

Effects of sucrose addition on the rheology and microstructure of κ -carrageenan gel



Zhi Yang^{a, b}, Huijuan Yang^{a, c}, Hongshun Yang^{a, b, *}

^a Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, Singapore 117543, Singapore

^b National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu 215123, PR China

^c Synergetic Innovative Centre of Food Safety and Nutrition, Key Laboratory of Meat Processing and Quality Control, Ministry of Education, Key Laboratory of Animal Products Processing, Ministry of Agriculture, Jiangsu Innovative Centre of Meat Production and Processing, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China

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ABSTRACT

The thermoreversible gelation behaviour of aqueous κ -carrageenan solution with addition of sucrose (up to 30 wt%) was investigated via small and large deformation oscillatory rheology. The structures of these κ -carrageenan/sucrose gels were characterised by Fourier transform infrared (FTIR) spectroscopy, small angle X-ray scattering (SAXS) and field emission scanning electron microscopy (FESEM). Incorporation of 30 wt% sucrose shifted both the gelation temperature (from 36.8 °C to 52.8 °C) and melting temperature (from 51.2 °C to 67.3 °C) to a higher level and made gel network stronger. The critical relaxation exponent n , critical gel strength S_g and normalised S_g obtained from Winter-Chambon equation varied as the concentration of sucrose increased. More specifically, with an increased addition of sucrose, n decreased while both S_g and normalised S_g increased. The shift of FTIR band of G4S in κ -carrageenan and loss of the vibration band of free hydroxyl group in sucrose indicated the interactions between sucrose and κ -carrageenan molecules. SAXS and FESEM results supported the rheology findings and revealed that denser and thicker carrageenan fibril structures formed when addition of sucrose increased. Moreover, upon 30 wt% sucrose, the average cross-sectional radius of gyration R_c of the carrageenan chains increased from 1.17 to 1.47 nm. In summary, a scheme was proposed to demonstrate how sucrose addition promoted the gelation of κ -carrageenan by facilitating the formation of denser and thicker fibril helical junctions.

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1. Introduction

Kappa-carrageenan, which forms thermo-reversible gel, is a sulphated polysaccharide extracted from different species of red seaweed (Hilliou, Wilhelm, Yamanoi, & Gonçalves, 2009). During the last 40 years, extensive research has been performed to investigate the gelation behaviour and mechanism of κ -carrageenan in aqueous solution extracted from different seaweed species (Hugerth, Nilsson, & Sundelöf, 1999). The gelation of κ -carrageenan is generally believed to involve two steps: the coil-helix transition and subsequent aggregation of double helices (Du, Brenner, Xie, & Matsukawa, 2016). Due to its excellent biodegradability and biocompatibility, κ -carrageenan is not only traditionally used as

texturing agent in food, cosmetic, and personal care industry, but also used in medical care, drug controlled release and encapsulation (Necas & Bartosikova, 2013).

The gelation of κ -carrageenan is influenced by temperature, concentration, type and amount of metal salts, and presence of food ingredients such as sugars (Azizi & Farahnaky, 2016; Stenner, Matubayasi, & Shimizu, 2016). To further expand the application of κ -carrageenan in real food systems e.g. confectionary products and beverages, it is extremely important to understand how the presence of common food ingredients like sucrose affects the gelling properties of κ -carrageenan. Indeed, in the past 20 years, the effect of sugar addition on the physico-chemical properties of κ -carrageenan gel was investigated (Al-Marhoobi & Kasapis, 2005; Kasapis, Mitchell, Abeysekera, & MacNaughtan, 2004; Loret, Ribelles, & Lundin, 2009; Oakenfull, 2000, pp. 277–284; Stenner et al., 2016). Most studies found that increasing sugar concentration up to 45 wt% enhanced the

* Corresponding author. Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, Singapore 117543, Singapore.
E-mail address: chmynghs@nus.edu.sg (H. Yang).

gel strength and gelation temperature of κ -carrageenan compared to their counterparts without sugar addition (Gekko, Mugishima, & Koga, 1987; Katsuyoshi; Nishinari, Watase, Williams, & Phillips, 1990). The effect of various sugars on the thermal properties of κ -carrageenan gel was also investigated by Differential Scanning Calorimetry (DSC). The finding shows that the increase of melting temperature is linearly related to the increase of weighted average number of equatorial OH-groups present on the sugars, which could be due to changed structure of water or direct interaction between OH-groups of sugar and κ -carrageenan (Nishinari & Watase, 1992). Computer simulation demonstrates that addition of sugar changes the structure of water and simultaneously changes the biopolymer hydration (Oakenfull, 2000, pp. 277–284). Through statistical thermodynamic simulation, the cosolvent exclusion and cosolvent-biopolymer binding were believed to be the dominant factor contributing to the enhanced κ -carrageenan gelation induced by sugar addition (Stenner et al., 2016). Therefore, the mechanism underlying the enhanced gelation and gel strength of κ -carrageenan upon sugar addition remains unclear.

Rheology is a sophisticated and powerful tool to investigate the sol-gel transition and viscoelastic behaviour of food gels. Recently, Winter and Chambon scaling laws have been successfully applied in the characterisation of critical gelling properties and rheological scaling behaviour of κ -carrageenan in aqueous solution (Liu, 2015), κ -carrageenan/ Ca^{2+} system (Liu & Li, 2016), and alginate hydrogels (Liu, Li, Tang, Bi, & Li, 2016; Lu, Liu, & Tong, 2006). However, to date, the Winter and Chambon scaling laws have not been employed to investigate the sol-gel transition behaviour in aqueous κ -carrageenan/sucrose system. Besides this, in the literature there is only limited information on the morphology and microstructure of κ -carrageenan gels affected by sucrose addition.

This study is aimed to investigate the rheological and microstructural properties of κ -carrageenan gel as affected by sucrose addition up to 30 wt%. The Winter and Chambon scaling law was employed to understand the critical gelling behaviour of κ -carrageenan gels near their sol-gel transition temperature. The oscillatory rheology was applied to examine the small and large deformation properties of the gels, while the interactions between sucrose and κ -carrageenan was determined by Fourier transform infrared (FTIR) spectroscopy. Moreover, the microstructure and morphology of κ -carrageenan gels were characterised using small-angle X-ray scattering (SAXS) and field emission scanning electron microscopy (FESEM). Finally, the possible mechanism for the enhanced gelation behaviour induced by sucrose addition was proposed and discussed based on their rheological and microstructural characteristics.

2. Materials and methods

2.1. Materials and sample preparation

κ -Carrageenan powder (Sigma-Aldrich, USA) and sucrose (Merck, USA) were used without further purification. κ -Carrageenan solution was prepared following the procedure similar as (Liu & Li, 2016). For 2 wt% homogenous solution, the κ -carrageenan powder was dissolved in Milli-Q water under mild magnetic stirring at 70 °C for 2 h until the powder was fully swollen and solubilised. To investigate the effect of sucrose addition on κ -carrageenan gels, different amount of sucrose was dry mixed with κ -carrageenan powder before hydration and solubilised upon heating. The final concentration of the sucrose was fixed at 10 wt%, 20 wt%, and 30 wt%.

