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Rheology of κ-carrageenan and β-lactoglobulin mixed gels

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Abstract

Gel formation and the melting of κ-carrageenan in the presence of β-lactoglobulin were investigated using dynamic rheological techniques as well as a sequence of experimental sweeps of time–temperature, frequency, and strain. The blends, initially prepared at 45°C, show homogeneous mixtures, which then lead to the formation of a gelled κ-carrageenan network containing inclusions of native β-lactoglobulin during the controlled cooling phase from 45 to 20°C. In its native state, the protein seems to weaken the polysaccharide network, particularly when present in high concentration. Upon subsequent heating to 90°C, mixed gels presented a biphasic profile: the first phase, characterized by a decrease in the storage modulus, involves a heat-induced meltdown of the κ-carrageenan network, whereas the second phase, exhibiting an increase in the storage modulus, corresponds to the build up of a protein network. The DSC analysis showed that each biopolymer undergoes specific conformational changes. These results are highly indicative of the lack of association between the biopolymers and suggest a phase separation and gelation in β-lactoglobulin and κ-carrageenan mixtures. The final cooling phase of the mixed gels, from 90 to 20°C, induces a consolidation of the protein network and a gelation of κ-carrageenan. This rheological behaviour suggests that gelation of κ-carrageenan in the presence of a gelled protein network would lead to the formation of a phase-separated bicontinuous network. The strain sweep results for mixed gels obtained at the end of the experiments support that hypothesis.

Keywords: β-Lactoglobulin; κ-Carrageenan; Mixed gels; Rheology; Phase separation

1. Introduction

In recent years, considerable interest has been given to the study of protein–polysaccharide mixtures in both the industrial and academic sectors. Current applications have shown that such blends can constitute new food ingredients, particularly as fat substitutes (Cain, Jones & Norton, 1987; Tolstoguzov & Vincent, 1997). In solution, the nature of protein–polysaccharide interactions has a great effect on the protein's functional properties (Tolstoguzov, 1991). When solutions of two different biopolymers are mixed together, three situations may arise. These are co-solubility, association, and incompatibility. Co-solubility is rather rare (Tolstoguzov, 1991). Complexing or association of the biopolymers occurs if biopolymer interactions are favourable (e.g. polyanion–polycation). As a result, a two-phase system is seen, one phase where both biopolymers are present in high concentrations while the other contains mainly water. On the contrary, thermodynamic incompatibility, which is the more usual situation, occurs if the interactions between the different biopolymers are repulsive and/or when the two types of polymer show varying affinity towards the solvent (Picullel & Lindmann, 1992; Tolstoguzov, 1991). The thermodynamic incompatibility, arising from the low entropy of mixing upon blending, results in demixing or phase separation when the overall biopolymer concentration exceeds a certain concentration, called the phase separation threshold. Then, a two-phase system is formed where each phase is characterized by the high concentration of one biopolymer. Such mixing behaviour is strongly influenced by pH, ionic strength, conformation, charge density, and biopolymer concentration (Tolstoguzov, 1991).

When gelation occurs, three basic types of gel structure can be observed, namely interpenetrating, coupled, and phase-separated networks. Interpenetrating networks represent the simplest situation, where the two components gel separately and form independent networks. Both networks are continuous throughout the sample but any interaction between them is only topological (Morris, 1986). Coupled networks are formed when there are direct associations (synergistic interactions) between polymers prior to network formation. In contrast, phase-separated gels are formed from incompatible polymers. In the latter system,

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a competition between the phase separation and gelation processes generally takes place, resulting in an increase in the complexity of the system. Here, an infinite number of stable phase distributions can be obtained: from a network containing both continuous and dispersed polymers to a phase-separated gel consisting of a bicontinuous network (Clark, 1995; Owen & Jones, 1998).

κ-Carrageenan is widely used as a thickening and gelling agent in the food industry. It is a fraction of the sulphated polysaccharide family extracted from certain species of red seaweed (algae), with a composition that consists of alternating 1,3-linked D-galactose-4-sulphate and 1,4-linked 3,6-anhydro-D-galactose monomers. The two-step gelation process, characterized by a coil (disordered) to helix (ordered) transition followed by aggregation and network formation (Hermansson, 1989; Morris, Rees & Robinson, 1980; Rochas & Rinaudo, 1980), occurs on cooling. Although a general consensus regarding this two-step process in the scientific community exists, its exact mechanism has not been well established. The gelation mechanism is strongly influenced by the type and the quantity of counter-ion present in the solution (Hermansson, 1989; Hermansson, Ericksson & Jordansson, 1991; Rochas & Rinaudo, 1980).

β-Lactoglobulin is the major protein constituent of whey and is considered as its primary gelling agent. In its native state, β-lactoglobulin exists as a globular protein folded into compact three-dimensional structures where the relative hydrophobic amino-acid side chains are located in the interior, while most of the polar side chains are exposed to the exterior. Heating β-lactoglobulin solutions above a certain temperature causes partial unfolding, exposition of interiorly located amino acid, and aggregation of partially or completely denatured protein (Kinsella & Whitehead, 1989; Ziegler & Foegeding, 1990). Above a certain concentration, a gel was formed, which was either transparent (fine-stranded) or opaque (particulate) (Stading & Hermansson, 1990). The properties of thermally induced gels made from β-lactoglobulin are influenced by several factors, including concentration, extent of denaturation, pH, temperature, concentration, heating rate, ionic strength, and the presence of specific ions (Aguilera, 1995; Kinsella & Whitehead, 1989; Mulvihill & Kinsella, 1988; Ziegler & Foegeding, 1990).

The functional properties of whey proteins, such as their gel-forming ability, emulsifying and foaming properties are extensively exploited by the food industry. An additional way to increase the potential of whey protein applications is to take advantage of their interactions with food hydrocolloids. As a matter of fact, it has recently been shown that the thermodynamic incompatibility of whey protein and polysaccharide mixtures may provide an alternative to high shear homogenization in the production of small homogeneous, and completely spherical whey protein gel particles, which could constitute potential food-texturizing or -whitening agents (Syrbe, 1997; Tolstoguzov & Vincent, 1997). This was achieved by optimization of the phase separation and whey protein gel formation process in a whey protein and anionic polysaccharide mixed systems. Recently, Mleko (1997) showed that mixtures of whey protein and κ-carrageenan can also be used to produce dairy desserts, having the same textural properties as milk desserts but with lower content of gelling agents.

The development of novel gelled products from biphasic systems of whey protein and polysaccharide and their control need a better understanding regarding gelation mechanisms and physical properties of mixed gels. The aim of this study is to investigate the rheological behaviour of κ-carrageenan and β-lactoglobulin mixed gels, under conditions where β-lactoglobulin exists both in its native and denatured states.

2. Materials and methods

2.1. Materials

Purified β-lactoglobulin lyophilized powder containing genetic variant A and B and κ-carrageenan sample from *Eucheuma cottonii* type III were purchased from Sigma (St Louis, MO, USA). The composition of κ-carrageenan powder, provided by the supplier, shows that it contains a mixture of the following cations: K^+ (6.8%), Ca^{2+} (2.4%), and Na^+ (0.6%).

