drying and grinding. The manufacturer reports that furcellaran contains $\leq 20\%$ and $\leq 16\%$ of cold water–insoluble matter and inorganic salts in dry matter, respectively. Loss on drying has been expressed by moisture content ($\leq 18\%$) and gel strength by Valenti (1500 g) in gel with 1.25% of furcellaran in dry matter and 70% sucrose.

2.2. Drying

Thermal stability of furcellaran was investigated by a uniform air-drying procedure carried out on Halogen Moisture Analyser HR 73 (Mettler-Toledo AG, Switzerland). The temperature range was 90–130°C with a drying duration of 15 min. The sample was heated up within 15 s to the drying temperature and held constant at this temperature. The instrument measured loss on drying with the thermogravimetric principle, i.e. loss on drying is expressed by apparent moisture determined from the weight loss of a sample dried by halogen heating. Every drying was duplicated.

2.3. Measurements of cations

The concentrations of Ca, Mg, K and Na in furcellaran were determined by flame atomic-absorption spectrometry (FAAS) and emission spectrometry (EAAS), respectively (SP9-700 Pye Unicam AA Spectrometer, Cambridge, UK). The dried sample (0.2–0.6 g) was mineralised by concentrated nitric acid and hydrogen peroxide (5:1) on a hot plate at 140°C for 6–8 h, as described by Viitak and Treumann (1996). The mineralised sample was cooled and diluted up to 100 ml with bidistilled water. The conventional parameters of acetylene–air FAAS and EAAS were applied (Price, 1979; Philips Scientific Atomic Absorption Data Book, 1988).

2.4. Measurements of conductivity

Conductivity measurements have been carried out with a Hand-Held Conductivity Meter model handylab LF 1 (Schott Glaswerke, Germany). A weighed sample (1.0 g) was suspended in 100 ml of bidistilled water at 25°C under intensive stirring for 5 min. Thereafter, the solution was filtered through a glass filter with a pore size of 160 μm and conductivity in filtrate was registered at 25°C. A minimum of three measurements were performed for each duplicated samples. Mean values were used for obtaining the graphs.

2.5. Preparation of gel

Gel strength measurements in our experiments have been carried out in gels without sugar. Sols were prepared by dispersing 2.5 g of furcellaran in dry matter in 100 ml of bidistilled water and left to swell for 1 h. After that, the mixture was heated up to 85°C and, maintaining this temperature, the mixture was intensively shaken for 10 min. After stirring and heating, the evaporated part was replaced up to 100 g by water. Gels were prepared by pouring the hot sols into suitable moulds with vertical standing cylindrical tubes inside. The samples were allowed to cool in refrigerator at +4°C before textural measurement.

2.6. Textural measurements

The tubes with gelled estagar were equilibrated at room temperature (22 ± 2°C) for 2 h. Gel plugs were gently removed from their tubes with blowing. The gel plugs were cut in 20-mm lengths with a knife to give straight cut for both ends. Each tube has cylindrical specimen with a uniform geometry (28 mm inside di-

ameter × 20 mm length) after the unusable portions were removed from both ends. The gelling test was performed with texture analyser TA-XT2I (Stable Micro Systems, Surrey, UK). The gel specimen was placed upright in the centre of the Valenti probe and subjected to force until it failed. Probe speeds of 2.0 mm/s for the pre-test, 0.2 mm/s for the test and 2.0 mm/s for the post-test were applied. Minimum quadruplicate measurements for each testing furcellaran sample were made.

3. Results and discussions

The typical drying curves of furcellaran are shown in Fig. 1. It is obvious that the drying temperature range of 100–130°C for 15 min proceeds by at least two different dynamics. The first, drying up to 115°C, results in a smooth non-linear curve, while drying over 115°C leads to a remarkable change of shape of the curve. It is also evident that the curves registered at 90, 100 and 110°C will result in different values of loss on drying (13.1%, 14.0% and 14.8%, respectively). The gradual increase in weight loss is probably caused by the decomposition of carbohydrates (Bradley, 1998).

The mathematical evaluation of the curves indicated that loss on drying of furcellaran (L_D, %), at temperatures below 115°C, is well prescribed by the polynom:

$$L_D = a + b\tau + c\tau^2 + d\tau^3 + et \quad (1)$$

where:

τ — time of drying (min);
t — temperature of drying (°C); and
a, b, c, d, e — empirical coefficients.

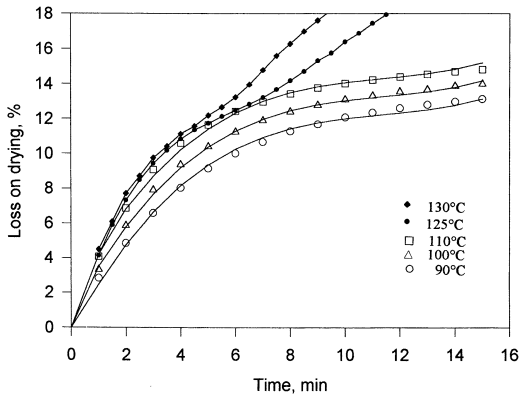

Fig. 1. Loss on drying as a function of time at temperatures of drying 90–130°C (symbols — experimental data; steady line with opened symbols — calculated curve).

By applying multiregression analysis, the calculated values L_D correlated well with experimental data ($R^2 = 0.9976$) when the following coefficients were used:

$a = -9.583$; $b = 2.989$; $c = -0.249$;
$d = 0.00729$; $e = 0.1034$;
S.D. = 0.229.

For the evaluation of heating of furcellaran on gel strength (G), the corresponding gels were made with a sample of furcellaran, dried for 5 and 15 min, respectively. In experiments with the 5-min processed furcellaran, the weight of samples was the same in all trials (2.8736 g) and the relative gel strength (G^{Rel}, %) was expressed as follows:

$$G^{Rel} = (G \times 13/G_o \times L_D) \times 100 \quad (2)$$

where

G — experimental gel strength (g);
G_o — gel strength obtained with the sample without subsequent drying (g);
L_D — experimental loss on drying (%); and
13 — the reference loss on drying (%).

In experiments with the 15-min processed furcellaran, the sample amounted to 2.5 g without the subsequent re-expression of gel strength upon presumption that all moisture had been removed (loss on drying > 13%) resulting in 100% of the total solid content. The corresponding relationships (Fig. 2) indicate that air-drying exerts an influence on gel strength under the experimental conditions allowing to linear regression. Upon the formation of a gel structure on cooling, random molecules transform into a helical structure, and the helices align side-by-side and form junction zones (Tanaka, Hatakeyama, Hatakeyama & Philipps, 1996). Drying above 90°C probably induces some conformational changes or defects in the ordered structure of polysaccharide molecules and it is difficult for a well-ordered junction zone to be formed.

From the atomic absorption measurements, the concentrations of Ca, Mg, K and Na in the air-dried furcellaran, in comparison with the untreated sample, were determined (Table 1). There is an obvious distinct decrease in Ca, K and Na contents bound to the matrix of polysaccharides, whereas the Mg concentration stays unchanged. Surprisingly, a reasonably high Ca content (17.5 mg/g) was monitored, as compared with a low K content (11.4 mg/g) and that of furcellaran (Truus et al., 1997) extracted from the algae (in 0.1 M KOH). This phenomenon is probably affected by the technological peculiarities of the manufacturing of furcellaran. It is assumed that the decrease in positively charged counterions is related to the weakening of intramolecular bonds. K^+ is known to be a structure-

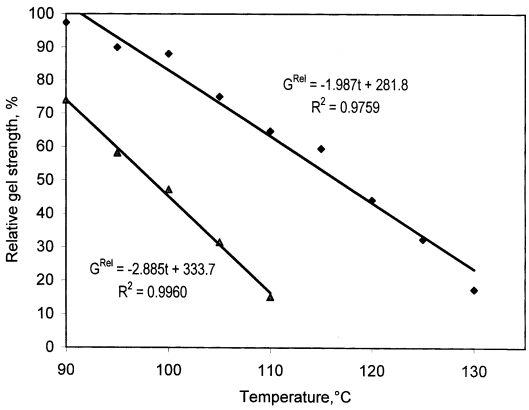

Fig. 2. Relative gel strength as a function of temperature of drying after 5 min (♦) and 15 min (△) processing.

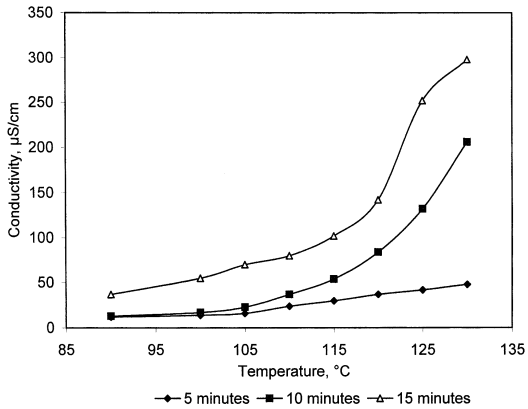

Fig. 3. Conductivity in filtrate after immersion of furcellaran in water depending on preliminary drying.

disordering ion (Watase & Nishinari, 1981). On the other hand, it has been proven that the thermal stability of guar gum can be substantially extended by crosslinking with transitional metal ions (Maier, Anderson, Karl, Magnuson & Whistler, 1993). Consequently, the ion binding to the polysaccharide matrix is liable to affect the structural features and processing conditions. By measuring conductivity in filtrate after suspending the sample in bidistilled water, the increase in conductance depending on applied head treatment can be easily monitored (Fig. 3). The corresponding values varied from 0.3 and 12 μS/cm up to 298 μS/cm for bidistilled water, commercial furcellaran and air-dried samples, respectively. Nevertheless, there is no evident explanation for the decrease in Ca, K and Na content in furcellaran in comparison with Mg due to the air drying procedure. Obviously, more investigations are needed to examine the furcellaran in its relation to thermal stability and degradation variables with particular interest on transition ions in this process. The thermal behaviour of polysaccharides will be of great importance in the assessment of technological renewals.

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Table 1
Ca, Mg, K and Na content in initial and air-dried[a] samples of furcellaran

	Initial mg/kg	90°C mg/kg	95°C mg/kg	100°C mg/kg	105°C mg/kg	110°C mg/kg	115°C mg/kg
Calcium							
Mean value	17526	16764	16460	16417	16228	14681	13692
S.D.	282	899	964	289	787	846	439
Magnesium							
Mean value	4568	4549	4534	4546	4501	4505	4441
S.D.	41.4	176	526	393	606	507	225
Potassium							
Mean value	11357	10497	10561	9858	9887	9189	9413
S.D.	34.9	102	176	37.2	51.4	1.14	58.6
Sodium							
Mean value	179	102	98.1	95.4	88.8	87.3	87.9
S.D.	21.4	44.9	16.7	22.3	11.4	12.9	22.9

[a] Drying time = 15 min.

References

Bradley, R. L. (1998). Moisture and total solid analysis. In: S. S. Nielsen, *Food Analysis* (pp. 119–139). Second edition. Gaithersburg, Maryland: Aspen Publishers, Inc.

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.

Friedenthal, M., Sirendi, M., Vokk, R., & Köster, M. (1999). High definition gelling agent from the local seaweed. NJF XXI Kongres, As, 28 Juni–1 Juli 1999. In Kongres Rapport Nordisk Jordbrugsforskning, Nr 2 argang 81, 180.

Glicksman, M. (1983). *Food Hydrocolloids*, 2 (pp. 73–113). Boca Raton, Florida: CRC Press, Inc.

Lai, 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.

Maier, H., Anderson, M., Karl, C., Magnuson, K., Whistler, R.L. (1993). In: R. L. Whistler, & J. N. BeMiller, *Industrial Gums. Polysaccharides and Their Derivatives* (pp. 181–226). Third Edition. San Diego, New York, Boston, London, Sydney, Tokyo, Toronto: Academic Press.

Nussinovitch, A. (1997). Hydrocolloid Applications. *Gum Technology in the Food and Other Industries* (pp. 40–62). London, Weinheim, New York, Tokyo, Melbourne, Madras: Blackie Academic & Professional.

Painter, T. (1983). Algal polysaccharides. In: G. O. Aspinall, *The Polysaccharides*, Vol. 2 (pp. 195–285). New York: Academic Press.

Philips Scientific Atomic Absorption Data Book (1988). Cambridge: Black Bear Press Ltd., 71.

Price, W. J. (1979). *Spectrochemical Analysis by Atomic Absorption* (pp. 287–357). London, Philadelphia, Rheine: Heyden & Son Ltd.

Research Report No 204 (1994). Institute of Chemistry. The Estonian Academy of Sciences. Unpublished.

Smewing, J. (1999). Hydrocolloids. In: A. J. Rosenthal, *Food Texture* (pp. 282–303). Gaithersburg, Maryland: Aspen Publishers, Inc.

Tanaka, R., Hatakeyama, T., Hatakeyama, H., & Philipps, G. O. (1996). Differential scanning calorimetric studies of Philippines natural grade kappa-carrageenan. *Food Hydrocolloids 10*(4), 441–444.

Therkelsen, G. H. (1993). Carrageenan. In: R. L. Whistler, & J. N. BeMiller, *Industrial Gums. Polysaccharides and Their Derivatives* (pp. 145–180). Third Edition. San Diego, New York, Boston, London, Sydney, Tokyo, Toronto: Academic Press.

Thomas, W. R. (1997). Carrageenan. In: A. Imeson, *Thickening and Gelling Agents for Food* (pp. 45–59). London, Weinheim, New York, Tokyo, Melbourne, Madras: Blackie Academic & Professional.

Truus, K., Vaher, M., Kukk, H., Pehk, T., & Kollist, A. (1996). Gelling galactans of red algae from the Baltic Sea (in Estonian). Eesti TA Toimetised. *Keemia 45*(1/2), 15–29.

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.

Usov, A. I. (1998). Structural analysis of red seaweed galactans of agar and carrageenan groups. *Food Hydrocolloids 12*(3), 301–308.

Vaher, M., Truus, K., Taure, I., Leito, I., Lahe, L., Kallavus, U., & Pehk, T. (1998). Composition and structure of summary polysaccharide from the red alga *Furcellaria lumbricalis*. Proceedings of the Estonian Academy of Sciences. *Chemistry 47*(2), 51–59.

Viitak, A., & Treumann, M. (1996). Electrochemical AAS for the determination of arsenic and selenium in hair. Proceedings of the Estonian Academy of Sciences. *Chemistry 45*(3/4), 91–96.

Watase, M., & Nishinari, K. (1981). Effect of alkali metal ions on the rheological properties of κ-carrageenan and agarose gels. *Journal of Texture Studies 12*, 427–445.

Appendix 2

Publication II

Friedenthal, M., Eha, K., Kaleda, A., Part, N., and Laos, K. (2020). Instability of low-moisture carrageenans as affected by water vapour sorption at moderate storage temperatures. SN Applied Sciences 2, 243. doi: 10.1007/s42452-020-2032-9.

