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***Thermal Properties of Mixed Gel System Formed by
 κ -Carrageenan and Casein***

Emako MIYOSHI

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Thermal Properties of Mixed Gel System Formed by κ -Carrageenan and Casein

Emako MIYOSHI

1. Introduction

Since agar, carrageenan, alginate, and pectin with a low degree of esterification are simple to use, they have been widely used for food products such as dessert jellies for a long time. However, agar gels that are formed on cooling become relatively turbid and the gel strength becomes weaker on addition of acid. Pectin with a low degree of esterification can form gels only in the presence of divalent cations. Seaweeds that are the source of alginate, carrageenan, agar, are subject to the uncertainties of nature and are not always available in sufficient quantities to satisfy market demand. Moreover, in most countries the range of gelling agents available for food use is limited by regulation. In the development of new textures and in the formulation of new products we have to rely on combinations of two or more of these permitted gelling agents. Mixed systems are therefore of great practical importance and there is much research in this area, although most of it is very empirical and unhelpful in understanding or illustrating general principles.

The carrageenans are sulfated polysaccharides, which are essentially alternating copolymers of 1, 3-linked β -D-galactose and 1,4-linked 3, 6-anhydro- α -D-galactose. They are extracted from marine algae of the class *Rhodophyceae* and, depending on source, differ in the extent to which they carry sulfate groups. Nevertheless, it is possible by selecting suitable genera of algae to extract at least two classes of carrageenan, which conform closely to the simple repeat formula. These are ι and κ -carrageenan and most research has been concentrated on these two types.

Milk proteins are classified as 'casein' and 'whey proteins'. Caseins are a group of phosphoproteins insoluble at pH 4.6 (the isoelectric point), where the whey proteins remain soluble. There are four primary proteins, α_{s1} , α_{s2} , β and κ in the approximate ratio 40:10:35:12, all relatively small molecules of about 20,000-24,000 Daltons. Because of their high content of phosphoserine residues, caseins bind polyvalent ions strongly, principally Ca^{2+} which promotes aggregation. In normal milk, about 95% of the casein exists as casein micelles. These are coarse colloidal particles with molecular weights of about 10^8 and mean diameters of about 100nm. Sodium caseinate, as used in this study, is prepared by adding acid to milk to

precipitate the casein at the isoelectric point. The washed protein is then redissolved by adding sodium hydroxide to restore neutrality. The high overall proportion of hydrophobic amino acid side chains throughout the casein primary structure causes sodium caseinate to form aggregates (or 'sub-micelles') in aqueous solution, with the bulk of the non-polar regions residing in a dense inner core with little associated (Farrell et al., 1990). But further association of sub-micelles to form the large casein micelles present in milk is prevented by the removal of most of the calcium.

Gelation and thickening of dairy products is one of the most important food applications of the algal polysaccharides, particularly ι - and κ -carrageenan. Milk gels formed by the addition of κ -carrageenan are of considerable practical importance. Thus, κ -carrageenan reacts specially with α_s -casein, stabilizing the protein to precipitation by calcium chloride (Hanse, 1968). It can also form a stable complex with κ -carrageenan with κ -casein (Grinsrod, 1968, Snoere, et al., 1975). For example, in chocolate milk, the weak gel network provided by addition of a small amount of κ -carrageenan (0.25~0.35 g/kg) prevents sedimentation of cocoa particles. Similarly small concentrations of κ -carrageenan can be added to give 'body' to skimmed milk; larger concentrations (2~3 g/kg) can be added to form gelled milk products such as pie fillings and custards. Addition of 1 g/kg gives a 10~20 % increase in the yield of cottage cheese base (Kilapthy et.al., 1992); addition of 0.25g/kg gives an approximately 10% increase in the yield of Cheddar cheese (Kanombirira et.al., 1995).

Despite this widespread use the mechanism of gelation is still not well understood, although it has long been known that ι - and κ -carrageenan and casein micelles (Hansen, 1968, Grinsrod & Nickerson, 1968, Dalgleish & Morris, 1988, Langendorff et al., 1997, Snoeren et al., 1975, Lin & Hansen, 1970; Sukura & Nakai, 1981, Ozaka et al., 1984, Oakenfull et al., 1999 & 2000). Dalgleish and Morris (1988) have studied the interactions between λ -, ι - and κ -carrageenan adsorb to casein micelles. They have measured electrophoretic mobilities and diffusion coefficients and showed that λ - and κ -carrageenan adsorb to casein micelle until the surface is covered and that κ -carrageenan also adsorbs strongly, but with less complete coverage. Whether or not the carrageenan is in the helix or random coil conformation appears to make little or no difference to the interaction. Xu and her colleagues (1992) have accordingly proposed that such interactions are the first step in the gelation process. But whereas Dalgleish and Morris (1988) envisaged the entire carrageenan chains wrapping around the casein micelle, Xu and her colleagues (1992) suggested that only part of the carrageenan chain is adsorbed. Most of it, they suggested, is free in solution in the form of loop or tails. As the solution is cooled to below the carrageenan's helix-coil transition temperature, the free κ -carrageenan chains link to form a gel network by forming double helices (Figure 1). This figure is consistent with electron microscopy studies (Hood & Allen, 1977), which showed aggregation of casein micelles

in milk with added κ -carrageenan but little or no aggregation with λ -carrageenan. λ -carrageenan binds to casein micelles (Dalglish and Morris, 1988) but it does not form gels (Glicksman, 1983). Langendorff et al. (1997) have studied the mixed gels formed by ι -carrageenan and sodium caseinate micelles. They found that ι -carrageenan added to milk (0.56g/kg) produced gels with higher elastic modulus and gelation temperatures. More recently, Alexander & Dalglish (2007) have investigated the interaction of casein micelles with κ -carrageenan using diffusing wave spectroscopy, and Arltoft et al. (2007) have studied the interaction between carrageenan and milk protein by micro-structural and rheological methods.

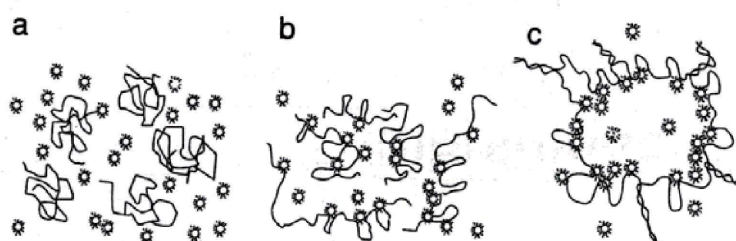


Fig. 1

Schematic representation of the gelation of casein and κ -carrageenan as proposed by Xu et al. (1992). (a) Casein micelle and random coil carrageenan in solution; (b) casein micelle-carrageenan interactions in solution; (c) gel formed from casein and carrageenan.