2.2. Preparation of solutions

Mixtures were prepared with a constant 1% (w/w) κ-carrageenan concentration and with varying amounts of β-lactoglobulin. Three β-lactoglobulin concentrations (0.5, 5, and 10%), which correspond to the following protein-to-polysaccharide ratios were, respectively, considered: 1:2, 5:1, and 10:1. Protein solutions were prepared by adding appropriate amounts of β-lactoglobulin powder to a portion of deionized water that contained 10 mM of $CaCl_2$, and then stirred for 2 h at room temperature. This calcium level corresponds to the optimum concentration that gives the maximum strength to β-lactoglobulin gels (Mulvihill & Kinsella, 1988). Solutions were then stored at 5°C overnight for a complete hydration of the protein molecules. Single κ-carrageenan solutions and mixtures were prepared by dispersing appropriate amounts of κ-carrageenan powder in a salt solution (10 mM of $CaCl_2$) and in protein solutions, respectively. After a complete dispersion of the κ-carrageenan powder, single κ-carrageenan solutions and mixtures were heated to 45°C for 20 min in order to ensure the dissolution of the polysaccharide. Hot κ-carrageenan solutions (45°C) had a clear aspect, indicating that the polymer had been well dissolved. This temperature allowed for good dissolution of the polysaccharide without protein denaturation. The pH of the solutions was adjusted to 7 using 1 N of HCl or NaOH.

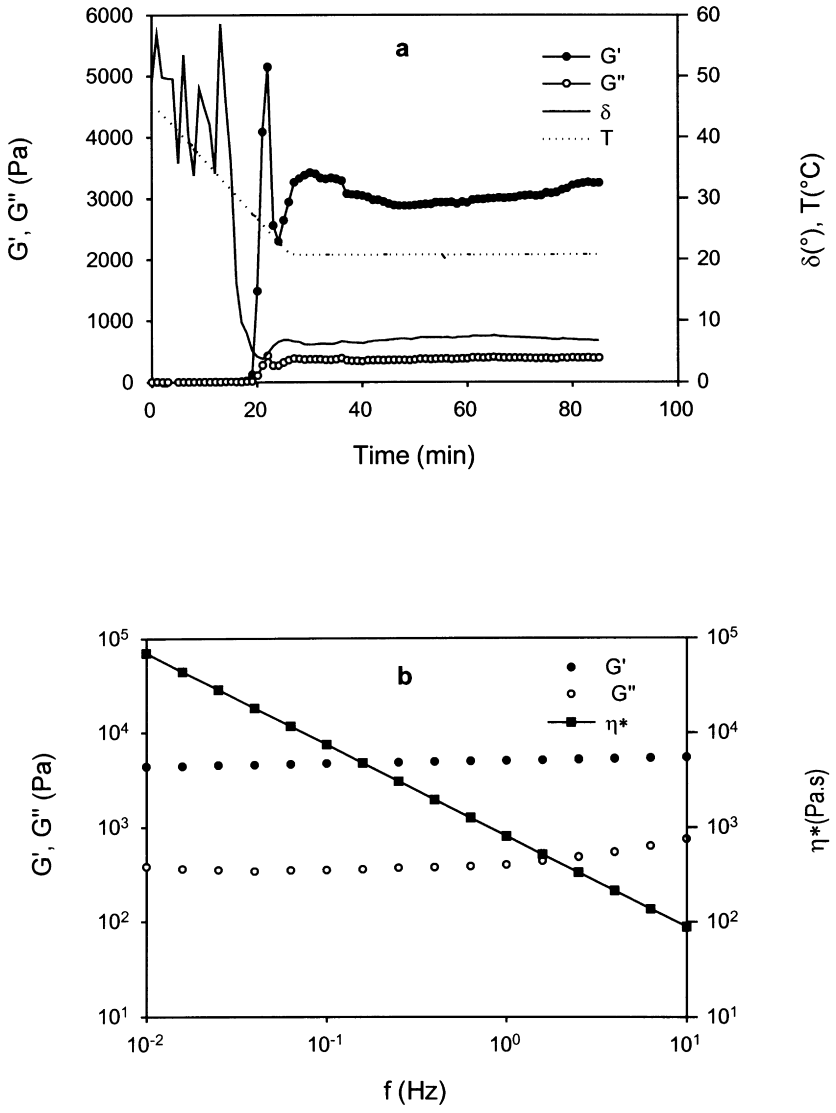

Fig. 1. Variation of G', G'', δ and η^* for 1% κ-carrageenan system as a result of: (a) cooling from 45 to 20°C at 1°/min and holding for 60 min; and (b) increasing the frequency of oscillation from 0.01 to 10 Hz ($T = 20$°C, strain = 0.5%).

2.3. Methods

2.3.1. Dynamic oscillatory measurements

Rheological measurements were performed on a controlled-strain rheometer (ARES 100 FRT, Rheometric Scientific, Piscataway, NJ, USA) using a parallel plate geometry (25 mm diameter, 1 mm gap), by small-deformation oscillatory measurements every 60 s at a frequency of 1 Hz and a strain of 0.5% unless specified. Hot (45°C) single κ-carrageenan or mixed solutions were poured directly into the measuring system of the rheometer, equilibrated before at the same temperature. The sample was covered with a thin film of paraffin oil to avoid evaporation during the measurements. Solutions were cooled to 20°C allowing κ-carrageenan to gel in the presence of native proteins and heated to 90°C in order to study the melting of the κ-carrageenan network and protein gelation. Finally, samples were cooled to 20°C to allow κ-carrageenan gelation in the presence of denatured proteins.

A sequence of the following sweeps was used: (1) a temperature decrease of 1°C/min from 45 to 20°C and measurements for 1 h at 20°C; (2) a frequency sweep between 0.01 and 10 Hz at 20°C; (3) a temperature increase at the same scan rate from 20 to 90°C and measurements for 30 min at 90°C; (4) a frequency sweep between 0.01 and 10 Hz at 90°C; (5) a final temperature decrease of 1°C/min from 90 to 20°C and measurements for 1 h at 20°C; (6) a frequency sweep between 0.01 and 10 Hz at 20°C; and (7) a strain sweep from 0.02 to 100% at 20°C. At least three replicates of each measurements were made. Single protein solutions were poured into the parallel plate geometry at 20°C and sequences (3) to (7) were performed.