2.2. Rheology

The rheological characterisation was conducted in a rotational Anton Paar MCR 102 stress-controlled rheometer (Anton Paar, Graz, Austria) equipped with a stainless-steel cone and plate geometry (angle: 1° and diameter: 60 mm). The sample for rheological characterisation was poured from a sample vial to a rheometer's bottom plate which was preheated to 70 °C and maintained for 5 min at 70 °C to relax the sample and melt all possible helical structures. Sunflower oil was placed around the perimeter of the sample to prevent water evaporation during measurement. The following three protocols for rheological characterisation were carried out. (1) A temperature sweep with a cooling and heating rate of 1 °C/min at a constant angular frequency of 1 rad/s and fixed strain of 1% was carried out from 80 °C to 20 °C and subsequently from 20 °C to 80 °C to obtain the gelation temperature and melting temperature, respectively. (2) A frequency sweep (100–1 rad/s) was conducted at various temperatures near sol-gel transition temperature obtained in protocol (1) at a fixed strain of 1%. (3) The temperature decreased from 80 °C to 20 °C at 1 °C/min until initial gel was formed. Then the gel network was formed at 20 °C for 5 h. During gelation, time sweep measurement was performed at a constant angular frequency of 1 rad/s with a constant strain 1% to monitor gelation evolution. At the end of time sweep (gelation), the frequency sweep tests were carried out at a fixed strain 1% with angular frequency ranged from 100 to 1 rad/s. Finally, the strain amplitude sweep measurements were conducted at a fixed angular frequency of 1 rad/s with strains in the range from 0.1% to 10000% to characterise the large deformation rheological properties. The storage modulus G' and loss modulus G'' were recorded during the above dynamic rheological measurements (Yang et al., 2015).

2.3. Fourier transform infrared spectroscopy

Various κ -carrageenan/sucrose hydrogel samples were freeze dried and milled with KBr powder. The Spectrum one FTIR spectrometer (PerkinElmer, MA, USA) was employed to record FTIR spectra. The scan range was set in the range of 4000 and 450 cm^{-1} at 4 cm^{-1} resolution. The background scan was performed before each sample measurement. All spectra were averaged from at least duplicate measurements with 32 scans before baseline correction and smoothing.

2.4. Small-angle X-ray scattering

Synchrotron small-angle X-ray scattering (SAXS) experiments were performed on the beamline BL16B1 at the Shanghai Synchrotron Radiation Facility (SSFR, Shanghai, China). The wavelength of monochromatic beam was 0.1240 nm, and the sample-to-detector distance was 2000 mm, covering the q ranges of 0.8–1.8 nm^{-1} . The FIT2D software was employed to transfer 2D scattering pattern to 1D scattering curve. The κ -carrageenan solutions were pipetted in a sample holder sealed with Kapton tapes on both sides. The κ -carrageenan solutions were set to form a gel for 5 h at room temperature (~25 °C) before SAXS measurement. All the data were background subtracted from the scattering of Kapton tapes and solvents.

2.5. FESEM observation

The microstructures of κ -carrageenan/sucrose gels were characterised using a field emission scanning electron microscopy (FESEM, JSM-6701F, JEOL, Japan) with accelerating voltage of 5 kV. The κ -carrageenan and κ -carrageenan/sucrose hydrogels with various sucrose concentrations were freeze dried at –80 °C for 5

days, and the platinum was sputter coated on the dry sample surface under vacuum before FESEM.

3. Results and discussion

3.1. Effect of sucrose addition on the thermoreversible behaviour of κ -carrageenan hydrogel

Fig. 1 demonstrates the viscoelastic modulus changes with temperature during cooling-melting cycles for pure κ -carrageenan and κ -carrageenan/sucrose hydrogels. During cooling, both storage and loss modulus (G' and G'') increased marginally with reducing temperature because of the loss of molecular chain mobility; and the G'' was still above G' , suggesting that the sample was still in solution state. Upon further temperature decreasing, both G' and G'' increased dramatically and G' increased much faster than G'' . Therefore, at certain temperature, G' and G'' crossover occurred and thus the gelation point (temperature) could be determined as the critical temperature at which $G' = G''$ (Michon, Cuvelier, & Launay, 1993). Beyond the gelation temperature, G' and G'' continuously increased with decreased temperature, and G' became higher than G'' , indicating an evolution from solution state to gel state caused by helix aggregation. In the successive heating and melting, the gel network was disrupted, as illustrated by the reduction of both G' and G'' . However, upon further increment of temperature, the gel to solution transition happened together with G' and G'' crossover, the temperature at which was defined as melting point. Beyond the melting point, the value of G'' was greater than that of G' , indicating that the gel network was disrupted and the gel became a viscous liquid solution, probably caused by the dissociation of helical aggregates and broken gel network (Liu & Li, 2016).

As the sucrose concentration increased, both the gelation temperature (T_g) and melting temperature (T_m) shifted to a higher level (Fig. 1), suggesting that the sol-gel transition was promoted by sucrose inclusion. Moreover, the T_g (coil-gel) was lower than the T_m (gel-coil) for all the carrageenan gels because of the hysteresis of the phase transition loops (Tari, Kara, & Pekcan, 2009). For G' value, at the lowest temperature studied here (20 °C), it increased with increasing sucrose concentration, suggesting the sucrose inclusion strengthened the κ -carrageenan network. It was concluded that sucrose addition improved the gel properties. The conclusion could

be related to the strong hydration of sucrose in aqueous solutions, which made it exclude from κ -carrageenan strands since it favoured hydration into bulk aqueous phase (Shimizu & Matubayasi, 2014). The exclusion of sucrose decreased the average water molecule population surrounding κ -carrageenan strands, leading to weakened κ -carrageenan strand-water interactions. Thus, the κ -carrageenan strands tended to self-associate with their neighbours (Oakenfull, 2000, pp. 277–284). The sucrose bound to κ -carrageenan molecules through formation of cross-linking hydrogen bonds between equatorial-OH groups of sucrose and κ -carrageenan molecules, which facilitated the formation of junction zone and gelation (Nishinari & Watase, 1992). In summary, the unfavourable interactions between κ -carrageenan and sucrose in the sol state shifted the sol-gel equilibrium to the gel state, where intermolecular hydrogen bonds between sucrose and κ -carrageenan stabilised the gel (Stenner et al., 2016). Therefore, the sol-gel transition, gel network strength and stability of κ -carrageenan were greatly improved upon sucrose addition.

3.2. Viscoelasticity of aqueous κ -carrageenan/sucrose solution during sol-gel transition

Although the gelling temperature of various κ -carrageenan/sucrose gels was obtained from the crossover points of G' and G'' during temperature sweep, it should be noted that these values were obtained under certain condition (cooling rate: 1 °C/min and frequency: 1 rad/s). It was reported that the cooling rate and frequency affected the gelling point (Liu, Bao, & Li, 2016). Thus, in order to obtain more accurate gelling temperature and understand the viscoelasticity of aqueous κ -carrageenan/sucrose during sol-gel transition, the Winter-Chambon and scaling laws were applied in the vicinity of gelling points.