Short Communication

Instability of low-moisture carrageenans as affected by water vapor sorption at moderate storage temperatures

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Abstract
Isothermal microcalorimetry was used to study the exothermic heat flow caused by the absorption of water into carrageenans and a furcellaran, leading to their thermodynamic instability under moderate storage conditions (35 °C, $\tau = 12$ h), where τ is the reaction time (h). The net heats evolved by furcellaran, ι-carrageenan, κ-carrageenan, and a mixture of κ/λ-carrageenan were 43.5 J/g, 45.9 J/g, 31.6 J/g, and 28.1 J/g, respectively. The pronounced exothermic behavior of furcellaran was attributed to the enthalpic association of water molecules with the thermally degraded carbohydrate matrix. The responses of a heat treatment of carrageenans at 55 °C, 85 °C, and 105 °C for 15 min on exothermic heat Q (J/g) at different water activities ($a_w = 0.26$, 0.51, 0.76, and 1.0) were measured. The dependence of the net recorded heat Q on water activity can be satisfactorily approximated by the equation $Q = a[1 - (1 - a_w)^b]$, where a and b are the coefficients found by the nonlinear least-squares method. It was concluded that carrageenans affected by excessive heat treatment should be preferably stored with limited water access.

Keywords Carrageenan · Instability · Water sorption · Isothermal microcalorimetry

1 Introduction

The success of any new product depends on the quality of its flavor, color, and texture, its stability under various storage conditions and its safety. These factors are intimately related to the ingredients in the food product and to the physical processes and handling procedures to which it has been subjected [1]. Particularly organic materials may involve problems because of the liability of macromolecules to structural alterations, resulting in different physical responses of the material: mechanical, thermal, optical, etc. This leads to the diminished functionality and undesired failure of the product [2].

The physical properties of any food are dictated by the complex behavior of its macromolecular constituents in their natural forms and as modified during processing and storage [3].

Industrial gums, which for the most part are water-soluble polysaccharides, are well-known ingredients in the marketplace and are used increasingly for both food and nonfood processing [4, 5]. Carrageenans, one of the most important algae polysaccharides originating from various Florideophyceae species, form a family of linear sulfated galactans built up of alternating 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose residues with a variable 3,6-anhydro ring [6]. The three main types of carrageenans are kappa (κ-), iota (ι-), and lambda (λ-)carrageenan. They have one, two, and three sulfate ester groups per dimer, respectively. κ- and ι-carrageenans contain 3,6-anhydrogalactose units, whereas λ-carrageenan has no 3,6-anhydrogalactose

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group [7]. Furcellaran is a type of κ-carrageenan whose major structural difference is a smaller degree of sulfation (17–18%) [8].

The principal property of these polysaccharides is their easy hydration to produce aqueous solutions possessing high viscosities and/or steady gels at low concentrations. The development of the desired properties, in general, is controlled by their structure–water associations in high-moisture systems [9].

Under normal humidity, galactans generally equilibrate to contain 8–12% moisture. With such limited water access, various food polysaccharides exist in an amorphous, metastable state, which has an effect on stability and rates of deteriorative changes [10, 11]. It is well recognized that the state diagram showing relationships between glass transition temperature *T*g and water content W_{H2O} (g/100 g of solids), depending on water activity a_w, has a fundamental significance in the characterization of the physical state of such materials [12]. In general, the sorption isotherms ($W_{H2O} = f(a_w)$) are recorded thermogravimetrically without a need for heat transferring (±) evaluation. If such an evaluation becomes necessary, *e.g.*, for sorption isosteric heat (Q^{st}) determinations, the Clausius–Clapeyron equation has been applied in most of the studies previously cited [13, 14].

Recently, it was noticed that some batches of the stored furcellaran powder showed an exothermic heat flow resulting in a local self-browning and limited burning as well.

In general, the pronounced exothermic behavior is attributed to the onset of pyrolytic degradation, which in fact takes place under very abusive conditions [15, 16]. Typically, differential scanning calorimetry (*DSC*) has been used to probe the thermal properties of polysaccharides at low moisture, whereas the primary thermal output has been registered as an endothermic event controlled by *T*g [17, 18]. To the contrary, Mitsuiki and coworkers [19] found a clear shift in exothermic heat flow and observed corresponding peaks in the derivative of the heat flow curve (*dDSC*) at ~ 90 °C and 40 °C for agar containing 22.8% of water and carrageenan with 25.1% of water, respectively. It has also been reported that galactans have no glass transition behavior [20]. Later it was proved that gel-derived agars exhibit a small endotherm in the range of 55–70 °C; at higher temperatures, a larger endotherm with an associated enthalpy change (32 J/g; 15.9% water) was observed. In contrast to the gel-derived agars, the roller-dried samples showed no corresponding endothermic transitions above 100 °C, while a small endotherm at 50–70 °C was still monitored [21].

So, no clear evidence of exothermic heat flow for galactans at moderate temperatures was found. However, it is known that the amorphous sugars may sorb large amounts of water from their surroundings, resulting in crystallization during storage above a critical, temperature-dependent relative humidity [22]. An exotherm occurring in DSC scans has been attributed to the instant crystallization of sugars [23]. Therefore, considering that a feature of the reported furcellaran was its dehydration by the roller (drum)-drying procedure, contrary to a typical gel-processed technique, the pronounced exothermic behavior was attributed to an enthalpic association of water molecules with the thermally modified carbohydrate matrix.

The purpose of the present study was to determine the effect of the exothermal phenomenon in isothermal conditions, considered here to be due to the structural transformation and following water interaction with the affected furcellaran/carrageenan powders, resulting in subsequent wetting and crystallization of the sugars.

2 Materials and methods

2.1 Materials

Commercial furcellaran was supplied by Est-Agar AS (Kärla, Estonia). It is a typical product extracted from *Furcellaria lumbricalis* (Gigartinales), with subsequent drum-drying in the final step. The three various carrageenan samples analyzed in this study were obtained from Sigma-Aldrich (USA).

High-purity standards of sodium chloride NaCl 6.0 molal in H_2O ($a_w = 0.76$) and lithium chloride LiCl 8.57 molal in H_2O ($a_w = 0.51$) and LiCl 13.41 molal in H_2O ($a_w = 0.26$) were purchased from Decagon Devices, Inc. (USA). For $a_w = 1.0$ distilled water was used. Values of water activities are given at temperature 35 °C.

2.2 Preparation of samples for measurement

The delivered samples of furcellaran and carrageenan were stored in sealed plastic containers at room temperature (20 °C) until used either for direct microcalorimetric measurements or optional preheating before the thermal analysis.

The water content of each sample was determined by loss on drying (L_D, %) after heating at 105 °C to constant weight. The measurements were carried out at least twice; when values were different by > 1%, the number of measurements was increased.

The optional preheating was carried out according to the method described by [24] with a Halogen Moisture Analyzer HR 73 (Mettler Toledo, Switzerland). The selected temperature range was 55–105 °C, and the time of treatment 15 min. The residual moisture content of the samples

was expressed by L_D if necessary. The preheated samples were stored in a desiccator over P_2O_5 until used, within 6 h.

2.3 The microcalorimetric measurement of heat flow

The calorimetric experiments were performed using a Thermal Activity Monitor TAM IV built up as an isothermal heat conduction microcalorimeter (TA Instruments, Delaware, USA). The furcellaran or carrageenan sample (of 100 mg) was quantitatively transferred into a standard 3 ml glass ampule. Additionally, a small glass container (*microhygrostat*) with a saturated salt solution was placed inside the glass ampule immediately before sealing it. The operated sample maintained well-defined relative humidity ($a_w = RH/100$) all through the reaction time of 12 h. The heat flow due to any changes in the powder as a consequence of the humidity was recorded. Four parallel experiments under various *RH* against blank with deionized water (2 ml) were run simultaneously at 35 °C. Power–time curves were registered using the data acquisition program Digitam 3.0 (TA Instruments, Delaware, USA).

Minimum duplicated measurements for each testing sample were made, and mean values were used for further analysis.

2.4 Statistical analysis

Curve fitting was performed with a brute force nonlinear least-squares method from an R 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria) package "nls2" version 0.2.

3 Results and discussion

The measuring principle of TAM is based on registering heat flow rate between the sample vessel and heat sink detected by Peltier effect plates [25, 26].

The TAM IV responses for a commercial furcellaran containing 11.2% w/w water and equilibrated at $a_w = 0.26$, 0.51, 0.76, and 1.0 are shown in Fig. 1. The absorption of the samples at water activity of 0.51, 0.76, and 1.0 produced exothermic responses, and the curves showed similar shapes. It could also be proposed that the absorption response is the sum of two exotherms, the absorption and the blank response, as is common for some salt solutions [27]. In the latter case, the hydration of water-binding sites along furcellaran polymer chains will dominate in the subsequent processes. The effect of increasing the water activity is clearly related to an increase in the amount of heat released during absorption process. The increase in the heat flow of absorption ranged from about 45 to 310 μW

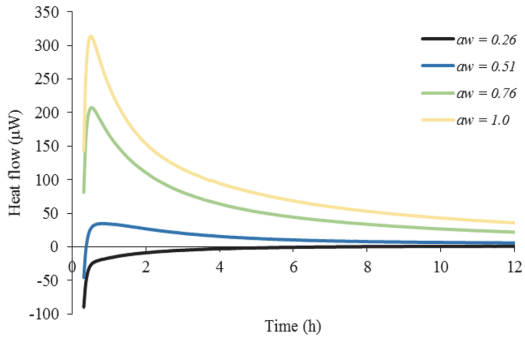

Fig. 1 Typical calorimetric curves on TAM for furcellaran at four different water activities. Experiment conditions: temp. 35 °C, $m = 0.890$ g

for water activity of 0.51 and 1.0, respectively. However, the sample at water activity 0.26 produced a slight endothermic response indicating the desorption process.

Although the calorimetric response for isothermal water sorption studies is balanced between the exotherm(s) and endotherm(s), heat emitted by furcellaran was evident. The released heat Q (J/g) was expressed as Eq. (1) [28]:

$$Q = \int \Phi \mathrm{d}\tau \tag{1}$$

where Φ—heat flow rate (μW) and τ—reaction time (h) are equal to the net area under the curve. The total heat evolved during an experiment is large enough to be detected and shows variation between furcellaran samples at different water activities. The value ranges from 0 to 43.5 J/g depending on applied a_w (Table 1).

In order to compare the calorimetric response for furcellaran with other glycans, three carrageenan samples (three replicates for each one) were examined under the same experimental conditions ($a_w = 0.26$, 0.51, 0.76, and 1.0). The example of corrected calorimetric response (the thermal power within the first 0.52 h was not registered) is shown in Fig. 2, in which the power–time curves for furcellaran and all carrageenan samples were found to be described by Eq. (2):

$$\mathrm{d}Q/\mathrm{d}\tau = a \cdot \mathrm{e}^{b\tau} \tag{2}$$

where $\mathrm{d}Q/\mathrm{d}t$ is the heat flow rate (μW), τ is the reaction time (h), a and b are the coefficients (Table 2) for each sample found by the least-squares method.

For example, the heat flow rate for κ-carrageenan monitored at $a_w = 0.51$ was described by $\mathrm{d}Q/\mathrm{d}\tau = 132.91 \times \mathrm{e}^{-0.38\tau}$.

Most of the experimental curves were well approximated by the simple exponential decay function (Eq. 2). However, it should be noted that in some samples, small

Table 1 Water content of furcellaran and carrageenans for isothermal calorimetry measurement (12 h at 35 °C) at different water activities and the net heat evolved

Sample	Water content L_D, % (w/w)	Experimental net heat evolved Q, J/g			
		$a_w = 0.26$	$a_w = 0.51$	$a_w = 0.76$	$a_w = 1.0$
Furcellaran	11.2	0	6.3	29.3	43.5
ι-carrageenan	9.4	0.41	15.1	37.5	45.9
κ-carrageenan	6.7	0	10.6	28.6	31.6
κ/λ-carrageenan	9.9	0	4.7	22.6	28.1

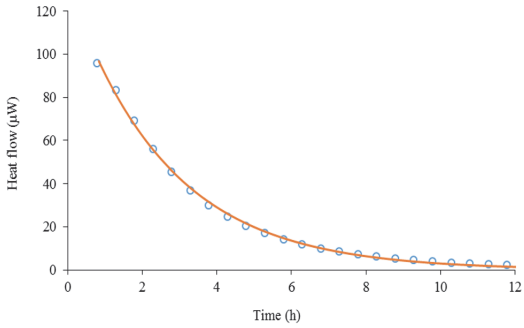

Fig. 2 Corrected calorimetrical curves on TAM for κ-carrageenan ("blue circle" denotes experimental data; "red solid line" denotes calculated curve). Experiment conditions: temp. 35 °C, $a_w = 0.51$, m = 0.1004 g

deviations were observed indicating that potentially more complex processes are taking place and can be investigated further.

Carrageenans have different thermal outputs depending on their specific compositions. As is evident from data in Table 1, no heat release was observed at $a_w = 0.26$, except for ι-carrageenan. The thermal outputs of all samples at higher water activities increased in the same shape of the curve; the highest values were obtained for ι-carrageenan. The thermal outputs of κ- and the mixture of κ/λ-carrageenan were similar, which was expected, as κ-carrageenan dominates the mixture.

It should be emphasized that in our research there were no strict criteria for the selection of carrageenans; for example, no processing or composition varieties were taken into account.

It is also important to stress that the experimentally measured net heat values shown in Table 1 form ca 85% of those calculated by fitting $dQ = (a \times e^{b\tau})d\tau$ because at the beginning of an experiment (0.52 h = 31 min) (Fig. 1) the heat flow cannot be recorded until the system has stabilized. The final moisture content in the sample was determined by moisture equilibrium with atmosphere at a given temperature. Practically, this means that if the sample was previously dehydrated below its equilibrium water content, it would ultimately absorb some water at the same relative humidity until equilibrium was reached. But it could also be expected that the equilibrium water content would depend on the thermal history relevant to the process of studied material. One possible explanation is that the furcellaran ($L_D = 11.2\%$) used in this study had more pronounced water uptake in comparison with carrageenans ($L_D = 6.7–9.9\%$) under the same environmental conditions, considering that it had passed through a previous severe heat treatment in drum-drying.

In the next series of experiments, furcellaran and carrageenans were subjected to optional heat treatment at 55 °C, 85 °C, and 105 °C for 15 min. The processed samples were monitored by isothermal microcalorimetry depending on a_w (Fig. 3). It became apparent that the calorimetric response depended on the depth of the applied optional heat treatment. The mathematical evaluation of the results expressed in Fig. 3 showed that the dependence of the total heat Q (J/g) on water activity a_w could be satisfactorily approximated by Eq. (3):

$$Q = a\left[1-\left(1-a_w\right)^b\right] \quad (3)$$

where a and b are the coefficients found by the nonlinear least-squares method.