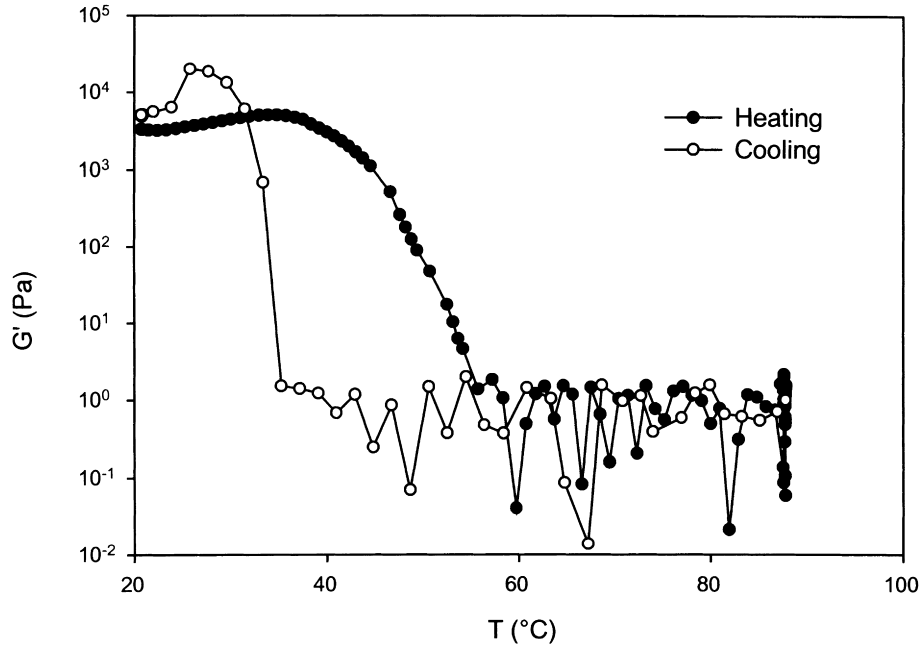

Fig. 2. Network melting and development of 1% κ-carrageenan sample during controlled heating and cooling phases, respectively. Sample was heated from 20 to 90°C at 1°/min, held for 30 min, then cooled to 20°C at the same scan rate and held for 60 min ($f = 1$ Hz, 0.5% strain).

2.3.2. DSC measurements

A DSC (model DSC 910, Dupont, Wilmington, DE, USA) equipped with an IBM computer and cooling system was used. Thermograms of single and mixed gels were recorded on heating and cooling over the range of 5–120°C at a scan rate of 10°C/min. An empty sealed pan was used as thermal reference. Thermal transition temperature and enthalpy were determined using an internal curve integration program. Two replications were measured for each experiment.

3. Results and discussion

3.1. Rheological behaviour of individual components

3.1.1. κ-Carrageenan gels

Fig. 1a shows the gelation of κ-carrageenan (1%, w/w) during initial cooling from 45 to 20°C. The onset of the aggregation, indicated by the increase in the storage and loss moduli (G' and G'', respectively) and the decrease in phase angle δ, occurred at about 31°C, which is in agreement with previous studies on κ-carrageenan gelation (Hermansson et al., 1991; Kohyama et al., 1996; Rochas & Rinaudo, 1980). After the gel point, a pronounced maximum in G' and G'' and a minimum in the phase angle were observed. Then a second little broad peak in G' was observed, and during the holding time (60 min) at 20°C, G', G'' and the phase angle tended to approach an asymptotic values. A maximum in G', during the sol–gel transition, has been previously observed for κ-carrageenan in the presence of potassium (Hermansson, 1989; Hermansson et al., 1991) as well as for mixed gels of κ-carrageenan and locust-bean gum (Stading & Hermansson, 1993). The peak in G' results from the formation of a transient state, consisting of a fine network structure. Furthermore, the decrease in G' after the peak comes from a partial breakdown of this transient structure and the formation of a coarse supermolecular network during κ-carrageenan gelation (Hermansson, 1989). According to this author, such behaviour is an indication of a dominant potassium form of κ-carrageenan and it can be seen in some, but not all, commercially available preparations of κ-carrageenan, depending on their cation composition. The κ-carrageenan sample, used in the present study, contains a mixture of potassium, calcium, and, to a lesser extent, sodium ions (see Section 2).

The existence of the peak could also be explained by the effect of slippage between the gel and the measuring device (Stading & Hermansson, 1993). However, in our case, a perfect sinusoidal torque was obtained on κ-carrageenan gel (results not shown), which is an indication that no sliding effect occurred (Arnaud, Choplin & Lacroix, 1989).

After a holding time of 60 min at 20°C, the frequency sweep performed on the carrageenan gels, showed a gel-like response with $G' > G''$, a small frequency dependence of G', and a linear variation of $\log \eta^*$ with $\log f$ (slope is equal to -0.96) (Fig. 1b).

The effect of subsequent heating to 90°C and further cooling to 20°C on the storage modulus of the κ-carrageenan sample is shown in Fig. 2. Under the present conditions, it was found that κ-carrageenan formed a thermoreversible gel. Heating above 40°C resulted in a pronounced decrease of the storage modulus and led to a complete meltdown of