Based on the Winter-Chambon equation (Winter & Chambon, 1986), at the critical gelling point a power-law dependence on the angular frequency ω for both the elastic and viscous modulus G' and G'' can be observed

$$G'(\omega) \sim G''(\omega) \sim \omega^n \quad (1)$$

The shear relaxation modulus $G(t)$ at the gel point exhibited a power law behaviour at the critical gelling point

$$G(t) = S_g t^{-n} \quad (2)$$

Where n and S_g are defined as the critical relaxation exponent and critical gel strength, respectively.

The loss factor which was defined as the tangent of the loss angle δ can be obtained from the following equation as

$$\tan \delta = \frac{G''(\omega)}{G'(\omega)} = \tan\left(\frac{n\pi}{2}\right) \quad (3)$$

Therefore, the accurate gelation point can be identified at the point when the loss factor is independent of angular frequency.

Fig. 2 shows the elastic and viscous modulus G' and G'' as a function of the angular frequency ω for the κ -carrageenan/sucrose samples containing various amounts of sucrose at various temperatures which governed the gelation process. To avoid overlapping, the data were shifted vertically by a factor of 10^3 . At high temperature beyond the gelation point, G' and G'' were proportional to $\omega^{1.4-1.5}$ and $\omega^{0.9-1.0}$, respectively towards low ω region. In addition, G'' dominated over G' within the full angular frequency, suggesting a typical viscoelastic fluid characteristic beyond the gelation point (Liu, 2015; Lu, Liu, Dai, & Tong, 2005). Some G' values at low frequency but high temperature were too small to be determined accurately due to reaching the lowest torque limit of

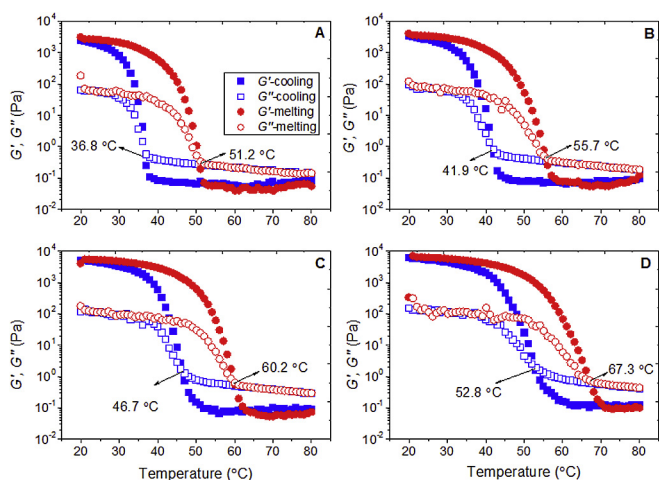


Fig. 1. Storage modulus (G') and loss modulus (G'') as a function of temperature during a rheological temperature sweep at the cooling and heating rate of 1 °C/min for (A) 2 wt% κ -carrageenan, (B) 2 wt% κ -carrageenan with 10 wt% sucrose addition, (C) 2 wt% κ -carrageenan with 20 wt% sucrose addition and (D) 2 wt% κ -carrageenan with 30 wt% sucrose addition.

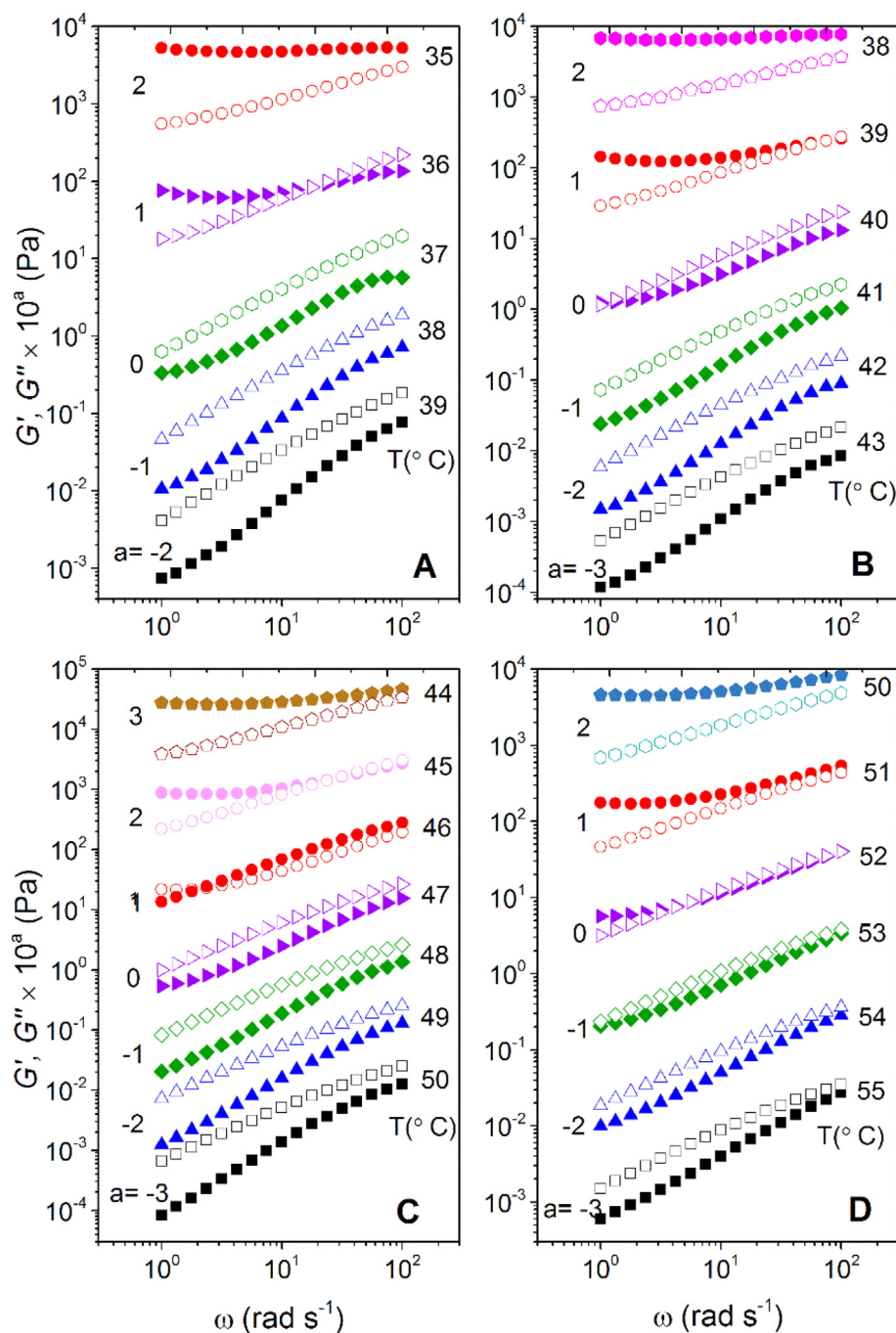


Fig. 2. Dependence of storage modulus G' (solid symbol) and loss modulus G'' (open symbol), as a function of angular frequency ω (rad/s) for 2 wt% κ -carrageenan solutions with addition of (A) 0 wt% sucrose, (B) 10 wt% sucrose, (C) 20 wt% sucrose, and (D) 30 wt% sucrose.

the rheometer (Dai, Liu, & Tong, 2010). When the temperature decreased, G' started to increase and became higher than G'' and finally a G' plateau appeared in low frequency region, suggesting the formation of viscoelastic gel. This similar phenomenon was observed in all κ -carrageenan samples containing various amount of sucrose. In order to obtain the gelation temperature, the loss factor was plotted against temperatures at different angular frequencies. The existence of critical gelation temperature, the specific temperature where all the curves with various frequencies crossed, suggested that the loss factor was independent of the frequency at the temperature. It was in a good agreement with the result achieved from crossover of G' and G'' in Fig. 1 and previous report (Liu &

Li, 2016).