Table 2 Coefficients a and b of furcellaran and carrageenans samples for Eq. 2

Sample	$a_w = 0.26$		$a_w = 0.51$		$a_w = 0.76$		$a_w = 1.0$	
	a	b	a	b	a	b	a	b
Furcellaran	–	–	44.30	−0.26	221.52	−0.32	303.80	−0.30
ι-carrageenan	–	–	158.23	−0.35	424.05	−0.43	613.92	−0.56
κ-carrageenan	–	–	132.91	−0.38	322.78	−0.42	449.37	−0.51
κ/λ-carrageenan	–	–	88.61	−0.62	170.87	−0.26	284.81	−0.36

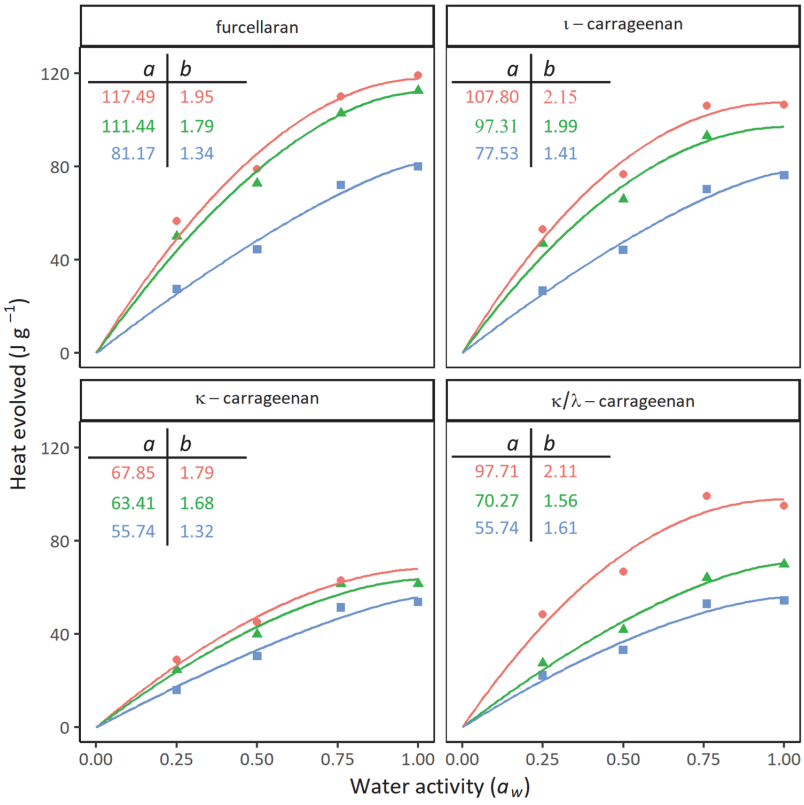

Fig. 3 Heat evolved as a function of water activity for furcellaran and different carrageenans at optional heat treatment temperatures for 15 min. "dashed lines" denote fitted curves; symbols "blue square," "green triangle," "red circle" denote experimental data: "blue square" 55 °C, "green triangle" 85 °C, "red circle" 105 °C. Values of the coefficients *a* and *b* from Eq. 3 obtained by curve fitting are shown with the corresponding colors. $Q_{55\,^{\circ}C} = 81.17\,[1-(1-a_w)^{1.34}]$, $Q_{85\,^{\circ}C} = 111.44\,[1-(1-a_w)^{1.79}]$, $Q_{105\,^{\circ}C} = 117.49\,[1-(1-a_w)^{1.95}]$

Curves of this type begin from the point [0,0] and asymptotically approach the maximum value *a* at water activity $a_w = 1$. Visual observation of the regression lines confirmed the goodness-of-fit of the proposed model.

The additional heat treatment reduced the moisture content in the examined samples to the level of 0–3.5%. Therefore, a joint contribution (structural rearrangement and lowered moisture level) to the exothermal heat effect was perhaps possible. But considering that $Q_{85\,^{\circ}C} = f(a_w)$ and $Q_{105\,^{\circ}C} = f(a_w)$ still had distinguishing curves, the influence of thermal disordering was probably dominant.

Water activity a_w was closely related to the chemical potential μ determining system at equilibrium, Eq. (4) [29]:

$$\mu = \mu_0 + RT \ln\left(a_w\right) = \mu_0 + RT \ln\left(p/p_0\right) \quad (4)$$

where μ is the actual chemical potential (kJ mol^{-1}), μ_0 reference state chemical potential (kJ mol^{-1}), R universal gas constant (8.314 J mol^{-1} K^{-1}), T temperature (K), p vapor pressure of water in the headspace above the sample at T (Pa), and p_0 vapor pressure of water in the headspace above the water at T (Pa).

It was predicted that applying heat for the additional processing of carrageenan would destabilize the galactans, thus opening more binding sites for water. Consequently, at higher a_w, the increase in actual chemical potential μ would lead to increased absorption energy. In general, the binding sites included hydroxyl groups of polysaccharides, carbonyl and amino groups of proteins, and other polar sites [2, 30]. Thus, the high-temperature heat treatment of low-moisture carrageenans leads to product instability, increasing water vapor absorption and causing significant increase in heat release even at moderate storage conditions. This process can lead to the possibility of temperature rise over product critical self-ignition temperature.

4 Conclusions

In conclusion, there was a noticeable similarity in the water absorption behavior of carrageenans and furcellaran monitored by the microcalorimeter. The results are: (1) furcellaran and carrageenans are affected by the excessive heat

treatment, so they should be preferably stored with limited water access, and (2) considering the structural similarities between furcellaran and carrageenan, the microcalorimetric assay is proposed as a potential method for the evaluation of the thermal history of the processed samples.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Brown M (2011) Processing and food and beverage shelf life. In: Kilcast D, Subramaniam P (eds) Food and beverage stability and shelf life. Woodhead Publishing, Sawston, pp 184–212
2. Fellows PJ (2017) Food processing technology–principles and practice, 4th edn. Elsevier, Amsterdam, pp 1–31
3. Ndob A, Melas M, Lebert A (2015) Physical-chemical properties of foods, 1st edn. Elsevier, Amsterdam, pp 1–17
4. Panda H (2010) The complete book on gums and stabilizers for food industry. Asia Pacific Business Press, Delhi
5. Kraan S (2012) Algal polysaccharides, novel applications and outlook. In: Chang C-F (ed) Carbohydrates–comprehensive studies on glycobiology and glycotechnology. InTech, Croatia, pp 489–532
6. Imeson AP (2009) Carrageenan and furcellaran. In: Phillips GO, Williams PA (eds) Handbook of hydrocolloids, 2nd edn. Woodhead Publishing, Sawston, pp 164–185
7. Campo VL, Kawano DF, da Silva DB, Jr Carvalho I (2009) Carrageenans: biological properties, chemical modifications and structural analysis–a review. Carbohydr Polym 77:167–180
8. Laos K, Ring SG (2005) Characterisation of furcellaran samples from Estonian *Furcellaria lumbricalis* (Rhodophyta). J Appl Phycol 17:461–464
9. Alzamora SM, Tapia MS, López-Malo A, Welti-Chanes J (2003) The control of water activity. In: Zeuthen P, Bøgh-Sørensen L (eds) Food preservation techniques. Woodhead Publishing, Sawston, pp 126–153
10. Liu Y, Bhandari B, Zhou W (2006) Glass transition and enthalpy relaxation of amorphous food saccharides: a review. J Agric Food Chem 54:5701–5717
11. Nurhadi B, Roos YH, Maidannyk V (2016) Physical properties of maltodextrin DE 10: water sorption, water plasticization and enthalpy relaxation. J Food Eng 174:68–74
12. Rahman MS (2015) Hurdle technology in food preservation. In: Siddiqui MW, Rahman MS (eds) Minimally processed foods: technologies for safety, quality and convenience. Springer, Berlin, pp 17–33
13. Staudt PB, Kechinski CF, Tessaro IC, Marczak LDF, Soares RD, Cardozo NSM (2013) A new method for predicting sorption isotherms at different temperatures using the BET model. J Food Eng 114:139–145
14. Tadapaneni RK, Yang R, Carter B, Tang JM (2017) A new method to determine the water activity and the net isosteric heats of sorption for low moisture foods at elevated temperatures. Food Res Int 102:203–212
15. Einhorn-Stoll U, Kunzek H, Dongowski G (2007) Thermal analysis of chemically and mechanically modified pectins. Food Hydrocoll 21:1101–1112
16. Roos YH, Karel M, Labuza TP, Levine H, Mathlouthi M, Reid D, Shalaev E, Slade L (2013) Melting and crystallization of sugars in high-solids systems. J Agric Food Chem 61(13):3167–3178
17. Zohuriaan MJ, Shokrolahi F (2004) Thermal studies on natural and modified gums. Polym Test 23:575–579
18. Jamaludin J, Adam F, Rasid RA, Hassan Z (2017) Thermal studies on arabic gum – carrageenan polysaccharides film. Chem Eng Res Bull 19:80–86
19. Mitsuiki M, Yamamoto Y, Mizuno A, Motoki M (1998) Glass transition properties as a function of water content for various low-moisture galactansJ Agric. Food Chem 46:3528–3534
20. Gidley MJ, Cooke D, Ward-Smith S (1993) Low moisture polysaccharide systems: thermal and spectroscopic aspects. In: Blanshard JMV, Lillford PJ (eds) The glassy state in foods. Nottingham University Press, England, pp 303–316
21. Cooke D, Gidley MJ, Hedges ND (1996) Thermal properties of polysaccharides at low moisture. II. Molecular order and control of dissolution temperature in agar. J Therm Anal 47:1485–1498
22. Makower B, Dye WB (1956) Equilibrium moisture content and crystallization of amorphous sucrose and glucose. J Agric Food Chem 4:72–77
23. Buera P, Schebor C, Elizalde B (2004) Effects of carbohydrate crystallization on stability of dehydrated foods and ingredient formulations. J Food Eng 67:157–165
24. Friedenthal M, Eha K, Viitak A, Lukas A, Siimer E (2001) Effects of drying on the gel strength and cation mobility of furcellaran. Innov Food Sci Emerg Technol 1:275–279
25. Liu JS, Rigsbee DR, Stotz C, Pikal MJ (2002) Dynamics of pharmaceutical amorphous solids: the study of enthalpy relaxation by isothermal microcalorimetry. J Pharm Sci 8:1853–1862
26. Wang J, Cheng D, Zeng N, Xia H, Fu Y, Yan D, Zhao Y, Xiao X (2010) Application of microcalorimetry and principal component analysis. Antibacterial evaluation of benzoinum and styrax on staphylococcus aureus growth. J Therm Anal Calorim 102:137–142
27. Buckton G (2000) Isothermal microcalorimetry water sorption experiments: calibration issues. Thermochim Acta 347:63–71
28. Suurkuusk J, Wadsö I (1982) A multichannel microcalorimetry system. Chem Scr 20(4):155–163
29. Reid DS (2007) Water activity: fundamentals and relationships. In: Barbosa-Cánovas GV, Fontana AJ Jr, Schmidt SJ, Labuza TP (eds) Water activity in foods: fundamentals and applications, 1st edn. Blackwell Publishing, New York, pp 15–28
30. Yousef AY, Balasubramaniam VM (2013) Physical methods of food preservation. In: Doyle MP, Buchanan RL (eds) Food microbiology–fundamentals and frontiers, 4th edn. ASM Press, Washington, pp 737–763

Appendix 3

Publication III

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Research article

Impact of short-term heat treatment on the structure and functional properties of commercial furcellaran compared to commercial carrageenans

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ABSTRACT

In the production of biopolymers, the processing operations (e.g. extraction and drying) involve some degradation of the polysaccharide-causing structural and functional changes in final products. In this study, short-term heat treatment (75–115 °C, 15 min) influence on commercial carrageenans' — furcellaran, κ-carrageenan, ι-carrageenan and a κ/λ-carrageenan — structure, molecular weight and gel rheology was studied. Compared with other carrageenans, commercial furcellaran that had undergone multiple heatings at high temperatures during production was found to be susceptible to polymer degradation. Heat caused the desulphation and degradation of furcellaran galactans and the molecular weight was significantly decreased, causing a drop in viscosity and gel hardness. The loss of the network cross-linking of furcellaran gels was confirmed by scanning electron microscopy. Carrageenan gel storage modulus values decreased with the increase in the temperature of the treatment. The greatest decrease in storage modulus values occurred with κ/λ-carrageenan gels, followed by ι-carrageenan > furcellaran > κ-carrageenan.

1. Introduction

Carrageenans are linear sulphated polysaccharides obtained from red algae. Their basic structural units are disaccharides consisting of alternating β-1,3- and α-1,4-linked galactose residues. The differences in the basic structure are due to the occurrence of 3,6-anhydrogalactose and sulphate groups' position and number in linked galactose residues [1]. The most important carrageenans are the κ– (KC), ι– (IC) and λ– (LC) types, which are differentiated by the occurrence of one, two or three sulphate ester groups per repeating disaccharide unit, respectively [2]. Commercially these types are often mixed or they are natural hybrid molecules consisting of different types of carrageenans [3].

Furcellaran (Fur) is similar in structure to KC, but the main difference is a lower sulphation level [4]. Structural complexity occurs when the hydroxy groups in D-galactose are replaced by sulphate, methyl and pyruvate groups [5, 6].

Carrageenans are used as gelling, thickening and stabilising components for various industrial purposes, such as pharmaceuticals, cosmetics and foods [7]. Different types of carrageenans give a wide spectrum of textures. Fur, KC and IC are gel–forming carrageenans, whereas LC is used only as a thickening agent. Carrageenan gelation is a two-step process: helical formation upon cooling and a further helical cation-specific aggregation [8]. Sulphate arrangements greatly influence the functional properties of carrageenans. Sulphation at the C2 position of (1→3) linked β-D-galactopyranose residues generally reduces the gel-forming ability of carrageenans by avoiding the formation of a helical structure [9]. The gel strength of different types of carrageenans depends on the presence and content of 3,6-anhydro-D-galactose residues [10]. Fur forms strong gels, similar to KC. The latter forms rigid and brittle gels, while IC forms soft and elastic gels [7].

The specific details of commercial carrageenan production processes are classified, but in general the production technology is rather similar. The extraction of carrageenans from weeds is carried out with hot water or an alkaline solution [11, 12, 13]. The alkaline treatment releases sulphate ester groups, causes the formation of 3,6-anhydro-D-galactose units and improves gel strength [14]. Several methods have been used to recover carrageenans from solutions. Higher quality products are obtained by the precipitation of carrageenans from solutions by alcohols [15]. However, as the alcohol precipitation method cost is higher than the direct drying method cost, spray drying or steam-heated rolls have been used extensively for concentrated carrageenan filtrates [16]. The elevated and uneven conditions during direct drying may initiate the

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degradation of polysaccharide macromolecules and consequently can affect the gels' rheological and mechanical properties. Robal et al. [17] have studied carrageenans' thermal stability in dry and sol states. They found that degradation during heat treatment in the dry state depends on the galactan's sulphur content and decomposition begins at lower temperatures for more sulphated preparations. They also found that thermal degradation is more intensive in the presence of divalent cations and decreases in the presence of methoxy groups. For drum-dried Fur, intense polymeric chain destruction was reported to begin at temperatures above 115 °C and the product survived brief heat treatment (15 min at 130 °C), after which a remarkable gel strength decrease was observed [18]. The same Fur powders dried at higher temperatures showed higher heat release [19], resulting in colour change and spontaneous combustion.