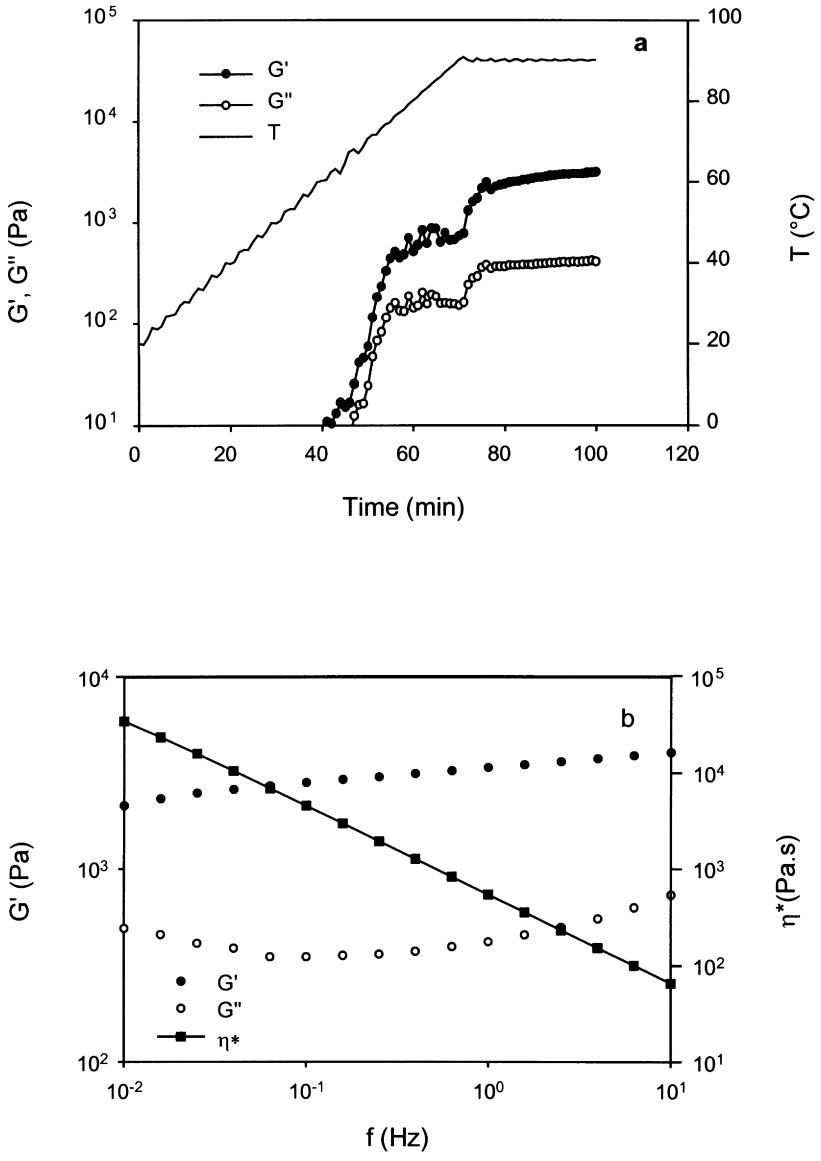

Fig. 3. Modulus development for 5% β-lactoglobulin sample as a result of: (a) heating from 20 to 90°C at 1°/min and held for 30 min; and (b) increasing the frequency from 0.01 to 10 Hz ($T = 90$°C, strain = 0.5%).

κ-carrageenan network at temperatures above 60°C. While, cooling induced gel formation, at temperatures below 30°C. A hysteresis in the rheological measurements between the heating and cooling curves was found, with the gel-to-sol transition temperature higher than that of the sol-to-gel transition. Such behaviour has been reported previously for κ-carrageenan in the presence of gelling cations (Hermansson et al., 1991; Kohyama, Sano & Nishinari, 1996; Rochas & Rinaudo, 1980). The melting curve of κ-carrageenan showed no pronounced peak in G' similar to that observed during gel formation, which is in agreement with previous results (Hermansson, 1989).

3.1.2. β-Lactoglobulin gels

Gel formation of 5% β-lactoglobulin sample during controlled heating (1°C/min) from 20 to 90°C and holding at this temperature for 30 min is shown in Fig. 3a. The onset of gelation, as indicated by the rise in G' and G'', occurred at temperatures above 65°C, which is in agreement with previous results found for β-lactoglobulin gelation with calcium cations (Twomey, Kieran Keogh, Mehra & O'Kennedy, 1997). A slight reduction in G' and G'' followed by a continuous increase was observed during the heating ramp. This unusual phenomenon could be caused by the presence of some air bubbles in the sample. During the holding time for 30 min at 90°C, both G' and G'' tended to approach asymptotic values (pseudo-equilibrium plateau). The frequency sweep performed at 90°C after the holding period (30 min) on β-lactoglobulin gels shows a 'true' gel-like spectrum (Fig. 3b), with a little dependency of G' over the entire range of frequency values and with a linear decrease in $\log \eta^*$ with increasing $\log f$ (slope is equal

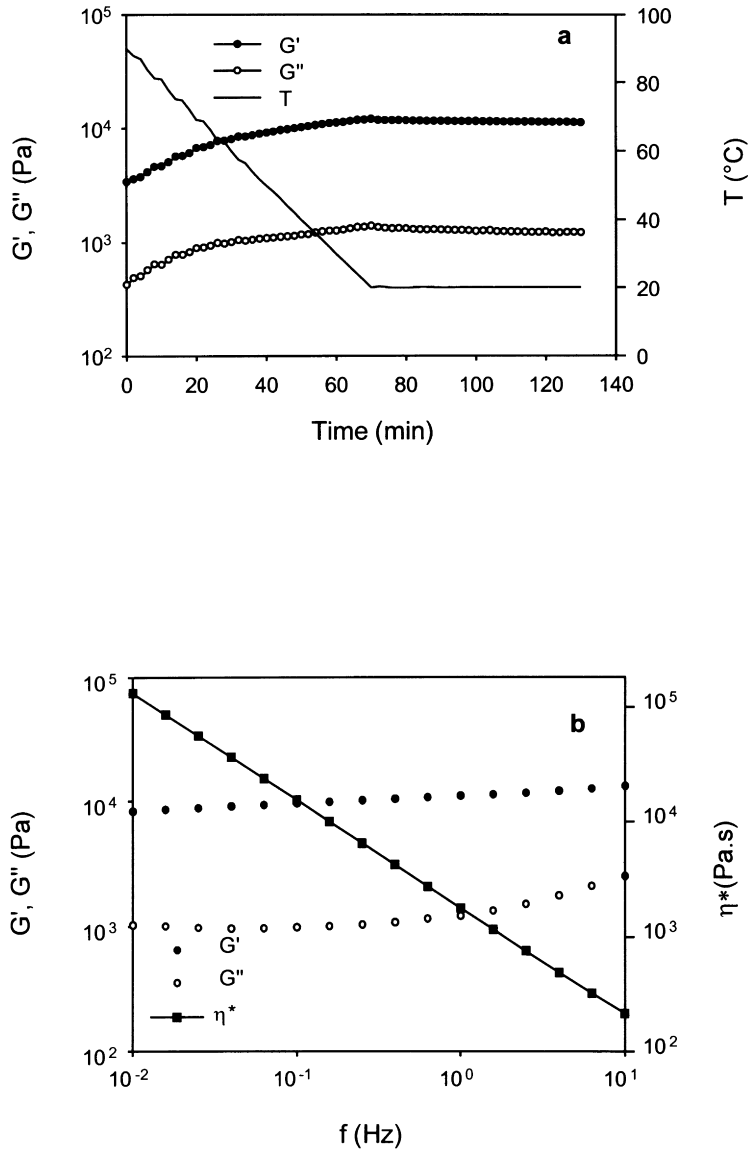

Fig. 4. Following the thermal treatment of Fig. 3, the 5% β-lactoglobulin gel was: (a) cooled to 20°C at 1°/min and held for 60 min; and (b) then subjected to a frequency sweep at 20°C (strain = 0.5%).

to −0.91). The initially clear solution turned into a white milky gel after the heat treatment.

Gelation of β-lactoglobulin in distilled water at different pH values has been previously studied and revealed that the critical gel concentration for β-lactoglobulin was highly pH-dependent. At neutral pH, a minimal concentration of 10% (w/w) is needed for β-lactoglobulin to form a self-supporting gel. While, at other pH values (5.5–4.5), it was possible to form a gel at concentrations as low as 1% (Stading & Hermansson, 1990). The critical gel concentration for β-lactoglobulin is also dependent on salt content and type. In the presence of 1% NaCl, Paulsson, Hegg and Castberg (1986) found that a 2% β-lactoglobulin solutions (pH 6.6) produced a gel. While at this pH and without salt, a minimal gel concentration of about 10% was found by Stading and Hermansson (1990). In our case, without added calcium chloride, a 5% protein solution did not gel, which is agreement with previous findings (Stading & Hermansson, 1990). But, with 10 mM calcium chloride, which corresponds to the optimum level that confers the maximum strength to β-lactoglobulin gels (Mulvihill & Kinsella, 1988), the minimal gel concentration for β-lactoglobulin was found to be about 1%. The presence of relatively high amounts of calcium in our solutions may explain the low minimal gel concentration needed for β-lactoglobulin gel formation. Calcium reduces repulsive negative charges between protein molecules and then increases their tendency towards aggregation. It is also involved in the formation of cross-linking between unfolded protein chain resulting in network formation (Mulvihill & Kinsella, 1988).