The critical loss factor corresponding to the critical gelation temperature was also identified and labelled in Fig. 3. The critical relaxation exponent n was calculated from equation (3) from the corresponding critical gel temperature, which was around 0.75, 0.68, 0.62 and 0.57 for κ -carrageenan solutions containing 0 wt%, 10 wt%, 20 wt%, and 30 wt% sucrose, respectively. Based on equation (1), the value of n varied between 0 and 1, which corresponded to the loss angle δ ranging from 0 to $\pi/2$. From a viscoelasticity point of view, $n = 1$ suggests a purely elastic behaviour, while $n = 0$ indicates a completely viscous behaviour. Thus, the lower the value n , the more elastic structure the sample could have (Liu & Li, 2016). As

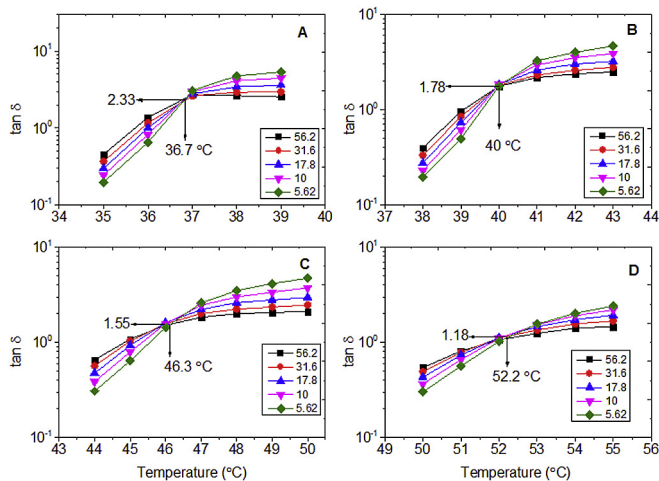


Fig. 3. Dependence of loss tangent, $\tan \delta$, as function of temperature at different angular frequencies (rad/s) as demonstrated for 2 wt% κ -carrageenan solutions with addition of (A) 0 wt% sucrose, (B) 10 wt% sucrose, (C) 20 wt% sucrose, and (D) 30 wt% sucrose.

indicated in Fig. 3, the value of n decreased as sucrose concentration increased. In other words, addition of sucrose made the sol-gel transition close to a fully elastic solid ($n = 0$) or far from a wholly viscous solution ($n = 1$). This finding suggested that sucrose addition facilitated coil-helix transition and further aggregated helices, which in turn promoted the gel network strength and elasticity.

It is worth noting that the value and the change of critical relaxation exponent n with gelation variables strongly depend on the gelling systems and their gelation mechanisms. Many groups reported that the n fell in the range of 0.65–0.75 for chemically crosslinked gel. In terms of physically crosslinked gel, the value of n varied much. For example, the n was 0.5 for pectin in water (Stang Holst, Kjønksen, Bu, Sande, & Nyström, 2006), and it was 0.08 for pectin in 10 wt% methanol under shear perturbation (Tho, Kjønksen, Nyström, & Roots, 2003). However, it was 0.69 in thermoreversible hydrogels formed by poly (ethylene oxide)-*b*-poly (propylene oxide)-*b*-poly (ethylene oxide)-*g*-poly (acrylic acid) (Bromberg, 1998). Some researchers indicated that n is a constant. For instance, n was found to be 0.75 in poly (vinyl alcohol) (PVA) physical gels and independent of its molecular weight (Li & Aoki, 1997). However, more research showed that n varied with the gelling conditions. For instance, n varied between 0.25 and 0.65, and depended on temperature in thermosensitive triblock copolymer hydrogel (Vermonden, Besseling, van Steenberg, & Hennink, 2006). By varying the methanol concentration from 0 to 10 wt%, the value of n varied between 0.11 and 0.05 in shear perturbed pectin gel (Tho et al., 2003). The finding of decreasing n with changing gelation variables in the current study was also reported in other gelling systems including κ -carrageenan/ Ca^{2+} (Liu & Li, 2016), alginate/ Ca^{2+} (Liu and Li, 2016) and alginate/ Cu^{2+} system (Lu et al., 2006).

The critical relaxation exponent n was connected to the fractal dimension d_f through the following equation, assuming the excluded volume effect and the hydrodynamic interaction can be totally ignored (Muthukumar, 1989),

$$n = \frac{d(d+2-2d_f)}{2(d+2-d_f)} \quad (4)$$

where d ($d = 3$) is the space dimension and d_f is the fractal

dimension.

The fractal dimension d_f was calculated and demonstrated in Fig. 4 (A). The result showed that the d_f increased as the concentration of sucrose increased. A looser structure resulted in a lower value of d_f while a denser structure contributed to a greater d_f (Beaucage, 1996). Thus, the observed rise of d_f indicated that the addition of sucrose strengthened the carrageenan gel network; the dense structure might be caused by the increment of helix aggregates density and/or the size of helix junctions. Consistently, the d_f observed here (around 2) was close to that reported in other natural polysaccharide systems (Lu et al., 2006; Lu, Liu, Tong, & Gao, 2006).

Except for n , the critical gel strength S_g that is related to the physical strength of gel network is also a crucial parameter to demonstrate the gelling behaviour at the gelation point. It can be obtained from the following expression

$$G'(\omega) = G''(\omega) / \tan\left(\frac{n\pi}{2}\right) = S_g \omega^n \Gamma(1-n) \cos\left(\frac{n\pi}{2}\right) \quad (5)$$

Where $\Gamma(1-n)$ is the Gamma function, the S_g can be calculated by knowing n and G' or G'' at the critical gelation point.