However, there have been no studies on the structural and functional changes in this drum-dried Fur after thermal processing at elevated temperatures. A better understanding of Fur properties will ultimately lead to better process control to obtain the best possible final product quality and increased consumer acceptance. The study's objective was to investigate short-term heat treatment effect on the structure and functional properties of drum-dried furcellaran compared with other carrageenans.

2. Materials and methods

2.1. Materials

Commercial furcellaran was extracted from *Furcellaria lumbricalis* (Gigartinales) (AS EstAgar, Kärla, Estonia). The furcellaran production process includes drying on rollers in the final step. Commercial κ-carrageenan, ι-carrageenan and a mixture of κ- and λ-carrageenan (kappa and lesser amounts of lambda carrageenan) preparations were purchased from Sigma (product codes 22048, C1138 and C1013, respectively).

2.2. Heat treatment

The heat treatment of the samples was performed as described by Friedenthal et al. [18] with a Halogen Moisture Analyzer HR 73 (Mettler Toledo, Switzerland) at 75–115 °C, with the treatment time being 15 min.

2.3. Chemical analysis

Monosaccharide contents were obtained by the hydrolysis of the polysaccharides in 2 M H_2SO_4 at 110 °C for 60 min, followed by neutralisation with 1 M NaOH; the monosaccharides were quantified by high-performance anion-exchange chromatography (HPAEC-PAD) [20], using the Shimadzu Prominence HPLC system (Shimadzu, Japan), equipped with an Antec II Decade electrochemical detector (ANTEC Leyden, The Netherlands). The analysis was carried out on a Dionex CarboPac MA-1 column, thermostated at 35 °C. Elution was performed using 450 mM NaOH at a flow rate of 0.4 ml min^{-1}, and the sample injection size was 10 μl.

The sulphate content was quantified using the $BaCl_2$-gelatin turbidity method [21] after hydrolysing the samples in 1 M HCl at 115 °C for 5 h.

The pH of 1% (w/v) polysaccharide sols was determined using a Mettler Toledo SevenEasy pH meter.

2.4. NMR spectroscopy

^{13}C NMR spectra were recorded on a Bruker AVANCE III spectrometer operating at 800 MHz. The spectra of 2.5% polysaccharide solutions in D_2O (w/w) were obtained at 60 °C, and 30000 transients were collected with a 1 s inter-pulse delay. The chemical shifts were calculated with reference to the C-6 signal from the galactose subunit, having a constant value of 61.3 ppm for these carrageenans [22].

^{13}C CP-MAS NMR spectra were recorded on a Bruker AVANCE-II spectrometer at 14.1 T magnetic field using cross polarisation, a proton decoupling pulse sequence and a home-made double resonance probe with magic-angle-spinning for 4 × 25mm Si_3N_4 rotors. The sample spinning speed was 12.5 kHz, the ramped polarisation transfer pulse duration was 1 ms, and the relaxation delay was 5 s.

2.5. Size exclusion chromatography

The molecular weight (Mw) determination was carried out in polysaccharides through size exclusion chromatography (SEC) analysis following the method of Saluri et al. [23], using a Shimadzu LC-30AD liquid chromatograph equipped with a RID-10A refractive index detector, a Shimadzu CTO-20AC column oven, an OHpak SB-G guard column, and two Shodex OHpak SB-806MHQ columns in series. Elution was conducted using a 0.1 M $NaNO_3$ solution as the mobile phase flow rate was set at 0.8 mL min^{-1}. The column oven temperature was set at 60 °C. To estimate the peak-average Mw, a calibration curve was obtained from 12 pullulan standards.

2.6. Lightness

The dry Fur sample lightness was evaluated with a CM-700d spectrophotometer (Konica Minolta, Japan), CIE D65/11 mm/2°.

2.7. Rheological characteristics of carrageenan sols and gels

2.7.1. Preparation of sols and gels

All hydrocolloid solutions were prepared by dissolving carrageenan powders in distilled water at 75 °C with a magnetic stirrer. After the complete solubilisation of the polysaccharides, the sols were used for viscosity and temperature sweep tests or poured into moulds (20 × 20 × 20 mm), cooled to +5 °C and stored at that temperature overnight for other rheological measurements.

The polysaccharide concentrations were 2.5% (w/v) in all cases, except for the temperature sweep test, where the polysaccharide concentration was 1.5% (w/v).

2.7.2. Viscosity

The flow properties of 2.5% (w/v) polysaccharide sols at 75 °C were measured with a RheolabQC rotating viscometer (Anton Paar, Germany), fitted with a concentric cylinder measuring set CC27 system, with a temperature-controlled water bath. The sample volume was 20 ml and the apparent viscosity η (Pa*s) was measured as a function of shear rate $\dot{\gamma}$ (10–50 s^{-1}) for 40 s.

2.7.3. Hardness

The hardness (first peak height) was determined using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, England). Gel samples were removed from the fridge and moulds (20 × 20 × 20 mm) and allowed to equilibrate to room temperature for 2 h before testing. A stainless steel cylindrical probe (25 mm in diameter) was used to compress the samples to 50% of their original height at a constant speed of 1 mm s^{-1}.

2.7.4. Rheology

A dynamic rheological measurement was performed with an MCR 301 rheometer (AntonPaar GmbH, Germany), using a serrated parallel plate of 50 mm diameter (profile depth: 0.5 mm) to minimise slippage at the gel-geometry interfaces. The gap between the plates was kept at 1 mm. All measurements were performed in duplicate, and data points were recorded at steady state.

The storage modulus (G′) and loss modulus (G″) of the gels at room temperature were determined by the modified method proposed by Chen et al. [24]. The study consisted of the following steps: time sweep (2 min) at 10 Hz and 0.1% strain (within the viscoelastic region), followed by a

Table 1. Monomeric composition (%, w/w dry weight) of commercial carrageenans.

Sample	Glucose	Sucrose	Galactose	3,6-anhydro-galactose	6-0- methyl galactose	SO_4
Fur	-	-	39.2 ± 0.5	29.4 ± 0.6	2.1 ± 0.2	16.2 ± 0.4
KC	-	26.3 ± 0.5	26.3 ± 0.4	25.4 ± 0.5	-	15.7 ± 0.3
KC/LC	1.2 ± 0.1	-	27.6 ± 0.5	26.0 ± 0.4	-	22.9 ± 0.3
IC	1.4 ± 0.1	-	28.5 ± 0.4	22.8 ± 0.4	-	36.1 ± 0.4

frequency sweep from 0.01 to 100 Hz at 0.1% strain. After another time sweep (2 min) at 0.1% strain 10 Hz measurement, an amplitude sweep at 0.01–100% strain 10 Hz, and a final time sweep (2 min) at 0.1% strain 10 Hz were recorded.

For temperature sweeps, the polysaccharide solutions were poured on a pre-heated (90 °C) Peltier plate and its borders were coated with low-viscosity silicon oil to prevent water loss. After 5 min, the samples were cooled down to 25 °C at a rate of 1 °C min^{-1} at 0.1% strain and 10 Hz frequency to follow the gelation process. The sample melting process was assessed during heating from 25 to 90 °C, at a constant rate of 1 °C min^{-1} and under the same strain and frequency conditions.

2.8. Scanning electron microscopy

2.5% (w/v) polysaccharide gels were frozen in liquid nitrogen and freeze-dried under vacuum at -60 °C. For scanning electron microscopy (SEM), samples were mounted on aluminium sample holders and were transferred to a SEM unit equipped with an EPSE detector (EVO LS15, Carl Zeiss, Milan, Italy), which was at ambient temperature and filled with water vapour at 70 Pa pressure.

3. Results and discussion

3.1. Characterisation of carrageenans

3.1.1. Chemical analysis

Table 1 shows the commercial carrageenan powder chemical compositions. The sugar analysis indicated that the most important sugar in all of the samples was galactose, followed by 3,6-anhydrogalactose. A high sucrose content was found in the KC samples. Such additives as sucrose and glucose are often used to improve commercial carrageenan preparations' functional properties (e.g. solubility, viscosity and gel strength). Small glucose amounts were found in IC and KC/LC samples, likely derived from floridean starch and 6-O-methylgalactose in the Fur samples. The highest ester sulphate content was found in the IC (36.1 % w/w, approximately two groups per repeating disaccharide unit) and the lowest in the KC (15.7 % w/w).

The KC sample sulphur content was lower than expected, but this can be attributed to the presence of sucrose additive. Excluding the additive, the KC sulphur content was approximately one group per repeating disaccharide unit, a value that is in agreement with the data reported elsewhere [25, 26].

3.1.2. ^{13}C-NMR

^{13}C-NMR spectra of the analysed samples are shown in Figure 1. The signal assignments (Table 2) are based on the carrageenan structure chemical shifts [26].

The commercial Fur main components were (1→3) linked β-D-galactopyranose, (1→4) linked 3,6-anhydro-α-D-galactopyranose, (1→3) linked β-D-galactopyranose 4-sulphate and (1→3) β-D-galactopyranose 6-sulphate, indicating that Fur is a hybrid of κ−, β− and ω−carrageenan, which has been reported elsewhere [27, 28, 29], with an approximate ratio of 5:4:1, respectively. Also, 3-linked 6-O-methyl-D-galactose residues were found, as these residues give specific signals for OMe at 59.0, for the substituted C-6 at 71.8, and for the neighbouring C-5 at 73.3 ppm in ^{13}C-NMR [30]. Additionally, several peaks between 10-35 ppm were registered (data not shown), indicating the presence of vegetable fats [31]. The fats could have got into the furcellaran during the production process when oil was added to the drums during the drum-drying process.

Commercial KC was found to be a blend of KC (the signals due to (1→4) linked 3,6-anhydro-α-D-galactopyranose and (1→3) linked β-D-galactopyranose 4-sulphate) and sucrose, with an approx. 1:1 M ratio. Also, 3-linked 6-O-methyl-D-galactose residues were found.

The spectrum of KC/LC shows the prevalence of KC but no signals of LC were detected. However, the signals of minor components indicate the presence of IC. Being common to KC, 6-O-methyl-D-galactose residues were again found.

The main commercial IC signals were due to 3-linked β-D-galactopyranose 4-sulphate and 4-linked α-D-galactopyranose 2,6-disulphate, corresponding to those of IC. Additionally, peaks corresponding to amylopectin were registered, indicating the presence of starch in the product, with branch-point residue C-6_b detectable at 65.5 ppm [32].

3.2. Effect of heat treatment on the structural properties of carrageenans

3.2.1. ^{13}C CP-MAS NMR

The ^{13}C CP-MAS NMR spectra of carrageenans show six signals (Figure 2) representing the galactopyranose and the anhydrogalactopyranose residues. The signal assignments (Table 3) are based on the carrageenan structure chemical shifts [33]. As seen with ^{13}C-NMR, the KC spectra showed an additional 12 peaks corresponding to sucrose chemical shifts [34, 35] (Table 3). Excluding the sucrose signals, the KC, KC/LC and Fur chemical shifts were very similar. The KC/LC spectrum showed chemical shifts corresponding only to KC; chemical shifts corresponding to LC [33] were not seen. Comparing the Fur with the KC spectrum, the resonance of the C-4 signal was of lower intensity, to the advantage of the C-5 signal, which was increased. As Fur is partially desulphated KC, due to the presence of the unsubstituted hydroxyl on 4G, this carbon resonates up field [36].

The relative changes in intensity of the peaks C-3, C-4 and C-5 between the spectra of KC and IC are based on the NMR substitution rules applied to sulphation [37]. Indeed, IC can be considered KC with the hydroxyl linked to carbon 2A replaced by a sulphate group.

No heat treatment influence at 115 °C for 15 min on the carrageenans' structure was seen (Figure 2), except for Fur, where the intensity of the peaks from C-3 to C-6 decreased, indicating polysaccharide degradation and galactan desulphation.

In order to further investigate the structural differences between non-treated and heat-treated carrageenans, the ^{13}C-NMR was applied. Again, no short heat treatment influence on the carrageenans' structure was seen (data not shown), except for Fur (Figure 3). Compared with non-treated Fur (Figure 1), three additional peaks, at 90.4, 87.2 and 82.8 ppm (DA-C1, DA-C4, and DA-C3, respectively), appeared, indicating the formation of oligosaccharides with decreasing terminal 3,6-anhydro-α-D-galactose residues [38]. Similarly to Robal et al.'s work [17], concurrent β-D-galactose and β-D-galactose-4-sulphate anomeric signals became segregated, with the latter showing slightly higher peaks in the spectrum. Also, a decrease in anomeric carbon of 3,6-anhydro-α-D-galactose residue at 95.0 ppm was observed, which can be explained by galactan desulphation [17].

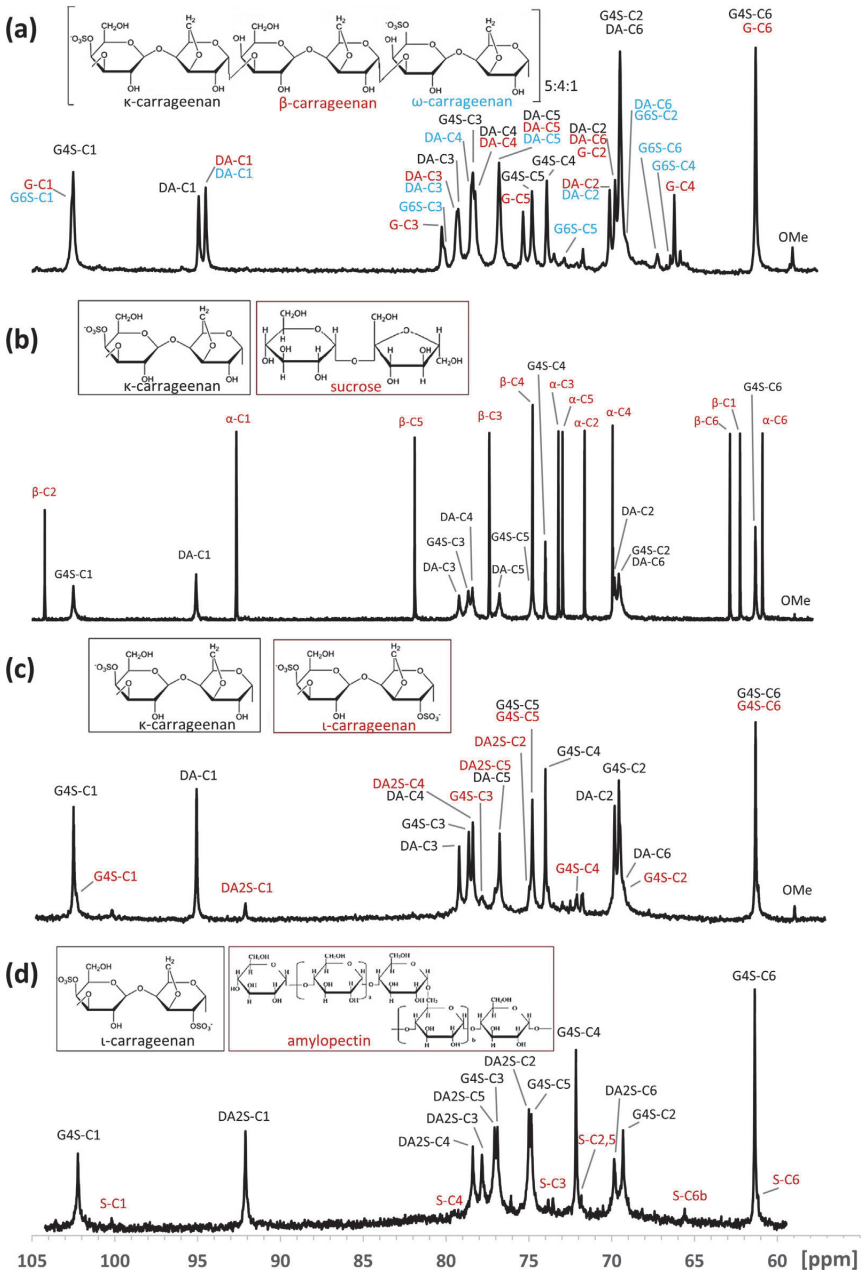

Figure 1. ^{13}C-NMR spectra of carrageenans. ^{13}C-NMR spectra of carrageenans. (a) furcellaran, (b) κ−carrageenan, (c) κ/λ−carrageenan, (d) ι−carrageenan.