On subsequent cooling from 90 to 20°C, both G' and G''

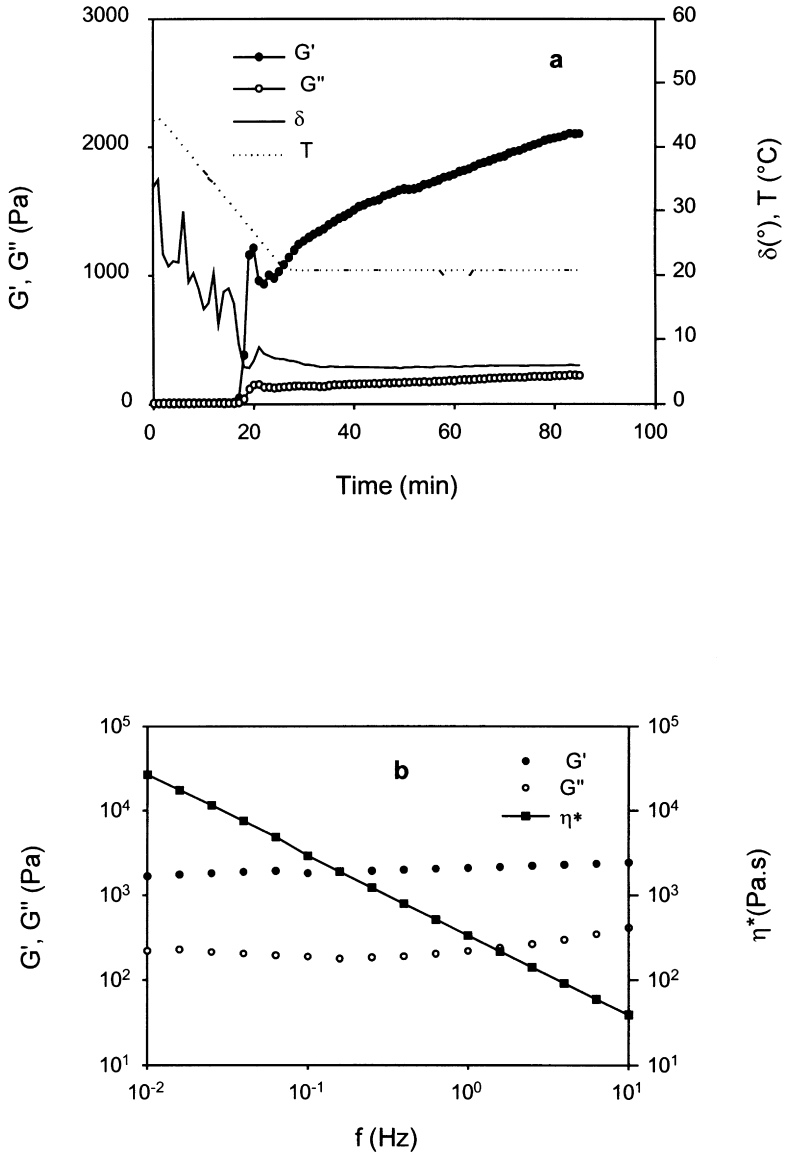

Fig. 5. Variation of G′, G″, δ and η^* for a mixture of 1% κ-carrrageenan and 5% β-lactoglobulin as a result of: (a) cooling from 45 to 20°C at 1°/min and holding for 60 min; and (b) increasing the frequency of oscillation from 0.01 to 10 Hz ($T = 20$°C, strain = 0.5%).

increased monotonically with decreasing temperature (Fig. 4a) and during the holding period (20°C, 60 min), they almost reached constant values. Linear increase of gel moduli with decreasing temperature has been previously observed for whey and soy proteins gels (Chronakis & Kasapis, 1993; Cooney, Rosenberg & Shoemaker, 1993; Manoj, Kasapis & Hember, 1997). This phenomenon was attributed to a reduction in entropy, which consolidated the attractive forces (hydrogen bonding, van der Waals forces) between the protein particles in the gel (Chronakis & Kasapis, 1993; Manoj et al., 1997). Mechanical spectra of β-lactoglobulin gel recorded at 20°C (Fig. 4b) appear similar to those obtained at 90°C (Fig. 3b) but with a smaller separation between G′ and G″ curves (the slope of log η^* vs. log f is equal to −0.93). This is in agreement with recent observations made by Manoj et al. (1997) on whey protein gels.

3.2. Rheological behaviour of mixed gels

The gelling and melting profiles of three κ-carrageenan and β-lactoglobulin mixtures were investigated using non-destructive oscillatory measurements. κ-Carrageenan, which forms thermoreversible gels, was first set during the initial cooling from 45 to 20°C. Subsequent heating to 90°C will induce meltdown (see above) of the κ-carrageenan network, denaturation, and gelation of β-lactoglobulin. Final cooling to 20°C will allow κ-carrageenan to gel in the presence of denatured protein.

An example of κ-carrageenan gelation in the presence of

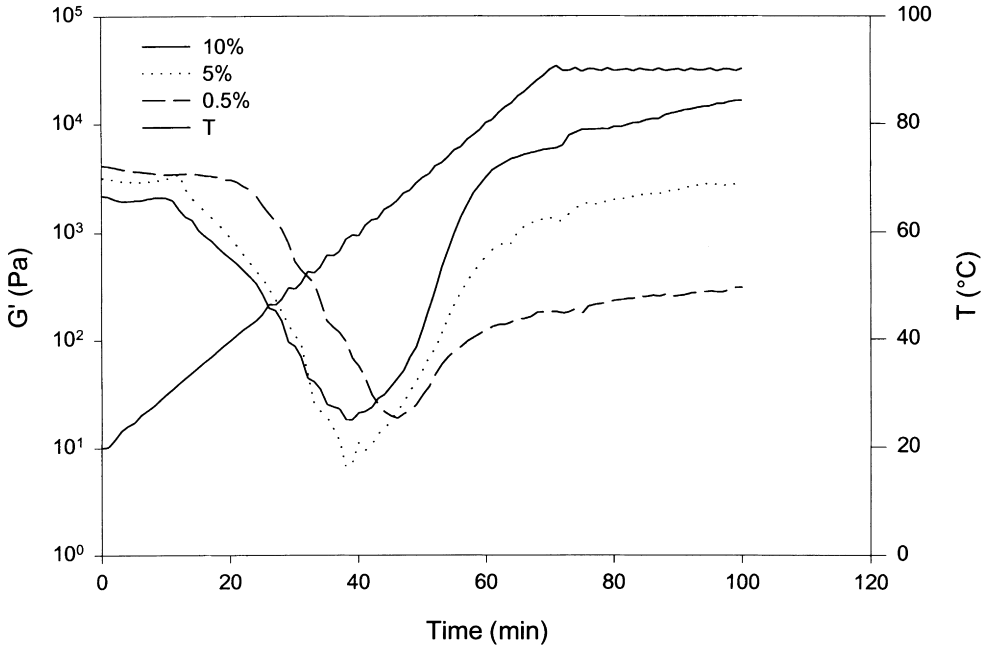

Fig. 6. Following the experiment of Fig. 5, mixed gels of 1% κ-carrrageenan and β-lactoglobulin (protein concentration is shown), were heated to 90°C at 1°/min and held for 30 min ($f = 1$ Hz, strain = 0.5%).