In their investigation, Xu and her colleagues used electron microscopy combined with rheological measurements, but confined to a single temperature (5°C). In this study, it has been extended the investigation of the κ -carrageenan-casein systems to thermal properties, measured by differential scanning calorimetry (DSC), and to oscillatory rheological measurement over temperature range encompassing gelation and melting. Moreover, we report here a parallel investigation of mixed gels of κ -carrageenan and skimmed milk powder (SMP) in view of their wide application on the dairy industry.

2. Materials and Methods

2.1. Material

κ -carrageenan was from Sigma. Analysis of the material by ICP-AES (carried out by the CSIRO Division of Exploration and Mining) gave the following percentages of calcium, potassium and sodium: Ca 1.96; K 20.93; Na 1.16.

Sodium caseinate, as used in this study, was a commercial product (Murray-Goulburn Cooperative Ltd) prepared by acid precipitation followed by neutralization with sodium hydroxide. Analysis of the material by ICP-AES gave the following percentages of calcium,

potassium and sodium: Ca 0.046; K < 0.02; Na 0.96.

Low heat skimmed milk powder (SMP) was also analyzed for calcium, potassium and sodium: Ca 13.9; K 17.5; Na 4.4.

2.2. Sample Preparation

Throughout this paper, concentrations are given by weight in grams of solute per kilogram of solution (g/kg). Sodium caseinate or SMP was dissolved in deionised water and heated for 30 min in a water bath at 95°C. κ -carrageenan was also dissolved in deionised water and heated for 30min in a water bath at 95°C to ensure complete dissolution. Appropriate weights of the two solutions were mixed hot with deionised water for the preparation of gels. The pH was 6.45 at 25°C.

2.3. DSC measurements

DSC measurements were carried out with a Setaram micro DSC-III calorimeter, Caluire, France. Approximately 900 mg of the sample solution was sealed hermetically into the DSC pan and the pan then accurately weighted. For each sample a reference pan was filled with distilled water to within ± 30 micrograms of the weight of the sample pan. The two pans were then placed inside the calorimeter, heated to 95°C and held at this temperature for 10min to annihilate the thermal history. The temperature was then lowered to 5°C at 1.0 K/min and raised again at the same rate to 95°C.

Transition temperatures (heating and cooling) were estimated from peak areas. The magnitude of ΔH estimated from DSC scans can be sensitive to how the base line is drawn. In each case we drew base lines within the range of possible positions and estimated the uncertainty in the results accordingly.

2.4. Rheological measurements

Oscillatory rheological measurements were made with a Dynamic Stress Rheometer DSR from Rheometrics Co. Ltd., NJ, USA. Parallel plate geometry was used, of diameter 50mm. The hot sample was poured directly onto the plate of the instrument. The temperature dependence of G' and G'' was observed at a frequency 1.0 rad/s. All measurements were made within the linear viscoelastic regime.

Difficulty was encountered with syneresis, which was very pronounced in the mixed gels. For concentrations of κ -carrageenan in excess of 4g/kg and of sodium caseinate in excess of 30g/kg, syneresis developed so rapidly as to caused slippage between the plates of the rheometer. We therefore also made complementary measurements of absolute shear modulus by the method of Oakenfull, Parker and Tanner (1989). This method is not affected by syneresis because it relies on insertion of a probe into a gel formed in a cylindrical container, as minutely described

in the previous study (Oakenfull et al., 1999). The absolute shear modulus (G) was calculated from the formula $G=0.0208Y$ (Oakenfull, 1989). This method is equivalent to measurement of G' at zero frequency. For κ -carrageenan alone, for which G' is almost independent of frequency (Morris, 1989), the two methods gave concordant results (data not shown).

3. Properties of Mixed Gels of κ -Carrageenan with Sodium Caseinate

In this study, it has been carried out the following two series of experiments.

- (i) Holding the concentration of sodium caseinate constant, the concentration of κ -carrageenan constant was varied.
- (ii) Holding the concentration of κ -carrageenan constant, we varied the concentration of sodium caseinate.

3.1. Differential Scanning Calorimetry

Figure 2 shows heating and cooling DSC curves for 20g/kg sodium caseinate with various concentration of κ -carrageenan. The cooling curves showed distinct single endothermic peaks; the heating curves showed multiple peaks (endothermic), which were broader and not well defined. For the cooling curves, the mid-peak temperatures (T_c) and the corresponding enthalpy values (ΔH_c) both increased with increasing concentration of κ -carrageenan (shown in Figure 3 (a) (b), both of which include for comparison the corresponding data for κ -carrageenan alone).

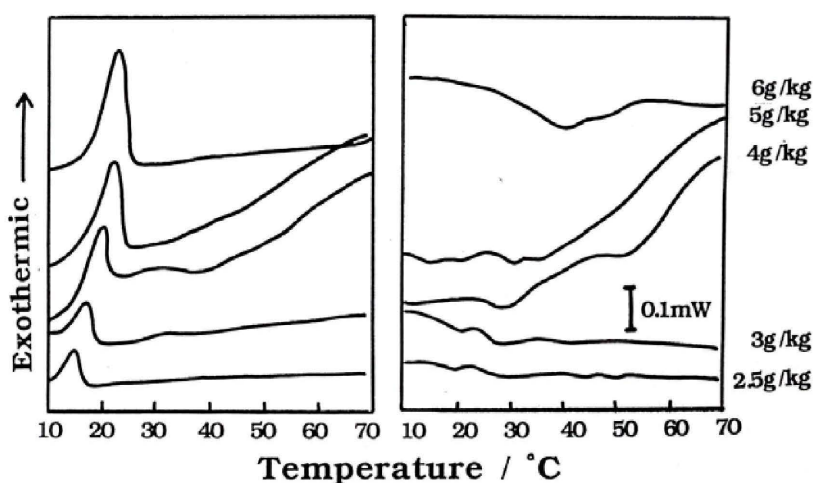


Fig. 2

DSC heating and cooling curves for sodium caseinate (20g/kg) with various additions of κ -carrageenan. The rate of heating and cooling was 1.0K/min.