3.2.2. SEC

The average molecular weight of the carrageenans as a function of heat treatment temperature is shown in Table 4. Of the non-treated samples, KC had the highest average molecular weight (1460 kDa), followed by IC (1201 kDa) and KC/LC (1059 kDa). Fur had the lowest average molecular weight (252 kDa). These molecular weight differences can be explained by the different production processes. It is known that elevated temperatures and prolonged extraction and drying times can cause carrageenan degradation [17, 39]. The results indicated that the carrageenan degradation rate was accelerated at higher temperatures, as lower molecular weight carrageenans were obtained.

Fur showed more sensitivity to temperature than any other carrageenan. The molecular weight of Fur showed a huge drop at temperatures above 90 °C, and a 91% of average molar mass decrease was observed at 115 °C, while the other carrageenans showed a less than 7% of average molar mass decrease at the same temperature. It can be assumed that carrageenan degradation depends on the production process thermal history. As Fur is dried on drum-driers, it may bind more

Table 2. ^{13}C-NMR chemical shift assignments (ppm) of commercial carrageenans.

Sample	Residue	Unit	Chemical shifts (ppm)					
			C1	C2	C3	C4	C5	C6
Fur	κ−carrageenan	G4S	102.5	69.5	78.5	74.0	74.8	61.3
		DA	95.0	69.8	79.2	78.3	76.8	69.5
	β−carrageenan	G	102.6	69.7	80.3	66.4	75.4	61.3
		DA	94.5	70.1	79.3	78.3	76.8	69.7
	ω−carrageenan	G6S	102.6	69.4	80.2	66.2	72.9	67.2
		DA	94.6	70.1	79.3	78.5	76.8	69.4
KC	κ−carrageenan	G4S	102.5	69.5	78.7	74.0	74.8	61.3
		DA	95.0	69.8	79.2	78.3	76.8	69.5
	sucrose	α−D-glucopyranose	92.9	71.9	73.4	70.0	73.2	61.0
		β-D-fructopyranose	62.2	104.5	77.3	74.8	82.2	63.2
KC/LC	κ−carrageenan	G4S	102.5	69.6	78.9	74.1	74.8	61.3
		DA	95.3	69.9	79.2	78.3	76.8	69.5
	ι−carrageenan	G4S	102.2	69.3	76.8	72.1	74.8	61.3
		DA2S	92.1	75.0	77.8	78.3	77.0	69.8
IC	ι−carrageenan	G4S	102.2	69.3	76.8	72.1	74.8	61.3
		DA2S	92.1	75.0	77.8	78.3	77.0	69.8
	starch	amylopectin	100.2	72.2	73.5	79.2	71.8	61.1

water compared with carrageenans [19], causing harsher degradation conditions.

3.2.3. pH and lightness

The sample degradation at high temperatures was likely the result of the acid hydrolysis produced by the release of the sulphate groups, rendering them acidic. The formation of acidic degradation products was shown by the decrease in pH at higher treatment temperatures. The pH values of the non-treated KC, KC/LC and IC were 9.14 ± 0.01, 9.58 ± 0.01 and 9.45 ± 0.02, respectively. After heat treatment at 150 °C for 15 min, the carrageenans' pH slightly decreased to 9.05 ± 0.01, 9.51 ± 0.01 and 9.05 ± 0.01, respectively, and no colour change was observed. Compared with carrageenans, the non-treated furcellaran sample pH was lower, only 7.42 ± 0.02 (Figure 4), and had very low molecular weight, indicating that acidic degradation products had already formed during the Fur production process. During heat treatment, furcellaran pH showed a sharp decrease from 100 °C up to 130 °C, and started to plateau beyond this temperature, at a pH value of 2.4 ± 0.01. The degradation was accompanied by a colour change, to a deep brown/black tone, of the highly degraded Fur (Figure 4).

3.3. Heat treatment effect on carrageenan gels' rheological and microstructural properties

3.3.1. Viscosity, hardness, gelling and melting temperatures

The non-treated and heat-treated (115 °C) 2.5% (w/v) carrageenan sols' viscosity at 75 °C is shown in Table 5. The highest viscosity was shown by KC, followed by IC and the KC/LC mixture. Fur had the lowest viscosity. These data correlate well with molecular weight (Pearson's r = 0.95). Higher molecular weight polymer coils occupy more solvent volume, resulting in an increase in solution viscosity.

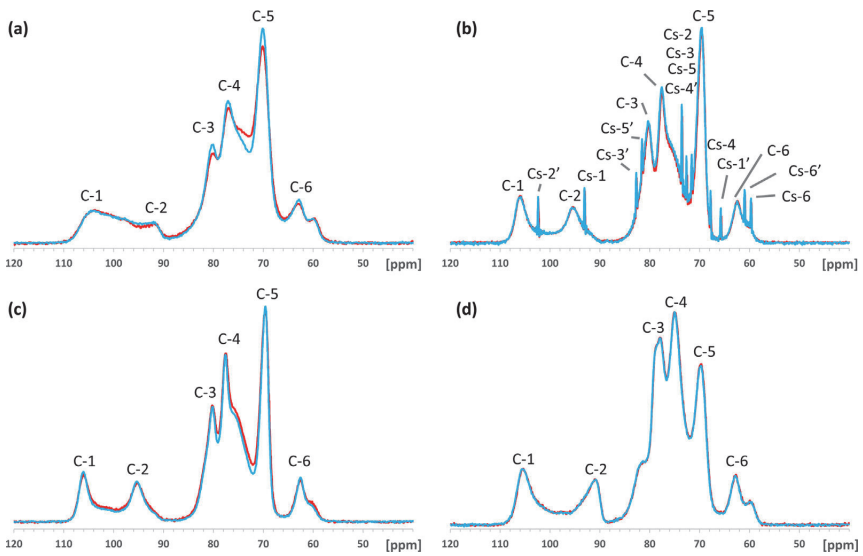

Figure 2. Comparison of the carrageenan spectra before (blue line) and after (red line) heat treatment. (a) furcellaran, (b) κ−carrageenan, (c) κ/λ−carrageenan, (d) ι−carrageenan. Chemical shifts from C-1–C-6 correspond to polysaccharide; chemical shifts from C_s-1–C_s-6 and C_s-1′–C_s-6′ correspond to sucrose.

Table 3. ^{13}C CP-MAS NMR chemical shift assignments (ppm) of commercial carrageenans and their additives.

Sample	Residue	Peak	Chemical shift
Fur	1G	C-1	104.5
KC	1A	C-2	91.9
KC/LC	3A, 3G, 4A	C-3	80.1
	5A, 5G, 4G	C-4	77.0
	2A, 2G, 6A	C-5	70.1
	6G	C-6	62.8
KC	1G	C-1	105.9
KC/LC	1A	C-2	95.5
	3A, 3G, 4A	C-3	80.2
	5A, 5G, 4G	C-4	77.6
	2A, 2G, 6A	C-5	69.6
	6G	C-6	62.5
IC	1G	C-1	105.7
	1A	C-2	90.8
	3A, 3G, 4A, 5A	C-3	77.9
	2A, 5G	C-4	75.0
	4G, 2G, 6A	C-5	69.7
	6G	C-6	62.9
Sucrose	α-D-glucopyranose	Cs-1	93.0
		Cs-2	73.6
		Cs-3	72.6
		Cs-4	67.8
		Cs-5	73.6
		Cs-6	59.7
	β-D-fructopyranose	Cs-1′	65.8
		Cs-2′	102.4
		Cs-3′	82.7
		Cs-4′	71.6
		Cs-5′	81.5
		Cs-6′	61.0

G – galactopyranose residues.
A – anhydrogalactopyranose residues.

The heat treatment didn't affect the carrageenans' viscosity except for Fur, where a drop in viscosity was noticed. Similar results were observed when determining the 2.5% (w/v) carrageenans' gel hardness (Table 5). No changes in hardness values were observed for KC, IC or KC/LC gels after heat treatment at 115 °C. However, Fur formed thermally unstable brittle gels as the hardness decreased after the heat treatment. At 115 °C, Fur lost its gelling ability, apparently due to the loss of the minimum polysaccharide chain length required for the formation of ordered structures.

A rheological temperature sweep test was used for the determination of the gelling and melting temperatures of the sols/gels of 1.5% (w/v) carrageenans. All samples showed two distinct crossover points corresponding to the sol-gel transition temperature (T_{sg}) during the cooling cycle and gel-sol transition temperature (T_{gs}) during heating, confirming their thermo-reversible property. The T_{sg} and T_{gs} values are shown in Table 6. The KC/LC transition temperatures were much higher than for KC, probably due to the inclusion of IC. The highest transition temperatures were obtained with IC. It is apparent that both T_{sg} and T_{gs} shift to lower temperatures with a heat treatment temperature increase, indicating that heat treatment impedes coil-helix transition. Also, the thermal hysteresis between T_{sg} and T_{gs} decreased with heat treatment for all samples except for KC/LC. Fur showed the greatest shift in transition temperatures and in thermal hysteresis, probably due to the greater degradation of polysaccharide. A positive correlation between molecular weight and melting and gelling temperatures has been previously reported [15, 40].

3.3.2. Dynamic rheology

Figure 5 shows storage modulus (G′) and loss modulus (G″) curves as a function of strain. The carrageenan gels present characteristic curves of well-structured systems with long linear viscoelastic regions reached up to 4% for KC and KC/LC gels and 10% for Fur gels, and decreasing trends over this threshold. The decrease can be explained by syneresis, which caused gel slippage between the plates of the rheometer.

No changes in G′ and G″ values were observed with IC. All G′ values were higher than G″, indicating a stable gel. However, an obvious difference appeared between the maximum G′ values of carrageenan gels due to their network strength differences. The highest G′ value was shown by KC/LC, followed by KC, Fur and IC. The gels with higher G' values had more compact structures and were more stable at rest.

All studied polysaccharide gels showed a G′ vs. frequency dependence and can be classified into physically cross-linked network gels (Figure 6). The slightly rising G′ curve of the KC/LC gel was above the G″ curve over the entire applied frequency domain and the frequency of oscillations was little affected by the gel's viscoelastic properties. For other studied carrageenan gels, the crossover took place at lower frequencies (Fur at 30

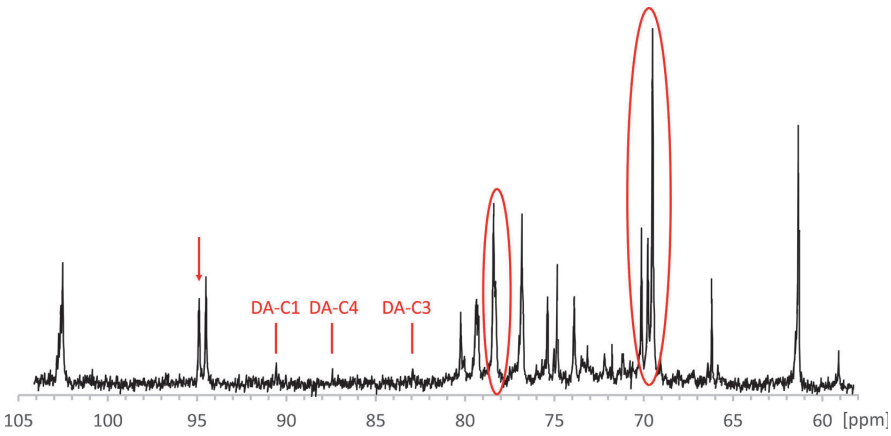

Figure 3. ^{13}C-NMR spectra of furcellaran dry-heated at 115 °C 15 min.

Table 4. Carrageenan molecular weight as a function of thermal treatment.

Sample	Molecular weight (Mw), kDa				
	Non-treated	75 °C	90 °C	105 °C	115 °C
Fur	252	194	179	91	22
KC	1460	1437	1420	1409	1389
KC/LC	1059	1047	1029	1028	1009
IC	1201	1183	1164	1133	1117

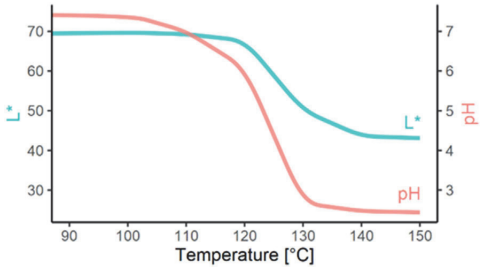

Figure 4. The dependence of furcellaran lightness (L*) and 1% (w/v) furcellaran solution pH on the drying temperature.

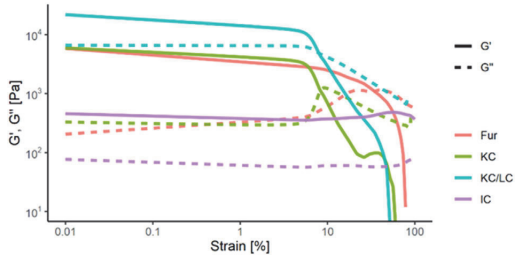

Figure 5. Storage modulus (G′) and loss modulus (G″) as a function of strain for 2.5% carrageenan gels.

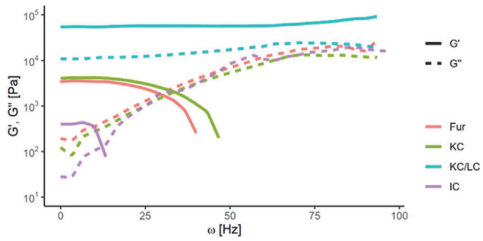

Figure 6. Storage modulus (G′) and loss modulus (G″) as a function of frequency for 2.5% carrageenan gels.

Hz, KC at 33 Hz and IC at 13 Hz). This means that these samples act as reversible networks and can form gels at low frequencies and viscous sols at high frequencies.