native β-lactoglobulin is shown in Fig. 5. The gelation profile (Fig. 5a) and mechanical spectra (Fig. 5b) obtained at the end of the isothermal run were almost similar to those of pure κ-carrageenan samples. The characteristic peak in G' and G'' and the minima in the phase angle observed for pure κ-carrageenan gel (Fig. 1a) also occurred in the mixture. However, the peak becomes less pronounced than for single κ-carrageenan samples. G' values at the peak and those obtained at the end of the isothermal run were also lower than those of single κ-carrageenan gels. Similar results were also observed with mixtures, containing 0.5 and 10% β-lactoglobulin. In its native state, the protein seems to weaken the polysaccharide network, particularly when present in high concentration.

The effect of subsequent heating on the storage modulus of κ-carrageenan and β-lactoglobulin mixed gel, containing three different protein-to-polysaccharide ratios is shown in Fig. 6. The temperature was increased from 20 to 90°C at a scan rate of 1°C/min and samples were set at 90°C for 30 min. The comparison of mixed gels behaviour with those of each individual component shows that the mixed gel profile could be viewed as a biphasic process. The first phase, where the storage modulus decreased, involves a heat-induced meltdown of κ-carrageenan gel since a progressive decrease in G' was also observed for single κ-carrageenan gel (Fig. 2). The second phase, where the storage modulus increased, corresponds to the build up of a protein network as revealed by an abrupt increase in G' and its slight continuous increase (pseudo-equilibrium plateau) observed during isothermal run at 90°C, a behaviour also observed for single β-lactoglobulin samples (Fig. 3a). Surprisingly, mixture containing 0.5% β-lactoglobulin formed gels. Protein solution of the same concentration did not gel but rather formed dispersion of visible white aggregates. The presence of 1% κ-carrageenan in that mixture induced β-lactoglobulin gelation at concentration below its minimal gel concentration, which is about 1% under the present condition. For the mixture containing 0.5% β-lactoglobulin, the onset of the increase in G', corresponding to the onset of protein gelation in the mixed system, occurred at about 68°C (Fig. 6). The temperature, at which this steep increase in G' started, decreased with increasing protein concentration, which is in agreement with previous observations made on mixed system of whey protein and maltodextrin (Manoj et al., 1997). As expected G' values, at the end of heating run, for mixed gels increased with increasing protein concentration, thus confirming that the second part of the biphasic profile is due to protein gelation.

The biphasic profile of mixed gels showed that the behaviour of both κ-carrageenan and β-lactoglobulin can easily be identified in that of mixed gels, thus suggesting that phase separation of the polymers occurred. In literature (Chronakis & Kasapis, 1993; Kasapis, Morris, Norton & Brown, 1993; Manoj, Kasapis & Chronakis, 1996; Manoj et al., 1997; Papageorgiou, Kasapis & Richardson, 1994; Walkenström & Hermansson, 1994, 1996), the cooling and heating ramps of composite gels in comparison with the temperature course of the individual components have proven useful in obtaining an idea of the phase distribution for various biopolymer mixtures. When the gel formation and/or melting steps of the mixed gels and those of individual components correlate with each other, one can conclude that phase separation has occurred.

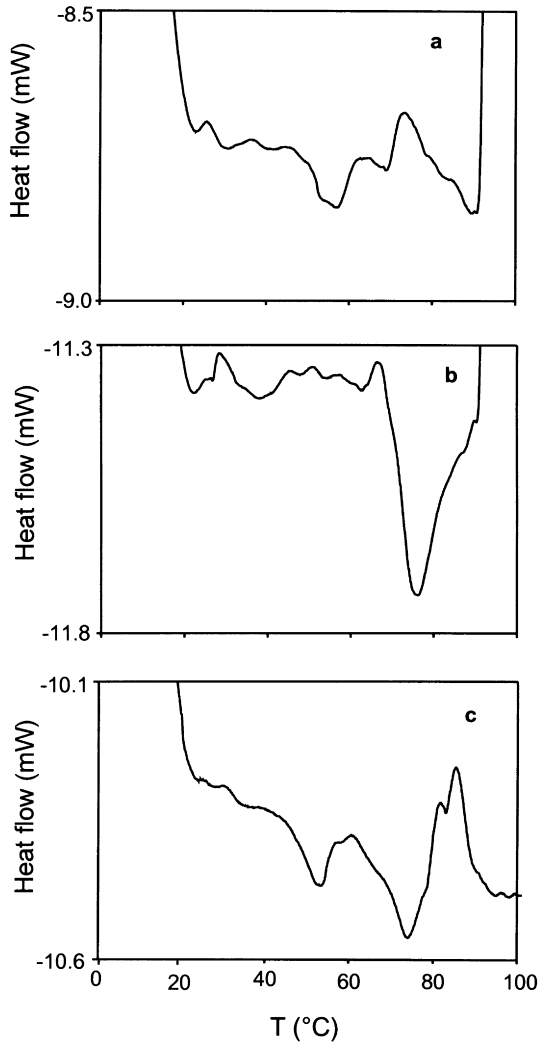

Fig. 7. Heating DSC curves for: (a) 1% κ-carrageenan; (b) 10% β-lactoglobulin; and (c) mixed system (1% κ-carrageenan +5% β-lactoglobulin). Scan rate is 10°/min.

Occurrence of the phase separation in mixtures of denatured β-lactoglobulin and κ-carrageenan was also supported by visual observation and DSC experiments. Phase separation of denatured β-lactoglobulin (80°C, 20 min) and κ-carrageenan was observed visually at room temperature and showed that mixtures of those biopolymers dissolved in deionized water formed a two-phase system at concentrations as low as 1% β-lactoglobulin and 0.4% κ-carrageenan. Centrifugation of mixture, containing 1% β-lactoglobulin and 0.4% κ-carrageenan, for 1 h at $10{,}000 \times g$ resulted in two distinct phases: an upper clear phase characteristic of κ-carrageenan and a lower white opaque phase, with high concentrations of β-lactoglobulin. In the presence of 10 mM calcium, it formed a two-gelled phase. In agreement with our observations, Syrbe, Fernandes, Dannenberg, Bauer & Klostermeyer (1995) showed that phase separation occurred in mixtures of denatured whey protein and κ-carrageenan at pH 7.0.