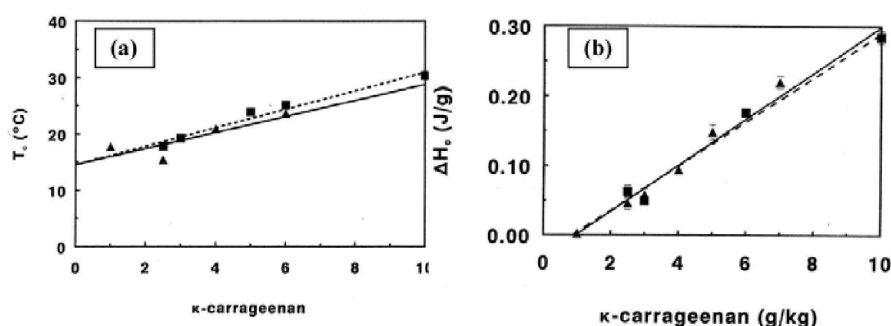


Fig. 3

DSC mid-peak cooling (gelation) temperatures (T_c) (a) or Enthalpy of cooling (gelation) transition (ΔH_c) (b) as a function of concentration of κ -carrageenan, with (broken line; ■) and without (solid line; ▲) addition of sodium caseinate.

In Figure 4, it was shown the equivalent heating and cooling DSC curves for κ -carrageenan at 5g/kg with various additions of sodium caseinate. The cooling curves again showed distinct single exothermic peaks. It was observed shallow minima in plots of T_c and ΔH_c vs. the concentration of sodium caseinate, as shown Figure 5 (a) (b). Minima were also seen with 3 and 6g/kg κ -carrageenan, but shifted to lower and higher concentrations, respectively (data not shown). The heating curves again showed multiple peaks (endothermic), which were broader and less distinct than those seen on cooling. They became progressively broader with increasing concentration of sodium caseinate.

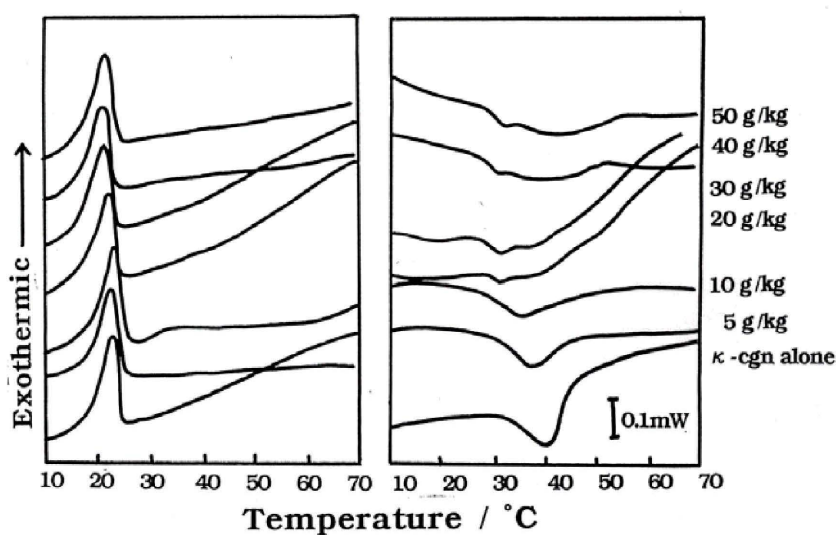


Fig. 4

DSC heating curves for κ -carrageenan (5g/kg) with various additions of sodium caseinate. The rate of heating and cooling was 1.0K/min.

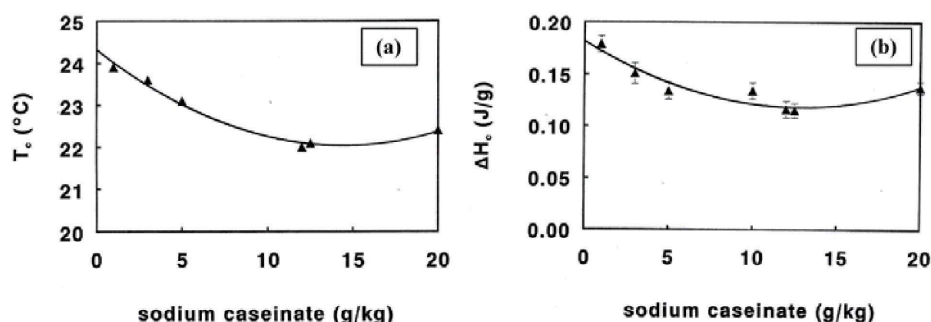


Fig. 5

DSC mid-peak cooling (gelation) temperatures (T_c) (a) or Enthalpy of cooling (gelation) transition (ΔH_c) (b) as a function of concentration of added sodium caseinate.

3.2. Rheometry

The variation of storage modulus (G') and loss modulus (G'') with temperature, with a cooling-heating cycle, is shown in Figure 6 for κ -carrageenan alone (5g/kg) and for mixtures of κ -carrageenan (5g/kg) with sodium caseinate at 1g/kg and 10g/kg. On cooling, G' and G'' both increased sharply at about 25°C (T_c) and G' became greater than G'' , indicating that gelation had occurred (Morris, 1989). On reheating, there was a sharp drop in both G' and G'' at around 45°C (T_m). There was clear hysteresis with T_m about 20°C higher than T_c . For the concentrations of sodium caseinate above 30g/kg syneresis caused slippage in the rheometer. T_m and T_c were still well defined but the absolute values of G' and G'' were unreliable.

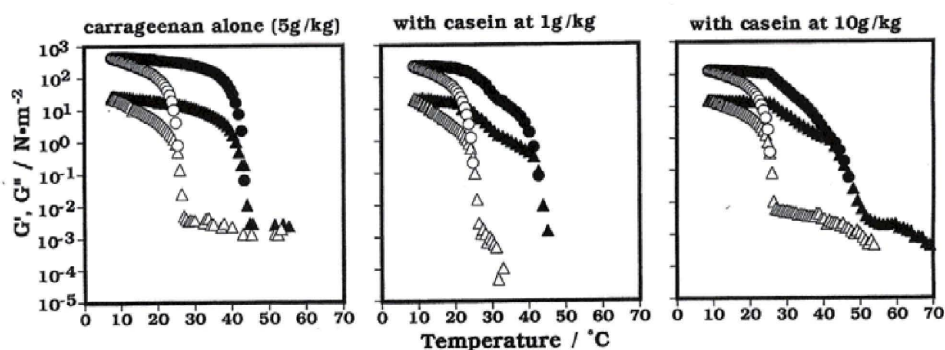


Fig. 6

Storage modulus (G' , ● ○) and loss modulus (G'' , ▲ △) at 1.0rad/s as a function of temperature for κ -carrageenan (5g/kg) with different addition of sodium caseinate (1 and 10g/kg). The rate of heating and cooling was 1.0K/min.