The dependence of S_g on the sucrose concentration is shown in Fig. 4B. Specifically, S_g increased with increasing sucrose concentration. S_g was believed to correlate to the amount and/or size of gel network junctions (Liu & Li, 2016). In previous reports, S_g increased with increased gelation temperature when κ -carrageenan concentration was the gelling variable (Liu, 2015) or Ca^{2+} addition was

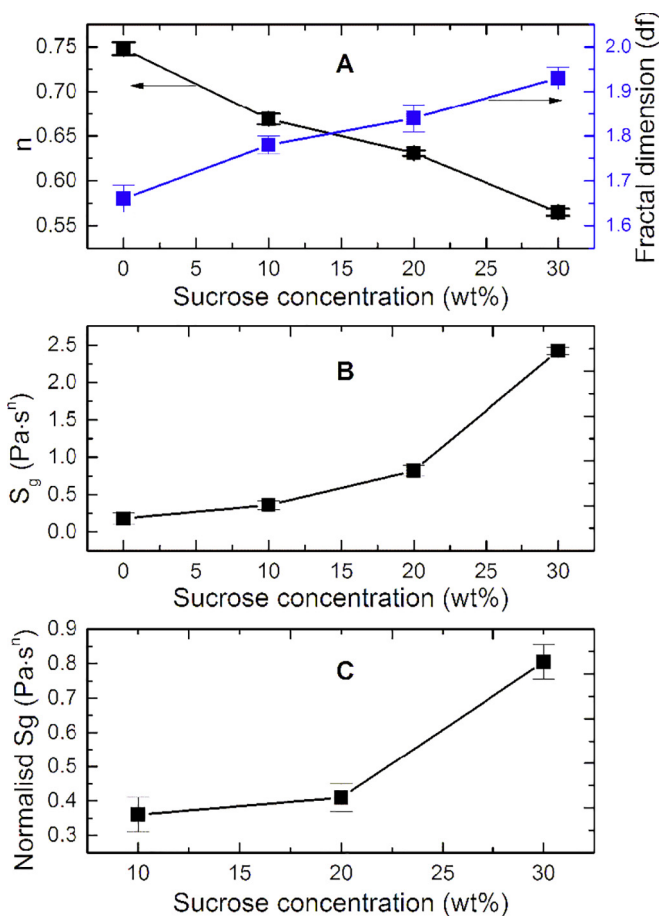


Fig. 4. Dependence of (A) the critical relaxation exponent n and fractal dimension d_f , (B) the critical gel strength S_g and (C) the normalised critical gel strength with sucrose concentration.

increased at fixed κ -carrageenan concentration (Liu & Li, 2016). The normalisation of S_g with gelling variables (e.g. concentration, Ca^{2+} concentration) was employed to further understand the gelling behaviour. For instance, Liu (2015) normalised S_g with critical gelation concentration C_g to exclude the temperature effect and found S_g/C_g was a constant. The normalised gel strength S_g/C_g was attributed to the fact that the increase in C_g mainly induced an increase of junction numbers while it had a faint impact on the junction size (Liu, 2015). In the current study, to examine how the sucrose concentration affected S_g , S_g was normalised by sucrose concentration to obtain S_g/S_{ucrose} . Fig. 4 (C) shows that S_g/S_{ucrose} was not a constant, which increased with increased sucrose concentration. Therefore, increasing sucrose concentration induced the increase of both number and size of helix junctions at the critical gelling point. Similar finding was also observed when Ca^{2+} was increasingly added to κ -carrageenan system (Liu & Li, 2016).

3.3. Small and large deformation rheological properties of κ -carrageenan hydrogel containing various amount of sucrose

In the above section, the viscoelastic behaviour of aqueous κ -carrageenan/sucrose solution during its sol-gel transition was investigated. In current section, the formed gel was kept for 5 h until a G' plateau was achieved (result not shown). After that, the small and large deformation rheological properties of the “mature” hydrogels were characterised by frequency sweep and strain sweep protocols, respectively.

Fig. 5A shows the G' and G'' with frequency dependency for the gel containing various amounts of sucrose. The strain was set as 1% to avoid perturbation of the gel networks. As seen in Fig. 5A, G' was nearly parallel with G'' and G' dominated over G'' by at least 100 times in the whole frequency investigated. This behaviour is a typical characteristic of strong gel system, suggesting a strong gel formation for all κ -carrageenan/sucrose hydrogels (Graessley, 1974). Moreover, the values of G' and G'' increased with increased sucrose concentration. To better understand the improvement of gel network strength by sucrose, the plot of complex modulus

$G^* = (G'^2 + G''^2)^{0.5}$ at 1 rad/s against sucrose concentration was conducted (Fig. 5B). As shown, G^* increased nearly linearly with the addition of sucrose, suggesting an enforcing effect of sucrose on the κ -carrageenan hydrogel network, a result consistent with previous reports (Gekko et al., 1987).

G' and G'' as the functions of strain are shown in Fig. 5C. All the samples demonstrated a similar pattern quantitatively. Within the linear viscoelastic region (LVR), both G' and G'' were nearly independent of strain accompanied with G' dominating over G'' , indicating a solid gel characteristic. After certain strain (critical strain), both G' and G'' began to decrease and eventually reached a cross-over point, the strain of which was defined as breaking strain. After breaking strain, G'' started to dominate over G' , indicating the gel network was destroyed and material started to flow. To better compare the large deformation rheological properties among the κ -carrageenan/sucrose hydrogels, the breaking strain was plotted (Fig. 5D). When the sucrose ranged from 0 to 20 wt%, the breaking strain increased gradually. However, from 20 wt% to 30 wt%, the breaking strain increased substantially from approximately 16.5%–31.6%, suggesting the κ -carrageenan gel network was significantly improved upon sucrose addition.

3.4. FTIR spectroscopy analysis

FTIR is a robust, non-destructive analytical method which has been employed extensively to distinguish various types of carrageenan (Pereira, Amado, Critchley, Van de Velde, & Ribeiro-Claro, 2009) and study the effect of ions on the structures of carrageenan (Belton, Wilson, & Chenery, 1986). It usually requires few milligrams of samples and recently has been applied to examine the effect of food additives (e.g. salt, sucrose, xylitol) on food biomacromolecules (Sow & Yang, 2015; Sow et al., 2017; Cai, Feng, Regenstein, Lv, & Li, 2017).

In the current study, FTIR was employed to investigate the effect of sucrose addition on the structures of κ -carrageenan through examining the position of FTIR absorption peaks. Fig. 6 shows the FTIR spectra of κ -carrageenan containing various amount of sucrose. The pure sucrose powder was included as control. The location and assignment of peaks are summarised in Table 1 and interpreted according to previous reports (Mathlouthi, Seuvre, & Birch, 1986; Pereira et al., 2009; Şen & Erboz, 2010). The spectrum of control sample without sucrose revealed the typical characteristics of commercial κ -carrageenan. A moderately strong band at around 845 cm^{-1} was assigned to C-O-SO₄ on C₄ of galactose-4-sulfate (G4S). The two bands at approximately 930 cm^{-1} and 1070 cm^{-1} indicated the presence of 3,6-anhydrogalactose (DA). And the presence of band at around 1260 cm^{-1} suggested the presence of sulphate ester (Pereira et al., 2009).

In general, the FTIR spectra of κ -carrageenan added with less sucrose (10 wt% sucrose) are similar to that of κ -carrageenan only. However, the spectra of κ -carrageenan containing more sucrose (30 wt% sucrose) are similar to that of pure sucrose. For instance, for control samples containing κ -carrageenan only and those containing 10 wt% sucrose, the sulphate ester groups were mainly reflected by the presence of large and broad peak at around 1260 cm^{-1} . However, for the samples containing 20 wt% and 30 wt% sucrose, the large and broad peaks disappeared, which were replaced by several small and sharp peaks characteristic of sucrose only samples.