The G′ values of all studied carrageenan gels decreased with an increase in the heat treatment temperature (Figure 7). The greatest decrease in G' values occurred with KC/LC gels, followed by IC > Fur > KC. As the hardness of the KC gels was higher than that of Fur, it can be concluded that Fur forms stronger but more brittle gels than KC does. IC forms soft and elastic gels, whereas KC/LC forms very strong and rigid gels.

3.3.3. SEM

Changes in the morphology of the Fur and carrageenan gels before and after heat treatment were assessed using the SEM technique (Figure 8). It was observed that the gel skeleton structures of the hydrogel samples formed porous networks, Fur was similar to IC, and KC was similar to KC/LC. The first mentioned samples had a characteristic honeycomb structure, whereas KC and KC/LC exhibited long cross-linked tubular structures with rectangular pores. The effect of heat treatment was observed only for the Fur gel network, which lost the cross-linking, resulting in a decrease in gel hardness.

Table 5. The viscosity and hardness of non-treated and heat-treated carrageenan sols and gels.

Sample	Viscosity, mPa*s		Hardness, N	
	Non-treated	115 °C	Non-treated	115 °C
Fur	13 ± 1	1 ± 0	3 ± 0	0 ± 0
KC	102 ± 5	102 ± 4	12 ± 1	12 ± 1
KC/LC	47 ± 2	47 ± 3	21 ± 0	21 ± 1
IC	81 ± 4	81 ± 4	2 ± 0	2 ± 0

Table 6. The gelling and melting temperatures of non-treated and heat-treated carrageenan sols and gels.

Sample	Sol-gel transition temperature (T_{sg}), °C			Gel-sol transition temperature (T_{gs}), °C		
	Non-treated	75 °C	95 °C	Non-treated	75 °C	95 °C
Fur	32 ± 0	28 ± 0	27 ± 2	53 ± 0	48 ± 0	45 ± 1
KC	33 ± 0	32 ± 0	32 ± 0	48 ± 0	44 ± 0	44 ± 0
KC/LC	45 ± 3	43 ± 1	42 ± 0	75 ± 0	73 ± 1	72 ± 1
IC	57 ± 0	55 ± 2	53 ± 1	60 ± 1	57 ± 1	56 ± 1

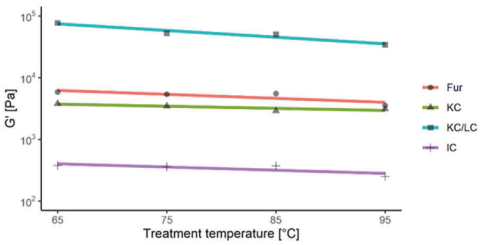

Figure 7. Storage modulus (G′) as a function of treatment temperature for carrageenan gels.

4. Conclusions

In this study, the influence of short-term heat treatment on the structure and rheological properties of commercial carrageenans was investigated. It was found that drying at 115 °C for 15 min had no influence on the structure of carrageenans except for Fur, where polysahharide degradation and galactan desulphation occurred. Heat decreased the molecular weight of carrageenans and the degradation was accelerated at higher temperatures. Fur had the lowest average molecular weight compared with the other carrageenans, as it had passed through previous severe repeated heat treatments in drum-drying during processing. It showed more sensitivity to heat as drying above 115 °C caused the loss of viscosity and gelling ability as the average molecular weight fell below the minimum value required for gelation. The viscosity and hardness of other studied carrageenan gels were not dependent on the drying temperature. All of the studied polysaccharide gels can be categorised as typical physically cross-linked network gels with thermo-reversible properties. Heat treatment decreases the storage modulus of carrageenan gels; also, the gelling and melting temperatures decrease with an increase in the heat treatment temperature, indicating that heat treatment impedes coil-helix transition. In order to improve the quality of carrageenans, the use of high temperatures in production must be avoided to prevent carrageenan degradation and functional property loss.

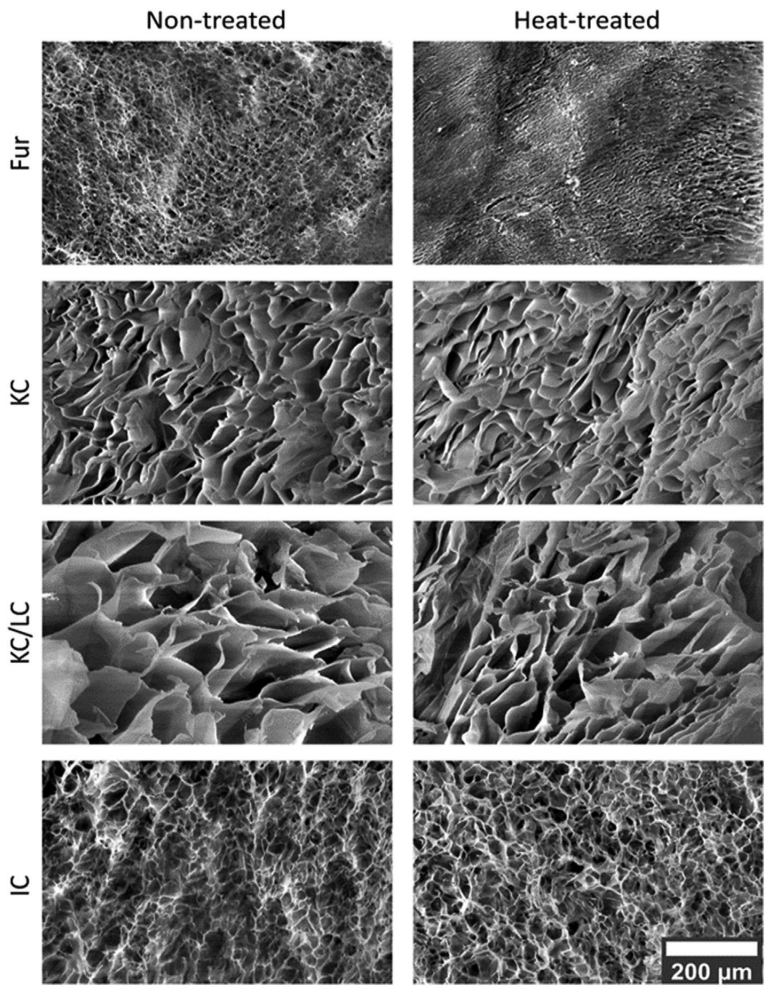

Figure 8. SEM images of 2.5% (w/w) gel network structures of non-treated and heat-treated (115 °C 15 min) carrageenans.

Declarations

Author contribution statement

Kairit Eha: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Tõnis Pehk, Ivo Heinmaa, Aleksei Kaleda: Performed the experiments.

Katrin Laos: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] S.H. Knutsen, D.E. Myslabodski, B. Larsen, A.I. Usov, A modified system of nomenclature for red algal galactans, Bot. Mar. 37 (1997) 163–169.

[2] I.M. Yermak, N.P. Mischchenko, V.N. Davydova, V.P. Glazunov, D.V. Tarbeeva, A.O. Kravchenko, E.A. Pimenova, I.V. Sorokina, Carrageenans-sulfated polysaccharides from red seaweeds as matrices for the inclusion of Echinochrome, Mar. Drugs 15 (11) (2017) 337.

[3] A. Hoefler, Hydrocolloid sources, processing, and characterization, in: Hydrocolloids, Eagen Press, St Paul, Minnesota, 2004, pp. 7–26.

[4] M. Glicksman, Red seaweed extracts (agar, carrageenan, furcellaran), in: M. Glicksman (Ed.), Hydrocolloids, CRC Press, New York, 1983, pp. 73–113.

[5] T. Painter, Algal polysaccharides, in: G.O. Aspinall (Ed.), The Polysaccharides, Academic Press, New York, 1983, pp. 195–285.

[6] A.I. Usov, Structural analysis of red seaweed galactans of agar and carrageenan groups, Food Hydrocoll. 12 (3) (1998) 301–308.

[7] A.P. Imeson, Carrageenan and furcellaran, in: G.O. Phillips, P.A. Williams (Eds.), Handbook of Hydrocolloids, second ed., Woodhead Publishing Limited, Cambridge, 2009, pp. 164–185.

[8] S. Ikeda, V.J. Morris, K. Nishinari, Microstructure of aggregated and nonaggregated K-carrageenan helices visualized by atomic force microscopy, Biomacromolecules 2 (2001) 1331–1337.

[9] E.R. Morris, D.A. Rees, E.J. Welsh, L.G. Dunfield, S.G. Whittington, Relation between primary structure and chain flexibility of random coil polysaccharides: calculation and experiment for a range of model carrageenans, J. Chem. Soc. Perkin Trans. 2 (1978) 793–800.

[10] F. Van de Velde, H.S. Rollema, R.H. Tromp, Coil-helix transition of ι-carrageenan as a function of chain regularity: the effect of counterion valency, in: E. Dickinson, T. VanVliet (Eds.), Food Colloids, Biopolymers and Materials, Royal Soc Chemistry, Wageningen, 2003, pp. 256–264.

[11] S. Mustapha, H. Chandar, Z.Z. Abidin, R. Saghravani, M.Y. Harun, Production of semi- refined carrageenan from *Eucheuma cotonii*, J. Sci. Ind. Res. 70 (2011) 865–870.

[12] N. Rhein-Knudsen, M.T. Ale, S. Rasmussen, S.K. Kamp, J.A. Bentil, A.S. Meyer, Alkaline extraction of seaweed carrageenan hydrocolloids using cocoa pod husk ash, Biomass Conver. Biorefin. 8 (2018) 577–583.

[13] R. Tuvikene, K. Truus, M. Vaher, T. Kailas, G. Martin, P. Kersen, Extraction and quantification of hybrid carrageenans from the biomass of the red algae *Furcellaria lumbricalis* and *Coccotylus truncatus*, P. Est. Acad. Sci. Chem. Chem. 55 (1) (2006) 40–53.

[14] L.E. Rioux, S.L. Turgeon, Seaweed carbohydrates, in: B.K. Tiwari, D.J. Troy (Eds.), Seaweed Sustainability, Academic Press, San Diego, 2015, pp. 141–192.

[15] G.H. Therkelsen, Carrageenan, in: R.L. Whistler, J.N. BeMiller (Eds.), Industrial Gums: Polysaccharides and Their Derivatives, third ed., Academic Press, London, 1993, pp. 145–180.

[16] J.G. Lewis, N.F. Stanley, G.G. Guist, Commercial production and applications of algal hydrocolloids, in: C.A. Lembi, J.R. Waaland (Eds.), Algae and Human Affairs, Cambridge University Press, 1988, pp. 205–236.

[17] M. Robal, K. Truus, O. Volobujeva, E. Mellikov, R. Tuvikene, Thermal stability of red algal galactans: effect of molecular structure and counterions, Int. J. Biol. Macromol. 104 (2017) 213–223.

[18] M. Friedenthal, K. Eha, A. Viitak, A. Lukas, E. Siimer, Effects of drying on the gel strength and cation mobility of furcellaran, Innovat. Food Sci. Emerg. Technol. 1 (2001) 275–279.

[19] M. Friedenthal, K. Eha, A. Kaleda, N. Part, K. Laos, Instability of low-moisture carrageenans as affected by water vapor sorption at moderate storage temperatures, SN Appl. Sci. 2 (2020) 243.

[20] B. Quemener, M. Lahaye, Comparative analysis of sulfated galactans from red algae by reductive hydrolysis and mild methanolysis coupled to two different HPLC techniques, J. Appl. Phycol. 10 (1998) 75–81.

[21] K.S. Dodgson, R.G. Price, A note on the determination of the ester sulphate content of sulphated polysaccharides, Biochem. J. 84 (1) (1962) 106–110.

[22] A.I. Usov, A.S. Shashkov, Polysaccharides of algae. XXXIV: detection of iota-carrageenan in *Phyllophora-brodiaei* (turn.) J. Ag. (Rhodophyta) using 13C-NMR spectroscopy, Bot. Mar. 28 (9) (1985) 367–373.

[23] M. Saluri, M. Robal, R. Tuvikene, Hybrid carrageenans as beer wort fining agents, Food Hydrocoll. 86 (2019) 26–33.

[24] M.H. Chen, L.L. Wang, J.J. Chung, Y.-H. Kim, P. Atluri, J.A. Burdick, Methods to assess shear-thinning hydrogels for application as injectable biomaterials, ACS Biomater. Sci. Eng. 3 (2017) 3146–3160.

[25] K. Truus, R. Tuvikene, M. Vaher, T. Kailas, P. Toomik, T. Pehk, Structural and compositional characteristics of gelling galactan from the red alga *Ahnfeltia tobuchiensis* (Ahnfeltiales, the Sea of Japan), Carbohydr. Polym. 63 (1) (2006) 130–135.

[26] B. Yang, G.L. Yu, X. Zhao, W.N. Ren, G.L. Jiao, L.H. Fang, Y.H. Wang, G.H. Du, C. Tiller, G. Girouard, Structural characterisation and bioactivities of hybrid carrageenan-like sulphated galactan from red alga *Furcelaria lumbricalis*, Food Chem. 124 (1) (2011) 50–57.

[27] F. Van de Velde, S.H. Knutsen, A.I. Usov, H.S. Rollema, A.S. Cerezo, ^{1}H and ^{13}C high resolution NMR spectroscopy of carrageenans: application in research and industry, Trends Food Sci. Technol. 13 (2002) 73–92.

[28] J.S. Craigie, Cell walls, in: K.M. Cole, R.G. Sheath (Eds.), The Biology of Red Algae, Cambridge University Press, Cambridge, 1990, pp. 221–257.

[29] K. Laos, S. Ring, Characterisation of furcellaran samples from Estonian *Furcellaria lumbricalis* (Rhodophyta), J. Appl. Phycol. 17 (5) (2005) 461–464.

[30] R. Tuvikene, K. Truus, M. Robal, O. Volobujeva, E. Mellikov, T. Pehk, A. Kollist, K. Tiiu, M. Vaher, The extraction, structure and gelling properties of hybrid galactan from the red alga *Furcellaria lumbricalis* (the Baltic Sea, Estonia), J. Appl. Phycol. 22 (1) (2010) 51–63.

[31] R. Zamora, G. Gómez, J.F. Hidalgo, Classification of vegetable oils by high-resolution ^{13}C NMR spectroscopy using chromatographically obtained oil fractions, JAOCS 79 (3) (2002) 267–272.

[32] Q.-J. Peng, A.S. Perlin, Observations on N.M.R. spectra of starches in dimethyl sulfoxide, iodine-complexing, and solvation in water-di-methyl sulfoxide, Carbohydr. Polym. 160 (1987) 57–72.

[33] C. Rochas, M. Lahaye, Solid state ^{13}C-NMR spectroscopy of red seaweeds, agars and carrageenans, Carbohydr. Polym. 10 (1989) 189–204.

[34] Y. Aso, S. Yoshioka, J. Zhang, G. Zografi, Effect of water on the molecular mobility of sucrose and poly(vinylpyrrolidone) in a colyophilized formulation as measured by (13)C-NMR relaxation time, Chem. Pharm. Bull. 50 (6) (2002) 822–826.

[35] R. Lefort, P. Bordat, A. Cesaro, M. Descamps, Exploring the conformational energy landscape of glassy disaccharides by cross polarization magic angle spinning ^{13}C nuclear magnetic resonance and numerical simulations. II. Enhanced molecular flexibility in amorphous trehalose, J. Chem. Phys. 126 (2007), 014511.