The form of the heating curves for G' and G'' changed markedly with the addition of sodium caseinate. G' and G'' decreased less sharply than for κ -carrageenan alone but in each case the decrease started at lower temperatures, suggesting the presence of a complex mixtures

of species with different melting temperatures. Lunch and Mulvihill (1994) have reported similar observations for gelation of mixtures of ι -carrageenan and sodium caseinate.

In Figure 7, we show G' (at 15°C) vs. the concentration of κ -carrageenan alone and κ -carrageenan with a fixed addition of sodium caseinate (10g/kg). The curve appears biphasic, with an abrupt change in slope at a concentration of κ -carrageenan of 6.8g/kg. The κ -carrageenan concentration at the breakpoint increased linearly with concentration of sodium caseinate, as shown Figure 8, and from the slope of the line, we estimate the adsorptive capacity of the sodium caseinate for κ -carrageenan to be 0.2g/g. This is consistent with an observation reported by Elfak et al. (1979). They studied the effect of sodium caseinate on the viscosity of solutions of κ -carrageenan and found a maximum effect at a concentration ration of 1:4. It is also qualitatively in good agreement with Dalgleish and Morris's electrophoretic mobility studies (1988) that indicate an adsorptive capacity of ca 0.28-0.4g/g.

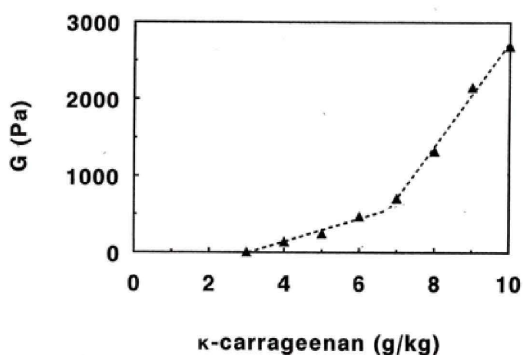


Fig. 7

Static shear modulus (G) at 15°C as a function of concentration of κ -carrageenan, with and without addition of sodium caseinate (10g/kg).

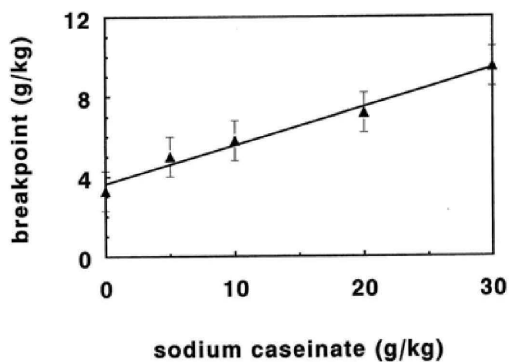


Fig. 8

Position of the breakpoint in plots of G vs. concentration of κ -carrageenan as a function of the added concentration of sodium caseinate (10g/kg).

In Figure 9, we show G' (at 15°C) vs. concentration of casein for a fixed concentration of κ -carrageenan (5g/kg). The curve shows a minimum, corresponding to the minimum in ΔH_c shown in Figure 5 (b). In addition, the magnitude of the hysteresis ($T_c - T_m$) initially increased with increasing concentration of casein to a plateau value at a concentration of casein coinciding with the minimum in G' .

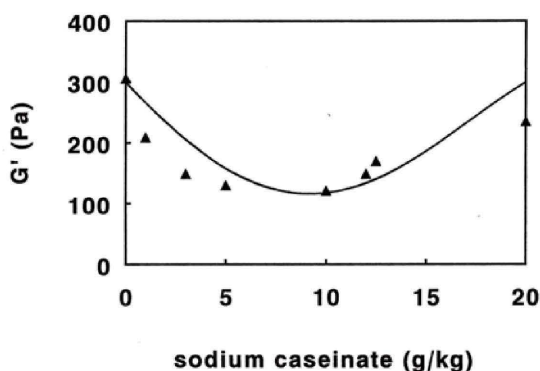


Fig. 9

Storage modulus (G') at 15°C and 1 rad/s for κ -carrageenan at 5g/kg as a function of concentration of sodium caseinate. The curve was calculated from Equations 6~8; see below.

Within experimental error, T_c determined rheometrically agreed with T_c determined calorimetrically. Thus the peaks observed in the DSC cooling curves correspond to full gelation, not simply the κ -carrageenan's helix-coil transition alone.

4. Gelation Mechanism of Milk Gel Formed with κ -Carrageenan

4.1. Thermal properties by DSC

Looking firstly at the DSC cooling curves (Figure 2), sodium caseinate has surprisingly little effect on the thermal properties of κ -carrageenan during gelation. When κ -carrageenan was added to a fixed concentration of casein (20g/kg), ΔH_c and T_c were indistinguishable from the corresponding values for κ -carrageenan alone, as shown in Figure 3 (a) (b). From the slope of ΔH_c vs. the concentration of κ -carrageenan, the enthalpy of gelation with 20g/kg casein was 32.8 ± 0.3 J/g (of κ -carrageenan the enthalpy of gelation with 20g/kg casein was 33.9 ± 0.05 J/g for κ -carrageenan alone. Our value for κ -carrageenan alone was very close to the range of values reported by Rochas and Rinaudo (1982) for melting of potassium-set gels of κ -carrageenan: 35~41 J/g.

Similarly, when sodium caseinate was added to a fixed concentration of κ -carrageenan, the changes in ΔH_c and T_c (Figure 5 (a) (b)) were small compared with the corresponding changes in

G' . The shallow minimum seen in ΔH_c can be explained by complexation with sodium caseinate; at low concentrations of casein the concentration of complex is below the gel threshold and the bound κ -carrageenan is withdrawn from the gel network.

