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Carbohydrate Polymers 56 (2004) 11–19
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Physical behaviour of fish gelatin-κ-carrageenan mixtures

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Received 11 March 2003; accepted 22 October 2003

Abstract

Fish gelatin is a possible alternative to, but not directly exchangeable with, mammalian gelatin due to low gel strength and low gelling and melting temperature. A possible approach to overcome these differences could be to mix fish gelatin and marine polysaccharides leading to systems with improved gel strength, gelling and melting temperature. Mixtures of fish gelatin and κ-carrageenan resulted in solutions and gels with varying degree of turbidity, depending on the concentration of polymers, pH, ionic strength and the nature of the added salt. The turbidity is most probably a result of phase separation in the system and was followed by measuring the optical density. Complexes of fish gelatin and κ-carrageenan at 60 °C were probably stabilised by electrostatic interactions, and the solutions were highly turbid. At room temperature and at 4 °C the turbidity of the mixed systems was much lower, and could be due to altered gel morphology. The system is believed to segregate when carrageenan adapts the ordered conformation and forms a gel network. Compression measurements revealed a considerable increase in Young's modulus when mixed solutions were allowed to gel at 4 °C compared to gelling at room temperature.

Keywords: Fish gelatin; Carrageenan; Phase separation; Turbidity; Excluded volume

1. Introduction

Gelatin is a widely used gelling agent in the food and pharmaceutical industry. The physical characteristics of gelatin, such as the melting close to the physiological temperature of humans, give the polymer the special 'melt-in-mouth' perception; a behaviour which is hard to mimic in other biopolymer systems. Gelatin has found a variety of applications. So far scientists have not been able to find a gelling protein or polysaccharide, which universally can replace gelatin as a gelling agent.

Skin and bone from bovine and porcine sources have usually been utilised in gelatin production (Veis, 1964; Ward & Courts, 1977). The out-break of the mad cow disease (BSE) in the 1980s accelerated the search for a gelatin alternative. Another motivation for finding an alternative to mammalian gelatin alternative is that Muslims, Jews and Hindus do not accept gelatin produced from bovine and/or porcine sources. An alternative to the mammalian gelatin, which is accepted as a food additive in these religious groups, is fish gelatin (FG). FG from cold water fish species gels at approximately 4 °C, when the concentration is higher than the minimum gelation concentration, c_0. The gels are, however, considerably weaker compared to mammalian gelatin gels (Haug, Draget, & Smidsrød, 2004). This makes it impossible to directly replace the mammalian gelatin with FG as a gelling agent.

The carrageenans is a family of linear, sulphated galactans extracted from marine red algae (Smidsrød & Grasdalen, 1982). The ideal kappa-carrageenan (CG) backbone has a repeating disaccharide unit of [→4) 3,6-anhydro-α-D-galactose (1→3) β-D-galactose-4-sulphate (1→]. Carrageenan undergoes a salt- and temperature driven conformational transition in solution (McKinnon, Rees, & Williamson, 1969; Rees, Steele, & Williamson, 1969). The carrageenan molecules form gels by the binding of monovalent cations in junction zones of ordered chain segments. When kappa-carrageenan is converted to different salt forms and compared at the same ionic strength, the shear and elastic moduli decrease in the order $Cs^+ > K^+ \gg Na^+ > Li^+$ (Morris & Chilvers, 1983; Smidsrød & Grasdalen, 1984; Watanase & Nishinari, 1981). The nature of the monovalent counterions also strongly influences the gelling and melting temperature of kappa-carrageenan (Morris & Chilvers, 1983; Rochas & Rinaudo, 1980).

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doi:10.1016/j.carbpol.2003.10.014

Most kappa-carrageenan samples are not pure and contain sequences of iota-carrageenan. Kappa-carrageenan is frequently used in the food industry as a stabiliser, thickener and gelling agent, and has been utilised in China back to ancient times (Piculell, 1995).

Biopolymers are in general used in a variety of applications as thickeners, stabilisers, fat substitute, taste release and structural components. In food applications biopolymers will be mixed with fat, minerals, vitamins and water, and there is usually more than one type of biopolymer present in a product. Mixed biopolymer systems are important because physical properties can be more finely controlled. When biopolymers or polyelectrolytes are mixed it is excepted that the systems will phase separate. Oppositely charged polyelectrolytes will associate into complexes, while equally charged polyelectrolytes will segregate into different phases (Piculell, Bergfeldt, & Nilsson, 1995). When these systems gel, complex morphologies can be developed, as described by Morris (1986) and Tolstoguzov and Braudo (1983).