[36] A.I. Usov, S.V. Yarotsky, A.S. Shashkov, ^{13}C-NMR spectroscopy of red algal galactans, Biopolymers 19 (1980) 977–990.

[37] A.I. Usov, NMR spectroscopy of red seaweed polysaccharides: agars, carrageenans, and xylans, Bot. Mar. 27 (1984) 189–202.

[38] R. Tuvikene, K. Truus, M. Robal, T. Pehk, T. Kailas, M. Vaher, T. Paalme, Structure and thermal stability of pyruvated carrageenans from the red alga *Coccotylus truncatus*, Carbohydr. Res. 344 (2009) 788–794.

[39] G.S.M. De Faria, L. Hayashi, A.R. Monteiro, Effect of drying temperature on carrageenan yield and quality of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) cultivated in Brazil, J. Appl. Phycol. 26 (2014) 917–922.

[40] Z.M. Al-Nahdi, A. Al-Alawi, I. Al-Marhobi, The effect of extraction conditions on chemical and thermal characteristics of kappa-carrageenan extracted from *Hypnea bryoides*, J. Mar. Sci. 2019 (2019). ID 5183261.

Appendix 4

Publication IV

Eha, K., Kaleda, A., Menert, A. and Laos, K. (2022). Water sorption behaviour of commercial furcellaran. Manuscript. Submitted to Heliyon.

Water sorption behaviour of commercial furcellaran

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ABSTRACT

Water sorption isotherms are important tool for designing the technological processes and predicting stability and shelf life of food. The aim of the work was to determine the water sorption isotherms of commercial furcellaran at different temperatures (20, 35 and 50 °C) using a gravimetric method under different levels of relative humidity (19–95%). The experimental data obtained have been interpreted in the terms of various isotherm models and it was found that the best results were obtained with the Peleg model. The obtained isotherms followed the type II pattern and, in all cases, the sorption capacity increased with increasing water activity and decreased with increasing temperature. The net isosteric heat of sorption decreased exponentially with increasing moisture content and it was slightly lower during adsorption than during desorption. The developed mathematical relationships can be used to optimize the drying processes and storage conditions of furcellaran.

Highlights

- Moisture sorption isotherms of furcellaran were determined.
- The optimal isotherm model for furcellaran is the Peleg model.
- An equation for the calculation of the net isosteric heat of the sorption of furcellaran was developed.

Keywords: furcellaran, sorption isotherm, modelling, net isosteric heat of sorption

1. Introduction

Furcellaran is sulphated polysaccharide extracted from the red seaweed *Furcellaria lumbricalis*. It is a hybrid of kappa- and beta-carrageenan (Eha et al., 2021), and is used in the food industry as a gelling and stabilising agent.

The physical and chemical properties of biopolymers are strongly dependent on their temperature and moisture histories. The high temperatures during production of furcellaran and the interaction between water can change the hydrocolloid structure and may make it sensitive to ambient humidity. To understand the water sorption characteristics among biopolymers and atmosphere the water sorption isotherms are usually determined that describes the relationship between the water content and water activity in certain temperature. These isotherms are important in order to optimise drying parameters, determine packaging requirements and estimate shelf life (Bazardeh & Esmaiili, 2014; Noriega et al., 2014; Bispo et al., 2015).

Several moisture sorption isotherm measurement techniques, such as vapour pressure manometric (Ajibola et al., 2003) and hygrometric (Demarchi et al., 2013; Pollatos et al., 2013),

are available, although the most widely used and recommended method is the static gravimetric technique (Stepien et al., 2020; Arslan-Tontul, 2021).

Water sorption isotherms are generated during wetting (water adsorption) or drying (water desorption) of food. These processes are not reversible and the gap between the curves is called moisture sorption hysteresis. Several different hysteresis loop shapes have been observed, depending on material and temperature (Sahin & Sumnu, 2006).

In the literature, different models can be found that make it possible to establish mathematical correlations between equilibrium moisture content and water activity, and that are commonly evaluated by the fitting of experimental data sets (Moreira et al., 2009). Among the most common models used for describing sorption in food products are GAB (Guggenheim, Anderson & de Boer), Caurie, Henderson-Thompson, Oswin, Peleg, and Smith.

The GAB model has a strong theoretical background and is an improvement on the BET (Brunauer et al., 1938) and Langmuir physical adsorption theories. The GAB model proposes multilayer adsorption assuming that molecules in liquid state are different from other layers (Andrade et al., 2011).

The Henderson model (Henderson, 1952) is semi-empirical model derived from the Gibbs adsorption theory. The Thompson modification of Henderson equation allows to describe processes at temperatures above absolute zero (Thompson et al., 1968).

Caurie, Oswin, Peleg and Smith are all empirical models. The Oswin model describes sigmoidal curves and was later modified by including a temperature term in the equation (Chen, 1990). Peleg is a four-parameter model that can be used for both sigmoidal and non-sigmoidal isotherms (Al-Muhtaseb et al., 2004). The Caurie's model is useful for dehydrated food as it takes account the moisture content, ensuring the stability of food during storage (Zapata et al., 2014). The Smith model has a good fit to polymers with high molecular weight. The model assumes that the second fraction of water has multilayers that prevent the first fraction from evaporating (Smith, 1947).

The sorption process involves changes in the isosteric heat of desorption or adsorption. The isosteric heat of sorption estimates the moisture absorbed by solid particles (Mulet et al., 1999) and is defined as the difference between isosteric heat and the heat of vaporisation at the system temperature. It can be used for drying process design and provides information on the state of water molecules in food matrices (Tadapaneni et al., 2017).

Every food has its own special isotherms and there is no available isotherm information on furcellaran. Understanding the water sorption characteristics of furcellaran is essential in industrial processing in order to optimise the drying process and ensure storage stability. So the aims of this work were to provide experimental data for the sorption characteristics of furcellaran, to find the best-fitting model to describe the sorption isotherms and to determine the net isosteric heat of sorption.

2. Materials and methods

2.1. Materials

The studied commercial furcellaran was an industrial product from AS EstAgar (Kärla, Estonia). Five saturated salt solutions (CH_3COOK, $Mg(NO_3)_2*6H_2O$, NaCl, KCl and KNO_3) were prepared to obtain constant relative humidity environments. All salt solutions were reagent grade.

2.2. Water sorption behaviour

Sorption isotherms measurements

The experimental water adsorption and desorption isotherms of furcellaran were determined at the temperatures 20, 35 and 50 °C at different water activities within the range of 0.19–0.95 (Table 1), using a static gravimetric method. First, samples for desorption processes were placed into an environment of 100% (H_2O) relative humidity until equilibrium was achieved. The samples with the initial water activity ($a_w = 0.235$) were used to study adsorption. The samples (0.5 ± 0.0001 g) were placed in sealed jars, which were provided with different saturated salt solutions to generate atmospheres of different relative humidity, until equilibrium was achieved. The samples were weighed at regular intervals until a constant weight (±0.0005 g) was established. The average time of sample equilibration was around three months.

Table 1. The water activities (a_w) of saturated salt solutions at various temperatures (Greenspan, 1977).

	Salt solutions				
	CH_3COOK	$Mg(NO_3)_2*6H_2O$	NaCl	KCl	KNO_3
a_w at 20°C	0.235	0.544	0.755	0.851	0.946
a_w at 35°C	0.208	0.499	0.749	0.830	0.908
a_w at 50°C	0.192	0.454	0.744	0.812	0.848

Modelling of sorption isotherms

The experimental data regarding the sorption isotherms of furcellaran were fitted to the GAB, Caurie, Henderson-Thompson, modified Oswin, Peleg and Smith mathematical models. Table 2 shows equations for the used models in this study. The non-linear regression function from package "nls2" 0.2 for R 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria) was used to fit the equations to experimental results and to estimate the parameters of the models.

Table 2. Models applied to the experimental water sorption data of furcellaran.

Name of the model	Model equation	Reference
GAB	$X_e = \frac{a\ b\ c\ a_w}{(1 - ca_w)(1 - ca_w + bca_w)}$	Van der Berg and Bruin, 1981
Caurie	$X_e = \exp(a + ba_w)$	Castillo et al., 2003
Henderson-Thompson	$X_e = \left[\frac{\ln(1 - a_w)}{-a(T + c)}\right]^{1/b}$	Thompson et al., 1968
Modified Oswin	$X_e = (a - bT)\left(\frac{a_w}{1 - a_w}\right)^c$	Chen, 2000
Peleg	$X_e = a(a_w)^b + c(a_w)^d$	Peleg, 1993
Smith	$X_e = a - b\ (\ln(1 - a_w))$	Smith, 1947

Where X_e is the equilibrium moisture content (g g^{-1} d.b.), a_w is the water activity, a, b, c, d are adjustable parameters and T is temperature (°C).

The goodness of fit of the different models to data was assessed by the mean relative percentage deviation modulus (P), the root mean square error (RMSE) and the coefficient of determination (R^2), determined by using equations 1–3 (Bahloul et al., 2008).

$$P = \frac{100}{N} \sum_{j=1}^{N} \left| \frac{Xe_{j\,cal} - Xe_{j\,exp}}{Xe_{j\,exp}} \right| \quad (1)$$

$$RMSE = \sqrt{\sum_{j=1}^{N} \frac{(Xe_{j\,cal} - Xe_{j\,exp})^2}{N - n_p}} \quad (2)$$

$$R^2 = \frac{S_t - SCE}{S_t} \quad (3)$$

where

$$S_t = \sqrt{\frac{\sum_{j=1}^{N}(\overline{Xe} - Xe_j)^2}{n-1}} \qquad \overline{Xe} = \frac{\sum_{j=1}^{N} Xe_j}{N}$$

$$SCE = \sum_{j=1}^{N}\left(Xe_{j\,cal} - Xe_{j\,exp}\right)^2$$

where $Xe_{j\,cal}$ and $Xe_{j\,exp}$ are calculated and experimental values of the equilibrium moisture content ($\overline{Xe}$), respectively. N is the number of data points and n_p is the number of free parameters in the model. SCE is the model sum of squares and S_t is the total sum of squares. Values of P below 10% are indicative of a good fit (Aguerre et al., 1989).

Net isosteric heat of sorption

The net isosteric heat of sorption (Q_{st}) was determined from the moisture sorption data using an equation derived from the Clausius-Clapeyron equation (Jamali et al., 2006) as follows:

$$\ln(a_w) = -\left(\frac{Q_{st}}{R}\right)\frac{1}{T} + K \qquad (4)$$

where a_w is the water activity, Q_{st} is the net isosteric heat of sorption (J g^{-1}), R is the water characteristic gas constant (0.4615 J g^{-1} K^{-1}) and T the absolute temperature (K).

The net isosteric heat of sorption can be calculated from equation 4 by plotting the sorption isotherm as $\ln(a_w)$ versus T^{-1} for the fixed moisture content of the material and determining the slope which equals $-Q_{st} * R^{-1}$ (Tsami, 1991). This procedure was repeated for all values of the equilibrium moisture content. The water activity values of the material for different temperatures were obtained using the equation that best fit the experimental moisture sorption data.

3. Results and discussion

Sorption isotherms

The moisture adsorption and desorption isotherms of furcellaran determined at 20, 35 and 50 °C are given in Figure 1. All sorption isotherms were Type II according to BET and IUPAC classifications and had sigmoidal shapes, which indicated multi-layers of sorption of water in a macroporous material. In all cases, the equilibrium moisture content increased with increasing water activity at each temperature and decreased with increasing temperature at water activity < 0.76. This thermal behaviour may be explained by the fact that at higher temperatures the

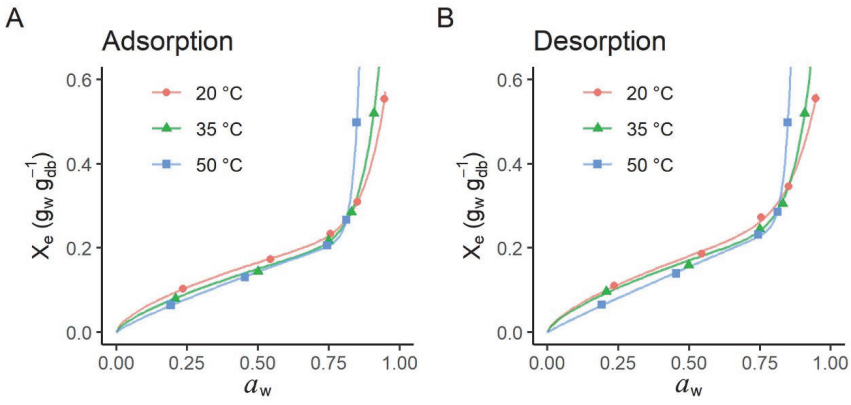

Figure 1. Water adsorption (A) and desorption (B) isotherms of furcellaran at different temperatures. X_e the equilibrium moisture content. Dots correspond to experimental data and lines to the Peleg model.

kinetic energy of water molecules increases, thereby increasing their distance and reducing the attractive forces between them. As a result, the sorption degree decreases with increasing water activity as the temperature increases (Bahloul et al., 2008).

A clear increase in moisture content can be seen in adsorption (Figure 1A) and correspondingly a decrease in moisture loss for desorption (Figure 1B) for water activity > 0.76 at all temperatures. This phenomenon has been observed with foods rich in soluble solids (Seth, 2018; Sormoli & Langrish, 2015) as soluble solids can bind water resulting the decrease of water activity. The intersecting of different isotherms can be observed. This is probably due to faster dissolution of soluble solids at higher temperatures. Also, more water binding sites in the furcellaran may be exposed due to the thermal effect.

Small differences in hysteresis can be seen in comparing moisture adsorption and desorption isotherms at each temperature (Figure 2). In all cases, the largest differences between the adsorption and desorption processes were observed at 20 °C, decreasing with increasing temperatures. These results may be related to the process of the solubility of some compounds in water, promoted by temperature and structural changes (Bell & Labuza, 2000). This thermal impact on hysteresis cycles has been previously found for agar (Iglesias & Bueno, 1999), high polysaccharide content red seaweeds, such as *Gracilaria chilensis* (Lemus et al., 2008), and starchy flours (Moreira et al., 2010).

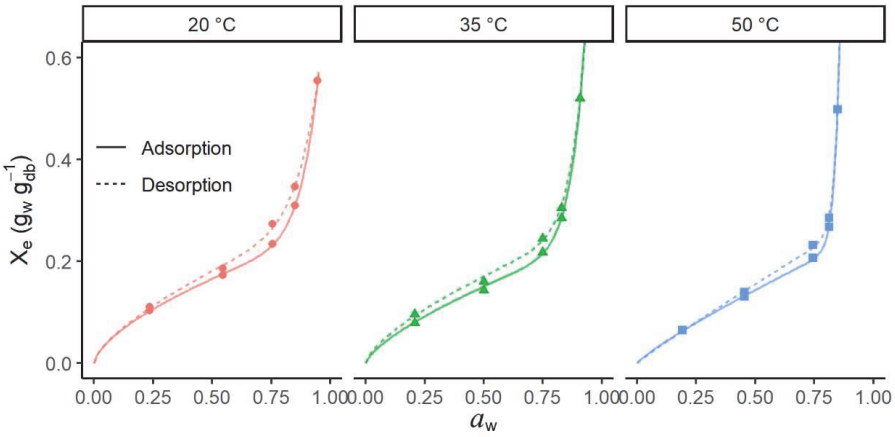

Figure 2. Sorption isotherms with a hysteresis between adsorption and desorption of furcellaran at different temperatures.