In summary, the thermal effects of added casein on the gelation of κ -carrageenan were very small-almost on the limit of detectability. Thus when κ -carrageenan binds to sodium caseinate, the thermodynamics of the coil-to-helix transition remain almost unchanged. This suggests that there is only, at most, very minimal contact between the κ -carrageenan and the sodium caseinate molecules (Dalglish and Morris, 1988). However, Dalglish and Morris (1988) showed that under the conditions of their measurements, the surface area of the sodium caseinate micelles was approximately equal to the area that could be covered by the carrageenan at the concentration of saturation binding (1988). They suggested therefore that the micelles are totally covered, with the carrageenan molecules intimately wrapped around them. If this were the case, we would expect casein to have a significant influence on the enthalpy of the coil-helix transition. Although it is of course possible that the less well-defined aggregates formed by sodium caseinate might behave differently from the sodium caseinate micelles of unprocessed milk studied by Dalglish and Morris (1988).

There were more obvious changes to the thermal behavior during gel melting. The melting peak becomes progressively broader with increasing addition of sodium caseinate (Figure 4). This effect can be interpreted in terms of the 'zipper' model (Nishinari et al., 1999). κ -carrageenan forms a gel network by association of double helices (Clark and Ross-Murphy, 1987, Oakenfull, 1987), and such a structure can be considered as a molecular zipper with N Links. Nishinari and colleagues (1999) showed that the heat capacity C of such a system of v zippers is given by:

$$\frac{C}{k} = v \left(\ln \frac{G}{x} \right)^2 \left[\frac{2x}{(1-x)^2} + \frac{N(N+1)x^N [-x^{N-1} + (N+1)x - N]}{[1 - (N+1)x^N + Nx^{N+1}]^2} \right] \quad (1)$$

where

$$x = G \exp(-E/kT) \quad (2)$$

In this last expression the terms have the following meaning: (1) when links 1, 2... ρ are open, the energy required to open the $(\rho+1)$ st peak is E and (2) each open link can assume G orientations (i.e. the open state of each link is G -fold degenerate). It follows from this model

that in a system of zippers with a range of values for E , the temperature distribution of heat capacity is correspondingly broad. Thus addition of sodium caseinate to κ -carrageenan appears to generate 'zippers' with a wide distribution of energy values and/or degrees of rotational freedom.

The model also explains the observed hysteresis and the much sharper peaks observed during the cooling (gelation) process. When the temperature is raised, G will start from the lower values (G_g) corresponding to the gel state. The gel expands, giving rise to an increase on the rotational freedom. Conversely, when the temperature is lowered from temperatures higher than the melting point, G will start from the higher value (G_s) corresponding to the sol state. Thus the opening of zippers starts at small G values during the heating process while during the cooling (gelation) process, G starts from higher G values at higher temperatures. The average effective value of G is therefore small during heating and large during cooling. As a first approximation, we can say that the melting temperature (T_m) is determined by a certain average G_g and the gelation temperature (T_c) is determined an average G_s of G for the sol state. $G_g < G_s$ hence T_m would be expected to be higher than T_c . During heating, the transition caused by the opening of zippers will start as soon as the temperature reaches the tail of the C-T corresponding to $G=G_g$. During cooling, the pair-wise coupling cannot start so easily because of the difficulty of a long molecule finding its partner in appropriate positions for zipper construction. A state like super cooling may therefore occur, explaining the sharp transitions invariably seen on cooling, compared with the much broader transitions seen on heating.

Gelation of κ -carrageenan is believed to proceed via a two-step mechanism, as shown in Figure 10 (Clark and Ross-Murphy, 1987, Oakenfull, 1987). Carrageenan chains associate by the formation of intermolecular double helices, but there do not in themselves produce a gel network. Gelation occurs with the subsequent aggregation of these helices mediated by specific binding of gel promoting cations (particularly K^+ and Ca^{2+}). Rochas and Rinaudo (1982) have shown that the enthalpy of gelation (ΔH_g) is greater than the enthalpy of the coil-helix transition by $\sim 9\text{J/g}$. The close agreement we observed between ΔH_g for κ -carrageenan alone and the mixed gel (with, for example, 20g/kg casein) therefore suggests that the formation of the mixed gel involves the same two-step mechanism. Such a process might occur as shown in Figure 11. In a hot solution, above the coil-to-helix transition temperature, κ -carrageenan molecules in the random coil conformation are either in free solution or bound to casein. Below the coil-to-helix transition temperature, helices form from both free and bound κ -carrageenan molecules; gelation then occurs by interaction of helices, as in the gelation of κ -carrageenan alone. It seems not unreasonable to suppose that the steric constraints imposed by the presence of casein could cause less than complete overlap of κ -carrageenan molecules in their subsequent aggregation to form a gel network. Hence the presence of "zippers" with a distribution of energy values in the mixed system.

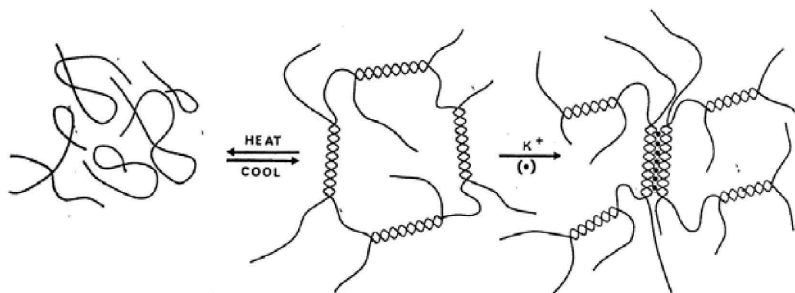


Fig.10

The two-step "domain" model for gelation of κ -carrageenan. Potassium or other gel-promoting cations are indicated by ● ((Glasstone & Lewis, 1962).

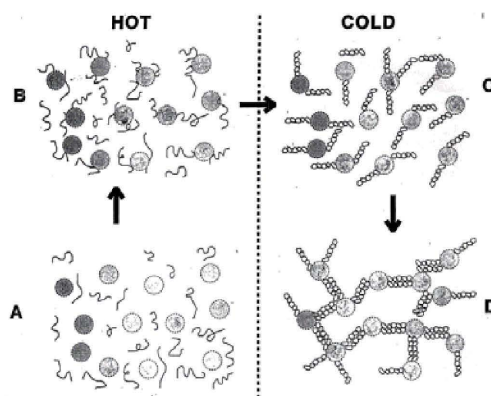


Fig.11

Proposed two-step mechanism for gelation of mixtures of sodium caseinate and κ -carrageenan.