By mixing FG and kappa-carrageenan one might be able to identify new and improved gelling systems, compared to CG and FG. Before such a system can be developed and commercialised it is important to know the behaviour of the mixtures with different composition, temperature and solvent parameters. The main object of this paper was to gain a basic understanding of the mixed solutions and gels of FG and kappa-carrageenan. The phase separation and gel strength in the systems were studied as a function of concentration of FG, pH, temperature, ionic strength and nature of the added salt. Turbidity measurements were used to relatively quantify the degree of phase separation in the systems, while compression and small-strain oscillatory measurements were used to monitor the mechanical properties and gelling kinetics.

2. Materials and methods

2.1. κ-Carrageenan

The κ-carrageenan (CG) sample, kindly provided by FMC Biopolymer A/S, Drammen, Norway, was approximately 91% (w/w) pure with 9% (w/w) in the iota-form, as determined by ^{1}H NMR analysis (Welti, 1977). The sample contained 90% (w/w) CG, 8% moist and 2% salts. The content of ions is; 6% (w/w) potassium ions, 2% (w/w) calcium ions and 1% (w/w) sodium ions. A 1% (w/v) CG solution/gel contains 13.8 mM K^+-ions, 3.9 mM Na^+-ions and 4.5 mM Ca^{2+}-ions (FMC Biopolymer, 2000).

The weight average molecular weight, M_w, (SEC-MALLS) is about 630 kDa. The sample was also found to contain 7.7% (w/w) sulphur. From the given data it can easily be calculated that there is not a significant surplus of inorganic salts.

2.2. Fish gelatin

The FG sample was produced from skins of cold water fish species and kindly provided by Norland Inc. The weight average molecular weight, M_w, as obtained from SEC-MALLS analysis was 170 ± 17 kDa for FG2 and 140 ± 9 kDa for FG3 (Haug et al., 2004). The isoelectric point, IEP, was 8.7 and 7.8, respectively.

2.3. Preparation of solutions

The CG solutions were made by heating for 30 min at 90 °C with stirring. After dissolving the solutions were incubated at 60 °C for 15 min. The pure CG gels were made from these solutions.

The FG was mixed with distilled water and dissolved at room temperature with stirring. The FG solution was then pre-heated to 60 °C for ~15 min and mixed with CG solution until the desired concentration of both polymers was reached.

The ionic strength of the mixtures was adjusted with 1.0 M NaCl or 1.0 M KCl in the CG solutions before mixing with FG. The ionic strength is reported as extra addition of salt, and does not include any contribution from the present counter ions. The pH of the mixtures was adjusted with 5, 1 and 0.1 M HCl or NaOH under vigorously stirring until the desired pH was reached. The volume of the added acid or alkali was <120 μl, imposing a negligible change the polymer concentration.

2.4. Preparation of gels

The solutions of CG and CG/FG were filled into wells of macro well plates (Costar) with care to avoid air bubbles. Each well is 16 mm in diameter and 18 mm height and with 24 wells/plate. The pure CG gels and the mixed gels of CG and FG were cooled to room temperature (~22 °C) and stored over-night in room temperature or at 4°.

The gels were stored at 4 °C in the refrigerator and compressed at room temperature immediately after they were taken from the fridge.

2.5. Turbidity measurements

The solutions for the turbidity measurements were prepared as previously described (Haug, Devle, Draget, & Smidsrød, 2002), and 100 μl of each sample was filled into eight wells of the micro well plate with care to avoid air bobbles. The absorbance of the 1% κ-carrageenan gels were used as a blank, and the relative turbidity is defined as $A_{405\text{-}TEST}$-$A_{450\text{-}CG}$. The absorbancy was read on a Novapath™ Mini Reader, BIORAD at 405 nm.