Although some peak positions were quite similar between κ -carrageenan only and pure sucrose samples, which made them difficult to identify, differences can still be observed. In the inserted graph of Fig. 6, the FTIR spectrum of pure sucrose shows a sharp vibration at 3563 cm^{-1} characteristic of a free hydroxyl group that did not take part in the hydrogen bonding in the sucrose crystal and

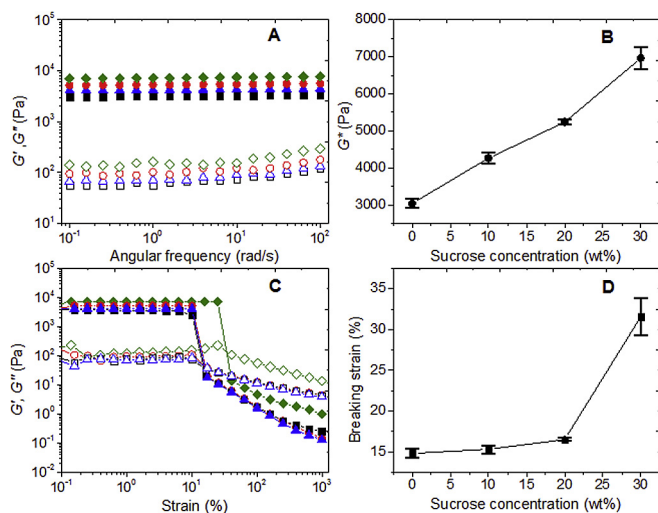


Fig. 5. The dependence of frequency on storage modulus G' (solid symbols) and loss modulus G'' (open symbols) for κ -carrageenan gel with various amount of sucrose addition (measured at 20 °C), (B) The complex modulus at 1 rad/s as a function of sucrose concentration, (C) The dependence of strain on storage modulus G' (solid symbols) and loss modulus G'' (open symbols) for κ -carrageenan gel with various amount of sucrose addition (measured at 20 °C), (D) The breaking strain values as a function of sucrose concentration. Sucrose concentrations are: 0 wt% (■, □); 10 wt% (▲, △); 20 wt% (●, ○); 30 wt% (◆, ◇).

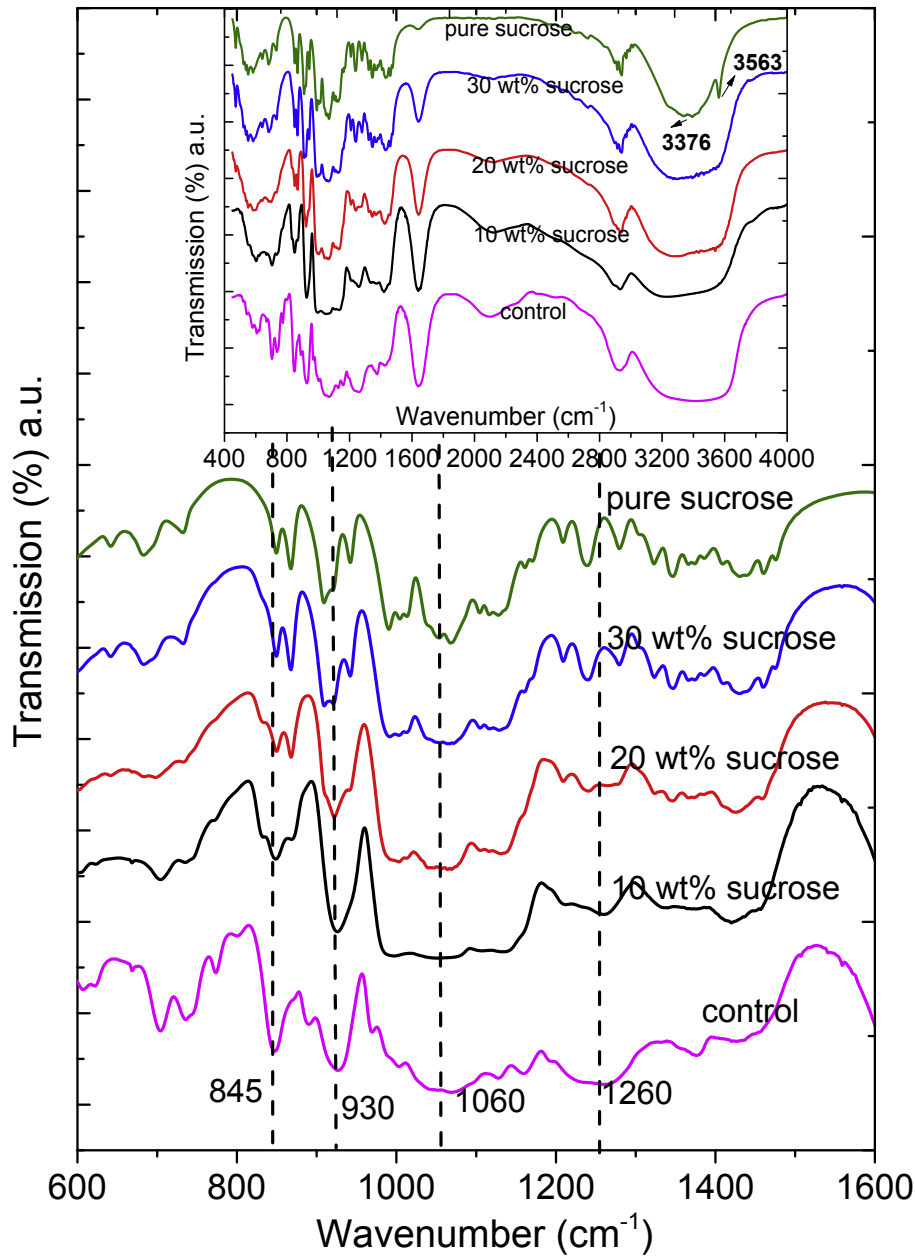


Fig. 6. FTIR spectra of κ -carrageenan with addition of 0 wt%, 10 wt%, 20 wt%, and 30 wt% sucrose. Pure sucrose is also included as a reference.

was assigned to the equatorial HO-4 while a broad peak around 3376 cm^{-1} indicated the hydrogen-bonded hydroxyl group (Mathlouthi et al., 1986). For all κ -carrageenan samples containing sucrose, the vibration band of free hydroxyl group disappeared.

This discrepancy probably was caused by that the free equatorial hydroxyl groups from sucrose formed cross-linking hydrogen bonds with κ -carrageenan molecule (Oakenfull, 2000, pp. 277–284). It also can be seen that the G4S band (around 845 cm^{-1})

Table 1

Location and assignment of the peaks identified in FTIR spectra for κ -carrageenan gel with sucrose addition.