4.2. Rheological Properties

The broadening of the DSC peaks for melting seen on addition of casein had its counterpart in the change of the shape of the curves of G' and G'' vs. temperature (Figure 6). Again, this result indicates that in the mixed gel, the junction zones are heterogeneous with a wide distribution of melting temperatures.

The minimum seen in G' when casein was added to fixed concentrations of κ -carrageenan (Figure 9) can be explained as follow:

- (1) When small amounts of are added, some of the κ -carrageenan is bound to the sodium caseinate micelles. Bound κ -carrageenan is no longer available to contribute to the κ -carrageenan network; sodium caseinate micelles are too few in number for themselves to form an equivalent network. Thus G' decreases.

- (2) As the concentration of sodium caseinate increases, sodium caseinate micelles with bound κ -carrageenan become sufficiently abundant to form a continuous network, as shown in Figure 11. G' then increases.

On this basis, we have developed a mathematical model relating G' to the concentration of casein. Considering firstly the binding of κ -carrageenan to casein, we make the simplest possible assumption, that there is a single association constant (a Langmuir-type model (Glasstone, 1962)). This is defined by

$$K = \frac{[C_1.B]}{[C_1][B]} \quad (3)$$

where $[C_1]$ is the concentration of free κ -carrageenan, $[C_1.B]$ is the concentration of bound κ -carrageenan and $[B]$ is the concentration of binding sites, all on a molar basis. If f is the fraction of bound κ -carrageenan, n the number of available binding sites (in moles) per gram of casein (i.e. the adsorptive capacity), C_2 the concentration of sodium caseinate (g/l) and $[C_1]^t$ the molar concentration of κ -carrageenan, it then follows that

$$K = \frac{f}{(1-f)(nC_2^t - C_1^t f)} \quad (4)$$

Since the κ -carrageenan is polydisperse, and we do not in any case know its molecular weight, it is convenient to incorporate this into K , redefining K on a weight concentration basis as K_w and n^w as the maximum weight (g) of κ -carrageenan bound per gram of casein, so that

$$K_w = \frac{f}{(1-f)(n^w w_2^t - w_1^t f)} \quad (5)$$

We next consider the separate contributions to G' from the networks of free κ -carrageenan (G'_{11}) and the casein- κ -carrageenan complex ($G'_{1,2}$). To a good approximation, G' for both κ -carrageenan and casein- κ -carrageenan gels increases with the square of the concentration (Oakenfull, 1989) so that

$$G_{11} = A_{11}(c_1 - c_1^0)^2 \quad (6)$$

$$G_{1,2} = A_{1,2}(c_{1,2} - c_{1,2}^0)^2 \quad (7)$$

Where the subscript 1 denotes κ -carrageenan and the subscript 1, 2 the κ -carrageenan/sodium caseinate complex, A_1 and $A_{1,2}$ are constants and c_1^0 and $c_{1,2}^0$ are the respective gel thresholds. In principle the polymer blending laws (Sperling, 1992) can be used to estimate the upper (isostrain) and lower (isostress) limits for the modulus of the mixed network:

$$\text{isostrain} \quad G_{\text{obs}} = G_1 V_1 + G_{1,2} V_{1,2} \quad (8)$$

$$\text{isostress} \quad \frac{1}{G_{\text{obs}}} = \frac{V_1}{G_1} + \frac{V_{1,2}}{G_{1,2}} \quad (9)$$

Where V_1 and $V_{1,2}$ are the respective volume fractions of the two networks. However, the evidence from electron microscopy (Xu et al., 1992, Hood & Allen, 1977) indicates that the two networks are interpenetrating, rather than phase separated. Thus only the isostress model (Equ 8) is applicable, with V_1 and $V_{1,2}$ equal (and equal to unity). For κ -carrageenan alone, A_1 and C_1^0 are known from the data (not shown); from the results shown in Figure 8 we know that $\eta_w = 0.20\text{g/g}$. Combining Equations 3, 6, 7 and 8 then gives a relationship between G'_{obs} and the concentrations of κ -carrageenan (w_1^1) and casein (w_2^1) with three unknown quantities – K_w , $A_{1,2}$ and $c_{1,2}^0$. Values for these were estimated by a standard curve-fitting procedure to best and $c_{1,2}^0 = 0.597$. The curve thus generated for κ -carrageenan at 5g/kg and varied concentration of casein is shown in Figure 12 (solid curve). Also in Figure 12 (dotted curves), we show the

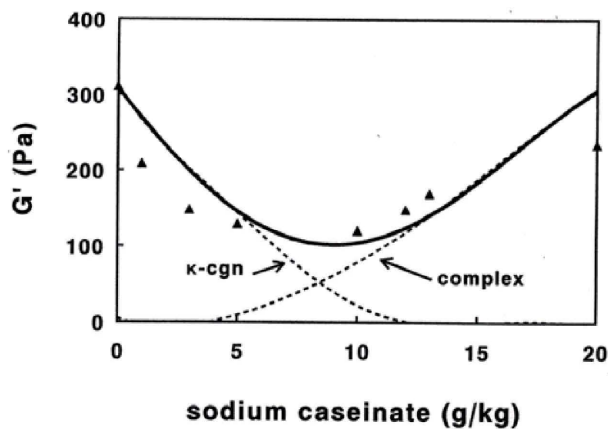


Fig. 12

Storage modulus (G') at 15°C and 1 rad/s for κ -carrageenan at 5g/kg as a function of concentration of sodium caseinate. The curves were calculated (see text). The solid curve shows calculated values of G' ; the dotted curves show the individual contributions of G' from the complex and free κ -carrageenan.

separate contributions to G' from the complex and κ -carrageenan. The contribution from κ -carrageenan decreases as, with increasing concentration of casein, the carrageenan forms the complex with casein; the contribution from the complex increases, once it has passed the gel threshold.

Using the same parameters, we also calculated the equivalent curves for 3 and 6 g/kg κ -carrageenan, which are shown in Figure 13. Despite its obvious crudity, the model successfully predicts the positions of the minima.