The typical DSC heating curves for single κ-carrageenan, single β-lactoglobulin and their mixtures are given in Fig. 7. The heating profile of single κ-carrageenan (Fig. 7a) showed a single broad endothermic peak, corresponding to the gel melting with a midpoint temperature of about 55°C. β-Lactoglobulin thermograms (Fig. 7b) showed an endothermic peak, corresponding to protein denaturation, with a midpoint of about 76°C. Mixed samples produced DSC heating curves (Fig. 7c), with two peaks, one corresponding to the melting of κ-carrageenan and the second due to the unfolding of β-lactoglobulin. In terms of general band-form and position, the peaks were almost identical to those of single components.

The above results indicate that each biopolymer undergoes independent conformational changes, in other words, there is no specific interaction between them. The same behaviour has been previously observed for gelatin/maltodextrin, gelatin/gellan and maltodextrin/sodium caseinate composite gels (Kasapis et al., 1993; Manoj et al., 1996; Papageorgiou et al., 1994). Such behaviour strongly differed from that observed for synergistic networks of κ-carrageenan and konjac-mannan where heterotypic bonding between blend components is responsible for the emergence of additional peaks or changes in the characteristic position and forms of single component transitions (Williams, Clegg, Langdon, Nishinari & Picullel, 1993).

Rheological results, DSC characterization, and visual assessment showed that phase separation and gelation occurred in β-lactoglobulin/κ-carrageenan mixtures during heating. Phase separation would arise from limited thermodynamic compatibility between unfolded protein molecules and disordered polysaccharide chains. During heating from 20 to 90°C, carrageenan undergoes an order–disorder transition and conformational changes occurred in the β-lactoglobulin structures. The presence of carrageenan as forms of random coils and β-lactoglobulin in an unfolded state would increase their mutual steric exclusion. Furthermore, the presence of calcium cations, which increase the tendency of aggregation between the protein molecules, would also favour a phase separation between the two polymers. Biopolymer incompatibility definitely increases in conditions that are favourable for self-association of macromolecules (Tolstoguzov, 1991). Steric exclusion would result in an increase in the effective concentration of β-lactoglobulin and consequently in the formation of a protein network at concentrations (0.5%) below its minimal gel concentration.

Finally, the experimental procedure was concluded by cooling blends from 90 to 20°C at a scan rate of 1°C/min and allowing the gels to mature at this temperature for 1 h. The G' profile with respect to time (or temperature) for mixed gels, with different protein concentrations, is shown in Fig. 8. This profile can be divided into two parts: an initial increase, between 90 and 30°C followed by a second increase during subsequent cooling to 20°C. The early development of the storage modulus of mixed gels is, in all likelihood, the result of attractive forces generated between adjacent polypeptides chains since a monotonic

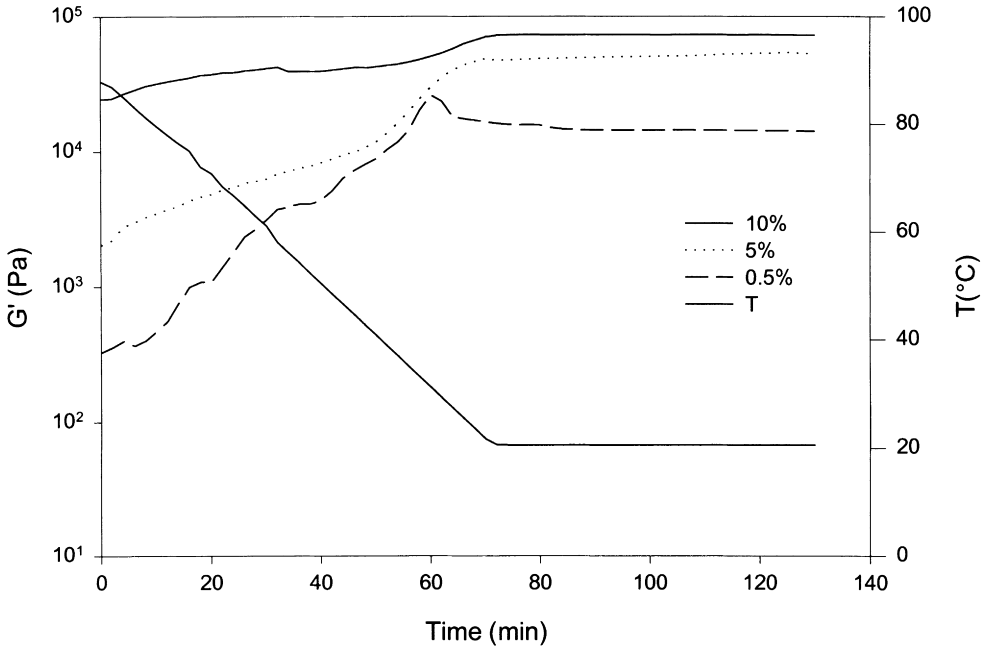

Fig. 8. Following the heating treatment of Fig. 6, the mixed systems were cooled to 20°C at 1°/min and held for 60 min ($f = 1$ Hz, strain = 0.5%).

increase in the storage modulus of single β-lactoglobulin gels is observed upon cooling. The second increase in G' for mixed gels, observed below 30°C, arises from carrageenan gelation since it appeared with the onset of gelation for carrageenan. A peak in the storage modulus was only observed for mixed gels containing 0.5% β-lactoglobulin just before the holding period (60 min) at 20°C, a phenomenon also observed during gel formation of single κ-carrageenan samples (Fig. 1a). During the holding period at 20°C, G' was practically constant in all mixtures. A higher rigidity of the mixed gel as compared with that of the protein gel was then observed for the three mixtures, which is in agreement with previous results on whey protein and κ-carrageenan mixed gels (Mleko, Li-Chan & Pikus, 1997; Syrbe et al., 1995). For instance, the G' of the 5% β-lactoglobulin + 1% κ-carrageenan mixed gel at 20°C was about 48 kPa, which is significantly higher than that of 11 kPa reported for 5% β-lactoglobulin single gel (Fig. 4).

The rheological behaviour of mixed gels during previous heating showed a complete meltdown of κ-carrageenan network followed by the aggregation and the gelation of protein for all mixtures, thus suggesting a phase separation of the polymers. The DSC spectrum comparison of the mixed systems with those of individual components indicated the absence of association between the two polymers. The subsequent gelation of κ-carrageenan during final cooling in the presence of such protein network would certainly lead to the formation of phase-separated composite gels. In such mixed gels, both κ-carrageenan and β-lactoglobulin are expected to form a continuous network. The evidence of a continuous protein network in composite gel is clearly demonstrated from the melting curve of mixed gel (Fig. 6), where a gelled protein network was formed for all mixture and from gel formation curves (Fig. 8), where the consolidation of such network is clearly observed. The appearance of mixed gels (white opaque gel with the presence of a clear zone) also confirms that finding. A white gel was formed by single β-lactoglobulin solutions while a clear transparent gel, observed for single κ-carrageenan sample. The existence of a continuous κ-carrageenan network in composite gel is also evident from the observations of gel formation curves (Fig. 8), where the κ-carrageenan gel formation can easily be detected in that of mixed gels. It is likely that upon cooling, the κ-carrageenan will gel in the pores of the protein network and as the pores are continuous, the κ-carrageenan network will be continuous.