The parameter values and statistical results at 20, 35 and 50 °C for all models used for adsorption and desorption isotherms are shown in Table 3 and Table 4, respectively.

Two of the proposed models did not provide satisfactory fits to the experimental data. The mean relative percentage deviation moduli (P, %) corresponding to the Caurie and Henderson-Thompson models were higher than 15% and cannot be used to simulate the sorption data of furcellaran. The Peleg model was found to be the most appropriate model to describe both the adsorption and desorption curves (Figure 1) for different water activities. The average P was 1.2% for adsorption and 2.1% for desorption; the coefficient of determination (R^2) was 1.00.

Table 3. Results of fitting the adsorption isotherms of furcellaran.

Adsorption isotherms								
Models names	T, °C	Parameters				P, %	RMSE	R^2
		a	b	c	d			
GAB	20	0.074	109.611	0.914	–	7.6	0.03	0.99
	35	0.058	148.202	0.978	–	7.9	0.03	0.99
	50	0.066	73.220	0.996	–	19.2	0.08	0.87
Caurie	20	-3.791	3.273	–	–	23.5	0.06	0.90
	35	-4.488	4.127	–	–	29.7	0.06	0.89
	50	-4.896	4.733	–	–	38.4	0.09	0.80
Henderson Thompson	20	0.017	1.060	322.396	–	18.0	0.06	0.95
	35	0.011	0.788	366.182	–	26.5	0.07	0.92
	50	0.021	0.687	115.805	–	36.7	0.10	0.81
Modified Oswin	20	-1.821	-0.099	0.422	–	5.8	0.03	0.99
	35	-1.853	-0.057	0.565	–	12.5	0.05	0.96
	50	-2.362	-0.049	1.051	–	34.8	0.09	0.86
Peleg	20	0.257	0.637	0.626	12.917	0.6	0.00	1.00
	35	0.246	0.711	1.298	15.529	1.8	0.00	1.00
	50	0.260	0.856	149.854	38.293	1.1	0,00	1.00
Smith	20	0.031	0.168	–	–	13.1	0.04	0.96
	35	0.001	0.190	–	–	20.6	0.06	0.91
	50	0.001	0.207	–	–	24.3	0.09	0.78

P is mean relative percentage deviation modulus; RMSE is root mean square error; R^2 is coefficient of determination; a, b, c, d are parameters of the equations; T is temperature (°C).

Table 4. Results of fitting the desorption isotherms of furcellaran.

Desorption isotherms								
Models names	T, °C	Parameters				P, %	RMSE	R^2
		a	b	c	d			
GAB	20	0.096	64.942	0.870	–	3.5	0.02	0.99
	35	0.073	41.956	0.946	–	8.3	0.03	0.98
	50	0.069	108.357	0.997	–	17.6	0.07	0.91
Caurie	20	-3.290	2.765	–	–	15.4	0.05	0.94
	35	-3.653	3.180	–	–	22.4	0.06	0.90
	50	-4.037	3.721	–	–	28.2	0.08	0.83
Henderson Thompson	20	0.048	1.150	104.263	–	12.3	0.04	0.97
	35	0.017	0.964	242.226	–	21.3	0.06	0.92
	50	0.016	0.753	165.822	–	31.7	0.09	0.84
Modified Oswin	20	-2.035	-0.111	0.333	–	7.4	0.05	0.96
	35	-2.686	-0.081	0.547	–	11.8	0.04	0.97
	50	-2.646	-0.055	0.788	–	24.9	0.08	0.89
Peleg	20	0.289	0.677	0.492	10.416	1.9	0.01	1.00
	35	0.275	0.689	1.104	14.753	2.8	0.01	1.00
	50	0.298	0.936	148.924	38.990	1.7	0.01	1.00
Smith	20	0.054	0.165	–	–	5.4	0.02	0.99
	35	0.027	0.184	–	–	14.2	0.05	0.93
	50	0.001	0.213	–	–	21.0	0.08	0.83

P is mean relative percentage deviation modulus; RMSE is root mean square error; R^2 is coefficient of determination; a, b, c, d are parameters of the equations; T is temperature (°C).

Net isosteric heat of sorption

The experimental data of sorption isotherms were used to determine the net isosteric heat of the sorption of furcellaran. The Peleg model with equation 4 was used to obtain the values of the water activity at constant moisture content at each temperature (Figure 3).

The net isosteric heat decreases as the moisture content increases, showing that the energy needed to adsorb or remove the water in the furcellaran decreases with moisture content. It is known that the sorption occurs at first on the most active polar sites having the greatest interaction energy (Tsami, 1991). When increasing the water content, the sites will occupied

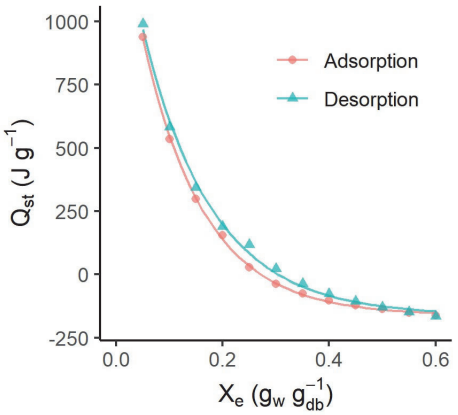

Figure 3. Net isosteric heat (Q_{st}) of furcellaran for adsorption and desorption for different moisture contents (X_e).

and sorption occurs on the less active sites and the net isosteric heat is decreasing. The net isosteric heat of desorption was slightly higher than that of adsorption, indicating that the energy required for the water desorption process was higher than that needed for the adsorption process.

The net isosteric heat of adsorption and desorption approached zero at 0.26 $g_w\ g_{db}^{-1}$ and 0.32 $g_w\ g_{db}^{-1}$, respectively. It shows that there is a critical moisture content where the net isosteric heat of adsorption and desorption is equal to the vaporisation of pure water, and the water molecules act as in the liquid state. So, at higher moisture content less energy is needed during material drying.

However, after a certain moisture content, the heat of sorption values became negative. This may be due to an increase in the moisture content by the dissolution of sugars and possibly of biopolymer at higher temperatures. Negative heat of sorption values has no physical meaning and is due to mathematical calculations.

The net isosteric heat of the sorption of water in furcellaran can approximated mathematically by equation 5:

$$Q_{st} = a * \exp(k * X_e) + b \quad (5)$$

For adsorption: P = 5.5; RMSE = 8.95; R^2 = 0.999.

$$Q_{st} = 1682.70 \exp\ (-8.616 * X_e) - 161.40$$

For desorption: P = 15.65; RMSE = 20.74; R^2 = 0.997.

$$Q_{st} = 1655.41 \exp(-7.632 * X_e) - 162.22$$

These mathematical equations can applied to predict the heat of the sorption of furcellaran for different moisture contents.

4. Conclusions

The moisture sorption behaviour of furcellaran exhibits the type II pattern. The sorption behaviour was found to decrease with temperature at fixed water activity with a crossover behaviour at water activity around 0.76. Hysteresis between the adsorption and desorption processes decreased with increasing temperature. The experimental sorption data of furcellaran was well expressed by the Peleg equation. The net isosteric heat of sorption was higher at low

moisture contents, indicating that water molecules more strongly interact with furcellaran at the lower moisture content. The net isosteric heat of adsorption was lower than that of desorption and they negatively correlated with moisture content. The developed heat of the sorption equations will be useful in the simulation of furcellaran during drying process and storage.

References

Aguerre, R.J., Suarez, C., & Viollaz, P.Z. (1989). New BET type multi-layer sorption isotherms. – Part II: Modelling water sorption in foods. Lebensmittel-Wissenschaft und Technologie 22(4), 192–195.

Ajibola, O.O., Aviara, N.A., & Ajetumobi, O.E. (2003). Sorption equilibrium and thermodynamic properties of cowpea (*Vigna unguiculata*). Journal of Food Engineering 58(4), 317–324.

Al-Muhtaseb, A.H., McMinn, W.A.M. & Magee, T.R.A. (2004). Water sorption isotherms of starch powders. Part 1: mathematical description of experimental data. Journal of Food Engineering 61, 297–307.

Arslan-Tontul, S. (2021). Moisture sorption isotherm and thermodynamic analysis of quinoa grains. Heat and Mass Transfer 57(3), 543–550.

Andrade, P.R.D., Lemus, M.R., & Perez, C.C.E. (2011). Models of sorption isotherms for food: Uses and limitations. Vitae-Revista De La Facultad De Quimica Farmaceutica 18(3), 325–334.

Bahloul, N., Boudhrioua, N., & Kechaou, N. (2008). Moisture desorption-adsorption isotherms and isosteric heats of sorption of Tunisian olive leaves (*Olea europaea L.*). Industrial Crops and Produtcs 28(2), 162–176.

Bazardeh, M.E., & Esmaiili, M. (2014). Sorption isotherm and state diagram in evaluating storage stability forsultana raisins. Journal of Stored Products Research 59, 140–145.

Bell, L.N., & Labuza, T.P. (2000). L.N. Bell (Ed.), Moisture Sorption: Practical Aspects of Isotherm Measurement and Use (2nd ed.) American Association Cereal Chemistry, St. Paul, Minnesota.

Bispo, J.A.C., Bonafe, C.F.S., Santana, K.M.O.V., & Santos, E.C.A. (2015). A comparison of drying kinetics based on the degree of hydration and moisture ratio. LWT – Food Science and Technology 60(1), 192–198.

Brunauer, S., Emmet, P.H., & Teller E. (1938). Adsorption of gases in multimolecular layers. Journal of the American Chemical Society 60(2), 309-319.

Castillo, M.D., Martinez, E.J., Gonzalez, H.H.L., Pacin, A.M., & Resnik, S.L. (2003). Study of mathematical models applied to sorption isotherms of Argentinean black bean varieties. Journal of Food Engineering 60, 343–348.

Chen, C.-C. (1990). Modification of Oswin EMC/ERH equation. Journal of Agricultural Research of China 39(4), 367–376.

Chen, C. (2000). Rapid model to determinate the sorption isotherms of peanuts. Journal of Agricultural Engineering Research 75, 401–408.

Demarchi, S.M., Ruiz, N.A.Q., De Michelis, A., & Giner, S.A. (2013). Sorption characteristics of rosehip, apple and tomato pulp formulations as determined by gravimetric and hygrometric methods. LWT – Food Science and Technology 52(1), 21–26.

Eha, K., Pehk, T., Heinmaa, I., Kaleda, A., & Laos, K. (2021). Impact of short-term heat treatment on the structure and functional properties of commercial furcellaran compared to commercial carrageenans. Heliyon 7, e06640.

Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. Journal of Research of the National Bureau of Standards – A Physics and Chemistry 81A, 89–96.

Henderson, S.M. (1952). A basic concept of equilibrium moisture. Agriculture Engineering 2, 29–32.

Iglesias, O., & Bueno, J.L. (1999). Water agar-agar equilibrium: determination and correlation of sorption isotherms. International Journal of Food Science and Technology 34(3), 209–216.

Jamali, A., Kouhila, M., Mohamed, L.A., Jaouhari, J.T., Idlimam, A., & Abdenouri, N. (2006). Sorption isotherms of *Chenopodium ambrosioides* leaves at three temperatures. Journal of Food Engineering 72, 77–84.

Lemus, R.A., Pérez, M., Andrés, A., Roco, T., Tello, C.M., & Vega, A. (2008). Kinetic study of dehydration and desorption isotherms of red alga Gracilaria. LWT - Food Science and Technology 41(9), 1592-1599.

Moreira, R., Chenlo, F., & Torres, M.D. (2009). Simplified algorithm for the prediction of water sorption isotherms of fruits, vegetables and legumes based upon chemical composition. Journal of Food Engineering 94(3–4), 334–343.

Moreira, R., Chenlo, F., Torres, M.D., & Prieto, D.M. (2010). Water adsorption and desorption isotherms of chestnut and wheat flours. Industrial Crops and Products 32(3), 252–257.

Mulet, A., Garcia-Reverter, J., Sanjuan, N. & Bon, J. (1999). Sorption isosteric heat determination by thermal analysis and sorption isotherms. Journal of Food Science 64(1), 64–68.

Noriega, M.D., Estrada, O., & López, I. (2014). Computational model to design plastic multi-layer films for food packaging to assure a shelf life at the best cost. Journal of Plastic Film & Sheeting 30(1), 48–76.

Peleg, M. (1993). Assessment of a semi-empirical four parameter general model for sigmoid moisture sorption isotherms. Journal of Food Process Engineering 16, 21–37.

Pollatos, E.P., Riganakos, K.A., & Demertzis, P.G. (2013). Moisture sorption characteristics of Greek durum wheat semolina. Starch-Starke 65 (11–12), 1051–1060.

Sahin, S., & Sumnu, S.G. (2006). Physical properties of foods. New York: Springer.

Seth, D., Dash, K.K., Mishra, H.N., & Deka, S.C. (2018). Thermodynamics of sorption isotherms and storage stability of spray dried sweetened yoghurt powder. Journal of Food Science and Technology 55(10), 4139–4147.

Smith, S.E. (1947). Sorption of wheat vapour by high polymers. Journal of the American Chemical Society 69, 646–651.

Sormoli, M.E., & Langrish, T.A.G. (2015). Moisture sorption isotherms and net isosteric heat of sorption for spray-dried pure orange juice powder. LWT – Food Science and Technology 62(1), 875–882.

Stepien, A., Witczak, M., & Witczak, T. (2020). Moisture sorption characteristics of food powders containing freeze dried avocado, maltodextrin and inulin. International Journal of Biological Macromolecules 149, 256–261.

Tadapaneni, R.K., Yang, R., Carter, B., & Tang, J.M. (2017). A new method to determine the water activity and the net isosteric heats of sorption for low moisture foods at elevated temperatures. Food Research International 102, 203–212.

Thompson, T.L., Peart, R.M., & Foster, G.H. (1968). Mathematical simulation of corn drying – a new model. Transactions of the American Society of Agricultural Engineers 24(3), 582–586.

Tsami, E. (1991). Net isosteric heat of sorption in dried fruits. Journal of Food Engineering 14, 327–335.

Van der Berg, C., & Bruin, S. (1981). Water activity and its estimation in food systems; theoretical aspects. In L.B. Rockland, & G.F. Stewart (Eds.), Water Activity: Influences on Food Quality (pp. 1–61). Academic Press, New York.

Zapata, M.J.E., Quintero, C.O.A., & Porras, B.L.D. (2014). Sorption isotherms for oat flakes (*Avena sativa L.*). Agronomía Colombiana 32(1), 52–58.

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