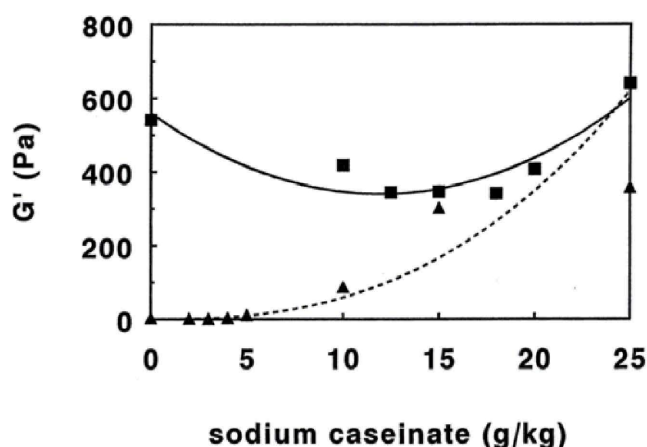


Fig. 13

Storage modulus (G') at 15°C and 1 rad/s for κ -carrageenan at 3 (▲) and 6 g/kg (■) as a function of concentration of sodium caseinate. The curves were calculated (see text).

The model was less successful in explaining the biphasic curves seen in plots of shear modulus (G) vs. concentration of κ -carrageenan with fixed concentrations of casein, as shown in Figure 14. Again, the curves are theoretical. The solid curve shows the calculated G and the dotted curves the separate contributions to G from the complex and κ -carrageenan. The contribution from the complex reaches a plateau value as the concentration of κ -carrageenan becomes high enough to saturate the binding sites on the sodium caseinate micelles; the contribution from κ -carrageenan continues to increase and dominates at high concentrations. The model fails quantitatively at high concentrations of κ -carrageenan – probably because calcium ions associated with the sodium caseinate enhance the contribution to G from the κ -carrageenan network (Morris and Chilvers, 1983). The parameters used to calculate the contribution from κ -carrageenan were calculated from data for κ -carrageenan in the potassium form.

The model also explains why only very small additions of κ -carrageenan are required to form a gel network in milk. The 'best-fit' values for the constants in Eqn 8 give a gel threshold for the complex ($c_{1,2}^0$) of 0.60 g/kg. Milk contains about 28 g/kg sodium caseinate (Webb,

1965). From Eqn 3, the concentration of κ -carrageenan required to give a concentration of complex at the gel threshold is ca 0.4g/kg – much the same as the concentration routinely added to chocolate milk to suspend the cocoa particles (0.25-0.35g/kg). Our estimated value for the gel threshold for the complex is very much lower than the gel threshold for enzyme-induced sodium caseinate gels, which is about 5.6g/kg (Clark & Ross-Murphy, 1987). However, in a study in which they compared the rheological properties of rennet-induced gels formed by casein micelles of different size, Niki and his coworkers (1994) found a lower gel threshold for small micelles. Another factor that may be significant is that enzyme-induced (and acid-induced) casein gels appear to have clusters of casein micelles, as would be formed by random aggregation (Roefs et al., 1990, Tombs, 1974). κ -carrageenan – induced gels appear to have a more filamentous structure (Hood and Allen, 1977), closer to a ‘string – of – beads’ network structure (Doi, 1993). The more random the aggregation process, the more protein that is required to form a gel network (Tombs, 1974).

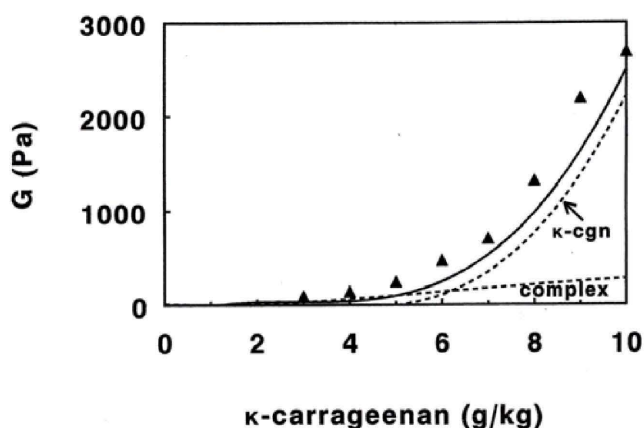


Fig.14

Static shear modulus (G) at 15°C and 1 rad/s as a function of concentration of κ -carrageenan with a fixed concentration of sodium caseinate (10g/kg). The curves were calculated (see text). The solid curve shows calculated values of G; the dotted curves show the individual contributions to G from the complex and free κ -carrageenan.

5. Applications: Gels prepared from Skimmed Milk Powder

Mixed gels of κ -carrageenan and SMP have been studied by differential scanning calorimetry (Miyoshi et al., 2010). Figure 15 shows heating and cooling DSC curves for 25g/kg SMP with various additions of κ -carrageenan. The cooling DSC curves showed single peaks, but these were broader and not well defined.

The enthalpy of gelation (ΔH_g) estimated from the sol-gel transition peak in DSC cooling curves (Miyoshi, 1996), appeared to be independent of the level of addition of SMP. This suggests that a gel network is formed either (a) from κ -carrageenan alone, with the casein micelles having no significant interaction with the polysaccharide and merely acting as a filler and a source of calcium-ions which promote gelation of the κ -carrageenan, or (b) by a mechanism analogous to that proposed by Xu and her colleagues (1992) in which κ -carrageenan molecules are adsorbed to casein micelles which they link to form a mixed gel network by association of carrageenan double helices – but with only small segments of κ -carrageenan chain adsorbed, leaving the bulk of the polysaccharide as loops and tails, free to form double helices such that the magnitude of ΔH_g is largely uninfluenced by proximity to the casein.

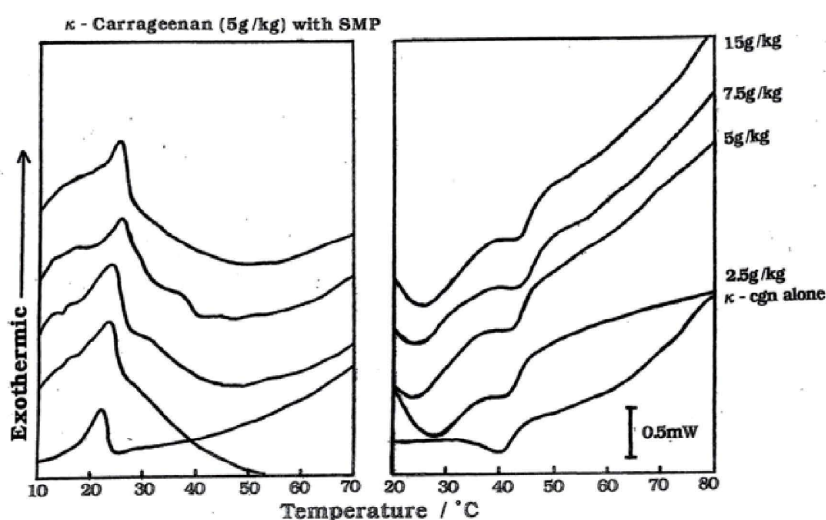


Fig. 15

DSC heating and cooling curves for κ -carrageenan (5g/kg) with different additions of SMP (0~15 g/kg).