The breaking profile of mixed gels, shown in Fig. 9a, also tended to confirm the hypothesis of a bicontinuous network. The strain sweep, performed at the end of the experiment, showed that the overall breaking profile of mixed gels is intermediate, in terms of critical strain, between those of β-lactoglobulin and κ-carrageenan gels. The linear viscoelastic region of β-lactoglobulin gels is large and extends up to more than 10% (Fig. 9b) while, that of κ-carrageenan gels is relatively narrow and extends up to only about 1%. This value is however higher than that used in the present study (0.5%), confirming that our experiments were well within the linear region. Increasing protein concentration resulted in an extension of the mixed gels' linear viscoelastic region towards higher strain values. Resulting phase-separated bicontinuous gels would have a morphology that is similar to that of interpenetrating networks (IPN). In an IPN, the two components gel separately, forming independent networks. Both networks are continuous throughout the sample but any interaction between them is only topological (Morris, 1986). The formation of a biphasic gel was reported

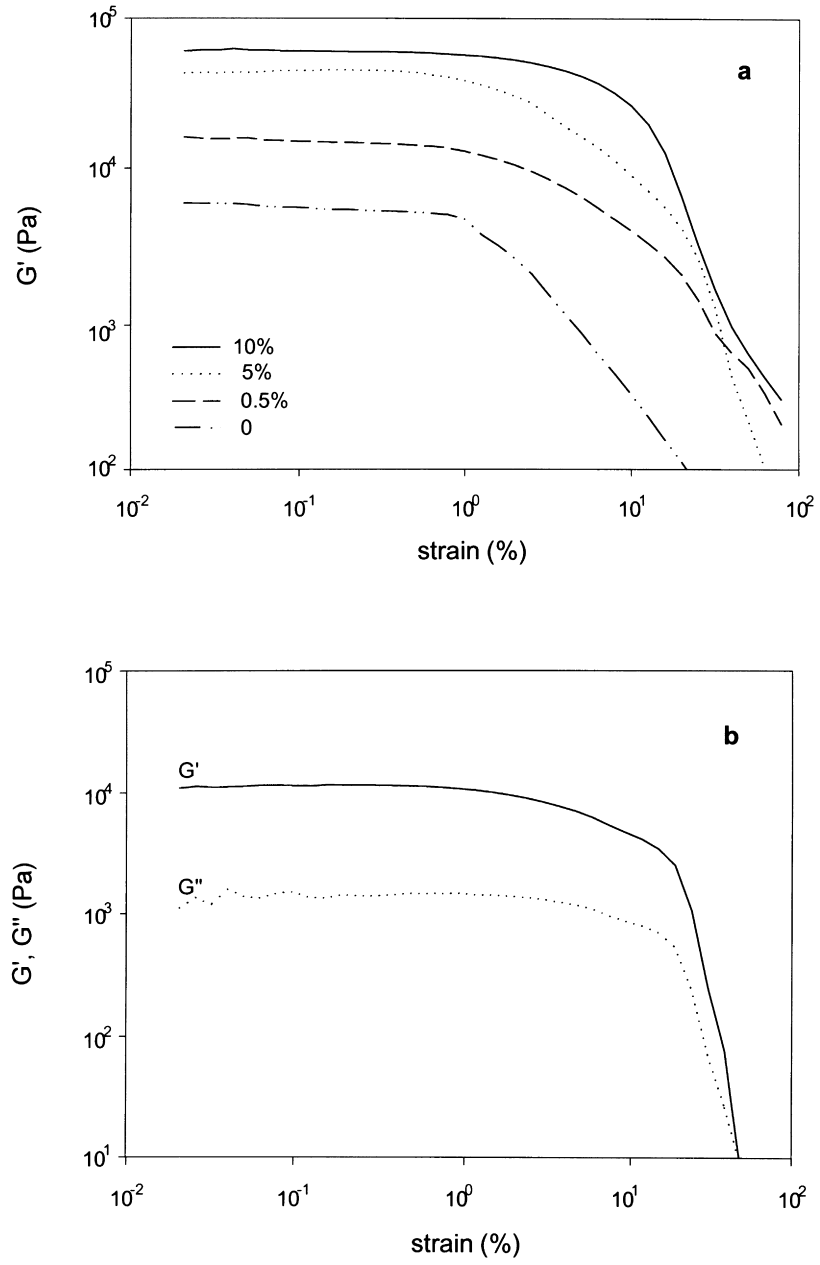

Fig. 9. Breaking profiles for (a) mixed gels and single κ-carrageenan gel (the protein concentration is shown); and (b) for 5% β-lactoglobulin gel ($f = 1$ Hz, $T = 20$°C).

previously for whey protein–gelatin systems (Walkenström & Hermansson, 1994, 1996).

4. Conclusions

This study shows that κ-carrageenan and native β-lactoglobulin form homogeneous blends upon mixing (45°C) and that the presence of the protein, in its native state, seems to weaken the κ-carrageenan cool-induced gel, particularly when present in high concentration. During gel formation, a characteristic peak in the storage modulus was found for single and mixed system. Such behaviour, previously observed by Hermansson et al. (1991), was attributed to the formation and breakdown of a transient structure, which occurred during κ-carrageenan gelation. During subsequent heating to 90°C, the storage modulus of mixed gels as a function of time–temperature showed a biphasic profile: an initial phase, between 20 and 65°C, where a decrease in G′ was found to correspond to the meltdown of the polysaccharide network and a second phase, above 65°C, where a rise in G' occurred, revealing protein gelation. The DSC heating curves for mixed system showed two peaks, one around 55°C and a second around 76°C, resulting from conformational changes that occurred for κ-carrageenan and β-lactoglobulin respectively. The peaks were, in

terms of general band-form and position, almost identical to those of individual components. The rheological behaviour and DSC characterization of mixed system indicate the absence of association between the two polymers and suggest rather that phase separation and gelation occurred in κ-carrageenan and β-lactoglobulin mixtures. The subsequent cooling of mixed systems to 20°C resulted in a first increase of the storage modulus, due to a reinforcement of the protein network, and a second increase, observed below 30°C, which arise from κ-carrageenan gelation. Such behaviour suggests that gelation of κ-carrageenan in the presence of a gelled protein network would lead to the formation of a phase-separated bicontinuous network. The strain sweep results for mixed gels obtained at the end of the experiments support that hypothesis. The overall breaking profile of mixed gel was indeed intermediate, in terms of critical strain, between those of κ-carrageenan and β-lactoglobulin respectively.

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