Peak wavenumber (cm^{-1})				Pure sucrose	Assignment and Remarks	References
None (control)	10 wt% sucrose	20 wt% sucrose	30 wt% sucrose			
845	835 (shoulder)	834 (shoulder)	–	–	C–O–SO ₃ on C4 of Galactose	(Pereira et al., 2009)
–	–	943	943	943	C–H deformation vibration (sucrose)	(Vasko, Blackwell, & Koenig, 1971)
–	–	909 (shoulder)	909	909	C–H deformation vibration (sucrose)	(Vasko et al., 1971)
1260	1262	–	–	–	O=S=O of sulphate esters	(Şen and Erboz, 2010)
–	–	–	–	3573	Non-hydrogen bonded (free) equatorial hydroxyl group	(Mathlouthi et al., 1986)

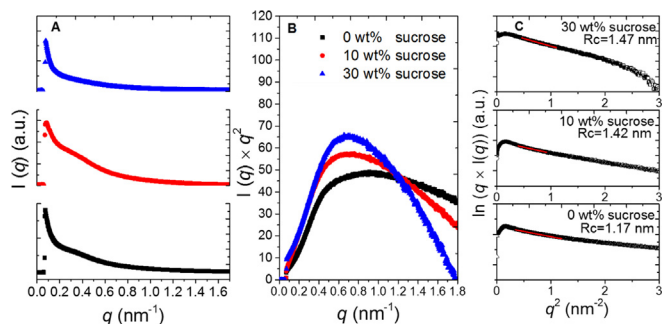


Fig. 7. (A) SAXS scattering profile $I(q)$ of 2 wt% κ -carrageenan solutions added with various amounts of sucrose. (B) Kratky plots of the SAXS scattering profiles. (C) Cross-sectional Guinier plots of the SAXS profiles. The red solid line indicates the Guinier region in which the equation (4) was applied to extract R_c .

tended to shift from 845 to 835 cm⁻¹ and from 845 to 834 cm⁻¹ by 10 wt% and 20 wt% sucrose, respectively. The similar FTIR peak shift upon sucrose addition was also reported in fish gelatine (Sow & Yang, 2015) and duck feet gelatine (Kuan, Nafchi, Huda, Ariffin, & Karim, 2016). The shifts by sucrose addition suggest the potential interactions between sucrose and κ -carrageenan molecules, e. g. the intermolecular cross-linked hydrogen bonding between κ -carrageenan and equatorial-OH groups of sucrose molecules, which played an important role in the formation of junction zone and gelation of κ -carrageenan (Stenner et al., 2016). To understand the sucrose- κ -carrageenan interaction in more detail, more robust analytical techniques like NMR may be needed in future research.

3.5. SAXS characterisation of network structures of κ -carrageenan and κ -carrageenan/sucrose hydrogels

Various microscopic techniques including scanning and transmission electron microscopy (SEM and TEM), atomic force microscopy (AFM) have been utilised to examine the microstructures of κ -carrageenan hydrogels. However, the sample preparation procedures of extensive dilution, etching, oxidised colouring and drying may have tremendous impacts on the native structure of κ -carrageenan hydrogels, leading to some artefacts. To avoid these, SAXS was employed to investigate the nanostructures of κ -carrageenan/sucrose hydrogels *in situ*.

The SAXS scattering patterns and corresponding Kratky plots are demonstrated in Fig. 7A and Fig. 7B, respectively. $I(q)$ and q represent the scattering intensity and the scattering vector, respectively, where $q = 4\pi\sin(\theta/2)/\lambda$ (θ and λ stand for the scattering angle and the wavelength of incident X-ray source, respectively). For κ -carrageenan only samples and κ -carrageenan containing 10 wt% sucrose, the SAXS profiles exhibited a broad and weak peak (Fig. 7A). Since κ -carrageenan is a polyelectrolyte carrying sulphate groups, the presence of peaks indicates repulsive electrostatic interactions between sulphate groups on the κ -carrageenan chains (Hassan, Fritz, & Kaler, 2003; Yuguchi, Thuy, Urakawa, & Kajiwara, 2002). However, when sucrose was increased to 30 wt%, the peak vanished. The discrepancy was caused by sucrose since it promoted the formation of κ -carrageenan junction and aggregation through cross-linking hydrogen bond between κ -carrageenan and equatorial-OH groups of sucrose, thereby the electrostatic repulsion interactions between κ -carrageenan molecules were overcome (Stenner et al., 2016).

Fig. 7B shows the Kratky plot that was employed extensively to characterise the hydrogel network structure and highlight the scattering characteristics in high q range. It is mainly used to distinguish the scattering from rod-like, Gaussian chains, and mass

fractal structures. More specifically, Kratky plots of scattering from rod-like structures could become linear at high q . In terms of scattering from Gaussian chains, Kratky plots increased monotonically with q until reaching a plateau at high q . For scattering from a three-dimensional structure, Kratky plots showed a peak and then decreased (Saffer et al., 2014). In the current study, all Kratky plots demonstrated a peak in the recorded q -regime, a scattering characteristic of a three-dimensional mass fractal structure (Hermansson, Schuster, Lindgren, Altskär, & Ström, 2016), suggesting that all the κ -carrageenan and κ -carrageenan/sucrose samples formed interconnected gel network before SAXS investigation. Compared to samples without sucrose, in κ -carrageenan/sucrose gels the peak of the Kratky plot shifted to smaller q values due to an aggregation of polymer chain junctions of greater dimensions and cross-sectional radii (Hermansson et al., 2016; Stokke et al., 2000).

To gain more insight of junction zone dimensions resulted from network assembly, cross-sectional Guinier plots ($\ln(qI(q))$ vs q^2) were employed to extract the cross-sectional radius of carrageenan chains (Fig. 7C). This Guinier plots were also employed to study the chain structures of alginate gel (Stokke et al., 2000), pectin (Schuster, Cucheval, Lundin, & Williams, 2011) and gelatine (Yang et al., 2015). In polymer physics, radius of gyration stands for the average distance of polymer from its centre of mass (Fixman, 1962). In the special case of rod-like structures, the two-dimensional analogue of radius of gyration R_g is called the radius of gyration of the cross-section R_c (Glatter & Kratky, 1982). Since the X-ray diffraction of κ -carrageenan suggests that the carrageenan chains consist of double-stranded helices arranged laterally in a trigonal unit, the crosslinking domain in carrageenan networks is regarded as a cylinder (Millane, Chandrasekaran, Arnott, & Dea, 1988; Yuguchi et al., 2002). Previously, the average bundle size/thickness of κ -carrageenan junction cylinder, of which the radius was assumed far smaller than its length, was physically characterised by the cross-sectional gyration of radius (Schuster et al., 2011).