The variation of storage modulus (G') and loss modulus (G'') with temperature, within a cooling-heating cycle, is shown in Figure 16. On cooling G' and G'' both increased sharply at about 25–30°C (T_c) and G' became greater than G'' , indicating that gelation had occurred. On reheating, there was a sharp drop in G' and G'' at around 45–55°C (T_h) as the gel melted. At low to intermediate concentrations of SMP (2–15g/kg), the heating curves showed broadening of the transition, as observed with DSC. However, at the higher concentrations of SMP (25–40g/kg), this effect disappeared and the curves reverted to the smooth, sharp transition observed with κ -carrageenan alone.

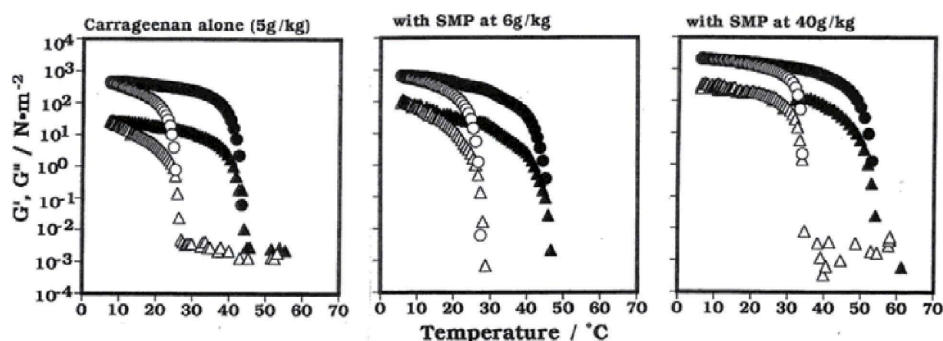


Fig. 16

Storage modulus (G' , ● ○) and Loss modulus (G'' , ▲ △) at 1 rad/s as a function of temperature for κ -carrageenan (5g/kg) with different additions of SMP.

Judging from our results, it is argued that the second mechanism (b) is more likely to be correct because:

- (1) κ -carrageenan is known to adsorb to casein micelles.
- (2) The peak broadening observed in DSC heating curves indicates increasing heterogeneity of the junction zones with addition of SMP.
- (3) Plots of storage modulus (G') and Loss modulus (G'') vs. temperature also indicate junction zone heterogeneity with addition of SMP.
- (4) Gels formed from SMP and low methoxyl pectin (which does not form a complex with casein) show very different relationships between modulus and concentration of polysaccharide and SMP compared with equivalent gels formed from SMP and κ -carrageenan.
- (5) When gels were formed from κ -carrageenan and SMP separated by a dialysis membrane, G' was substantially less than for equivalent gels with the components intimately mixed – again indicating that in the mixed gel, SMP is more than an inert filler and a source of calcium ions. At high ratios of κ -carrageenan to SMP, a purely – carrageenan network also appears to form, presumably interpenetrating the network formed from concentration of casein micelles.

Our results suggest that the gelation mechanism proposed for mixed gels of κ -carrageenan and sodium caseinate are also appropriate for SMP. In broad outline this is the same as the mechanism proposed by Xu and her colleagues who were the first to propose (1992) that only part of the κ -carrageenan chain is adsorbed to the casein micelle, with the rest free in solution as loops or tails, able to form a gel network by forming double helices. We are suggesting, however, that perhaps more of the κ -carrageenan remains free to form helices than is indicated by Xu and her colleagues 'model. At high ratios of κ -carrageenan to SMP, a purely

κ -carrageenan network also appears to form, presumably interpenetrating the network formed from concentration of casein micelles.

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Thermal Properties of Mixed Gel System Formed by κ -Carrageenan and Casein

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Gelation and thickening of dairy products is one of the most important food applications of the carrageenans, particularly κ -carrageenan. In this study, mixed gels of κ -carrageenan and sodium caseinate or skimmed milk powder (SMP) were examined by differential scanning calorimetry (DSC) and rheological measurements.

DSC showed that during gelation (i.e. cooling) the thermal behavior of κ -carrageenan was almost uninfluenced by the presence of sodium caseinate. Thus the interaction of κ -carrageenan with sodium caseinate has little (or no) effect on the carrageenan's coil-to-helix transition. In contrast, during melting, the added sodium caseinate strongly modified the thermal behavior. The DSC peak became progressively broader with the addition of sodium caseinate, indicating that the junction zones are highly heterogeneous in the mixed gel. Rheometry showed that sodium caseinate strongly influences the storage modulus (G'). Experiments in which the concentration of sodium caseinate was fixed and that of κ -carrageenan varied, the plot of G' vs. the concentration of κ -carrageenan was biphasic, with an abrupt change in slope at a concentration that increased linearly with the concentration of sodium caseinate. When the concentration of κ -carrageenan was constant and that of sodium caseinate varied, G' as function of concentration of sodium caseinate passed through a minimum. This behavior could be modelled quantitatively by assuming that: (a) the sodium caseinate adsorbs κ -carrageenan, but with a limited adsorptive capacity, (b) sodium caseinate aggregates (sub-micelles) with adsorbed κ -carrageenan can associate and form a gel network and the network formed by κ -carrageenan alone is additive. At low ratios of κ -carrageenan to sodium caseinate, the sodium caseinate and κ -carrageenan combine and form a mixed gel. As the ratio of κ -carrageenan to sodium caseinate increases, the sodium caseinate becomes saturated and no further association with κ -carrageenan can occur – the increase in G' as further κ -carrageenan is added comes from a gel network formed by κ -carrageenan alone.

κ -carrageenan forms a complex with casein micelles and it appears to act as a molecular "Velcro" – interaction between the free ends of bound κ -carrageenan molecules linking casein micelles to form a gel network. At high ratios of κ -carrageenan to SMP, a pure κ -carrageenan network also appears to form, presumably consisting of more extended cross-linked κ -carrageenan structures within which the casein micelles are enmeshed.