The cross-sectional radius of gyration R_c of the carrageenan chains can be extracted via fitting of the equation (Glatter & Kratky, 1982).

$$qI(q) \cong I(0)\exp\left(-\frac{1}{2}R_c^2q^2\right) \quad (6)$$

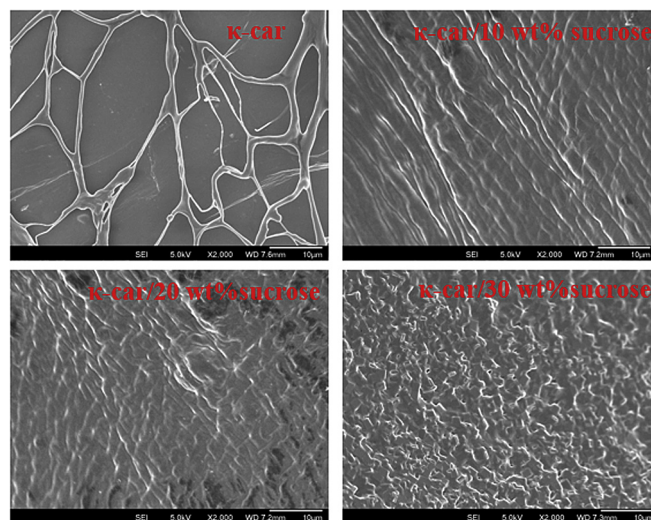


Fig. 8. FESEM images of 2 wt% κ -carrageenan hydrogels with various amounts of sucrose additions, which were prepared through freeze drying.

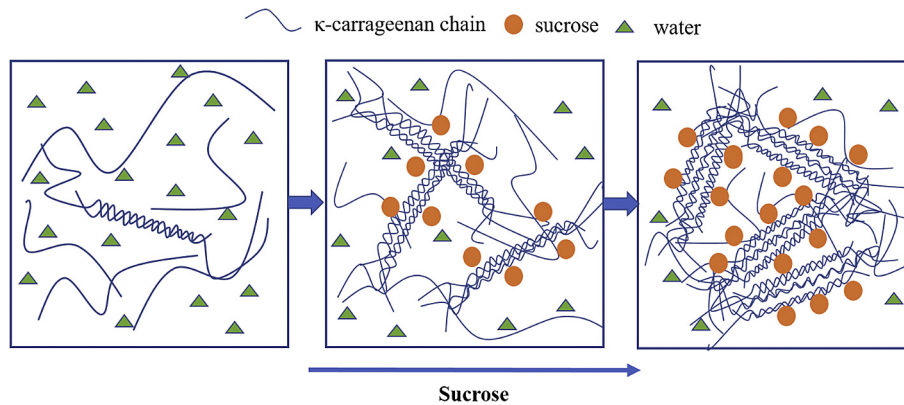


Fig. 9. A schematic presentation of the sol-gel transition of κ -carrageenan hydrogels with various amounts of sucrose additions.

In the Guinier region the qR_c is smaller than 1. The R_c can also be carefully evaluated from the steepest slope where the peak due to electrostatic interaction in smaller angle was avoided (Yuguchi et al., 2002). In the current study, the R_c was 1.17 nm for 0 wt% sucrose, 1.42 nm for 10 wt% sucrose, and 1.47 nm for 30 wt% sucrose; the R_c increased with the concentration of sucrose.

3.6. FESEM characterisation of network structures of κ -carrageenan and κ -carrageenan/sucrose hydrogels

FESEM and cryo-SEM have been employed recently to characterise Ca^{2+} induced κ -carrageenan gel (Liu & Li, 2016) and κ/ι -hybrid carrageenan gel, respectively (Hilliou et al., 2009). Here, FESEM was applied to examine the network structures of κ -carrageenan gel carrying various concentrations of sucrose (Fig. 8). The darker areas in the image indicate the voids caused by water sublimation due to freeze drying during sample preparation, while the lighter items correspond to carrageenan structures. The κ -carrageenan gel without sucrose addition shows interconnected, cell wall-like, porous structures with relatively large voids in between. The cell wall-like structure (Fig. 8) was believed to be composed of hundreds of stacked helices (Hilliou et al., 2009), since the average bundle size of helix aggregates is in nanometre scale as revealed by AFM and SAXS (Denef, Mischenko, Koch, & Reynaers, 1996; Schefer, Adamcik, Diener, & Mezzenga, 2015).

With the addition of sucrose, the gel strands became more and more densely packed and the open region in between turned much smaller, leading to increased density of gel strands and homogenous gel network. This finding agreed well with the TEM results of the κ -carrageenan gel (from 0.25 wt% to 3.0 wt%) at a fixed ion concentration. It has been reported that at lower κ -carrageenan concentration (0.25 wt%), the gel network was few and scattered with large voids in between. Whereas at higher κ -carrageenan gel concentration (3.0 wt%), the gel strands were more closely packed and the void regions in between gel strands were smaller (Lorén et al., 2009). In summary, FESEM results provide us the evidence that the κ -carrageenan gel was composed of fibrillar bundle like structures and it became denser and more homogeneously packed upon sucrose addition. These structural characteristics were believed to be the reason of greater gel network strength observed in various hydrogel systems (Hermansson et al., 2016; Liu and Li, 2016).

Based on the rheological and microstructural results, a pathway was proposed to elucidate the effect of sucrose addition on the gelation of κ -carrageenan (Fig. 9). The gelation of κ -carrageenan is generally suggested to include two key steps, the transition from

random coil to double helix and subsequent helix aggregation (Hermansson, 1989). By addition of sucrose into κ -carrageenan aqueous solution, the favourable hydration of sucrose in the bulk aqueous phase made it exclude from κ -carrageenan's surroundings. In the meantime, the average water molecule surrounding the κ -carrageenan strand reduced because of sucrose exclusion (Oakenfull, 2000, pp. 277–284). As a result, the κ -carrageenan-water interactions were weakened, and the κ -carrageenan strands were changed from self-association to association with their neighbours (Stenner et al., 2016). It is expected that upon more sucrose addition, more self-association of κ -carrageenan and coil-helix transition occurred, leading to formation of more junctions (aggregates). In addition, sucrose binding to the helices of κ -carrageenan through formation of intermolecular cross-linked hydrogen bond between κ -carrageenan and sucrose facilitated the aggregation of surrounded junctions to form large junctions or aggregates, thereby increasing both number and size of junctions (Nishinari & Watase, 1992). Therefore, the κ -carrageenan gel network became strengthened and its mechanical properties were largely improved.

4. Conclusions

In this study, the effect of sucrose addition (up to 30 wt%) on the thermoreversible gelation behaviour and microstructural characteristics of κ -carrageenan gels was systematically studied. More specifically, the presence of sucrose increased both the gelation temperature (from 36.8 to 52.8 °C) and melting temperature (from 51.2 to 67.3 °C). When the sucrose concentration increased, the gel network became stronger as suggested by a higher G^* value achieved. For all κ -carrageenan-sucrose gels, the gel point obtained by Winter's criteria was similar to that obtained from temperature sweep. With increased sucrose concentration, at the critical gel point, n decreased but S_g increased. The normalised gel strength S_g also increased with increased sucrose concentration. Structurally, the interactions between sucrose and κ -carrageenan molecules resulted in the shift of FTIR band of G4S within κ -carrageenan molecules and loss of free hydroxy group vibration band within sucrose molecules. The result of SAXS was consistent with that of FESEM, suggesting the formation of denser and thicker κ -carrageenan fibril bundle network structures upon sucrose addition. In terms of the average cross-sectional radius of gyration R_c of the carrageenan chains, the value of it increased from 1.17 to 1.47 nm upon 30 wt% sucrose additions. Based on the rheology and microstructural results, a schematic mechanism was proposed explaining the enhanced network strength upon sucrose addition.

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