2.6. Compression measurement

The compression measurements were performed on a Texture Analyzer, TA.XT2i (Stable Micro Systems, Surrey,

UK). The gels ($d = 16$ mm, $l = 18$ mm) were positioned in the centre below the probe (P25 Aluminium—25 mm in diameter) of the Texture Analyzer, and the compression was performed at 0.1 mm/s until breakage of the gel. The mechanical properties of the gels were evaluated at room temperature by measuring the initial linear slope of the force-deformation curve of the gels (N/m) giving Young's modulus, E (Smidsrød, Haug, & Lian, 1972).

2.7. Small-strain oscillatory measurements

The small-strain oscillatory measurements were performed on a StressTech Rheometer from Reologica, Lund, Sweden. All measurements on mixtures of CG and FG were carried out on a 40 mm serrated plate/plate geometry with 1 mm gap. The temperature gradient was 0.5 °C/min both on cooling and heating, while the frequency was 1 Hz. The applied shear stress varied with the properties of the final gel. The start and end temperature was 60 °C, while the maturing temperature was 22 or 4 °C. A sample of 2.45 ml was applied to the rheometer, and the sample was covered with low viscosity silicone oil (BDH Silicone Products, KeboLab-10cSt at 20 °C) to prevent evaporation.

3. Results and discussion

3.1. Gel properties—effect of fish gelatin concentration

Fig. 1 gives the results from the compression measurements of 1% CG and increasing concentrations of FG with no additional salt, and with 20 mM KCl and 20 mM NaCl added. As can be seen, mixtures with 20 mM KCl exhibit a maximum gel strength at approximately 2% FG, where $E \sim 25$ kPa. For the systems without salt and with 20 mM NaCl, the gel strength was at its minimum value at approximately the same concentration of FG.

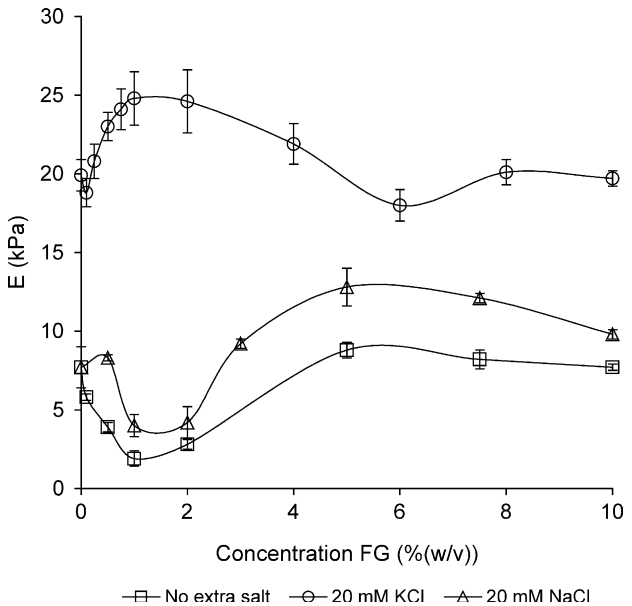

Fig. 1. Young's moduli at room temperature for mixed system of 1% (w/v) κ-carrageenan with increasing concentration of fish gelatin (FG2), with no extra salt, 20 mM KCl or 20 mM NaCl (average ± SD).

3.2. Phase separation and turbidity

By visual observations it was found that solutions of CG and FG alone gave optically clear solutions and gels. The mixed systems of 1% CG and FG gave, on the other hand, turbid solutions and gels. It is reasonable to believe that the system undergoes associative phase separation promoted by the release of counter-ions (Piculell et al., 1995) when both biopolymers are in a random coil conformation and at the present pH and ionic strength. This could explain the turbidity in the mixtures above the carrageenan ordering temperature. Association has also been found for net negatively charged mammalian gelatin and kappa-carrageenan (Antonov and Gonçalves, 1999), and iota-carrageenan and mammalian gelatin (Michon, Cuvelier, Launay, Parker, & Takerkart, 1995; Michon, Konaté, Cuvelier, & Launay, 2002). The association is possible due to the existence of patches of positively charged regions, in the gelatin primary structure (Michon et al., 2002). Tromp, van de Velde, van Riel and Paques (2001) also found from confocal scanning light microscopy, that mixtures of 5% gelatin and 1% kappa-carrageenan did coacervate.

It could, however, also be observed that the systems with CG and FG were turbid at lower temperatures and in the gelled state, but that the turbidity was reduced compared to the associated state. This change in turbidity could, however, be caused by variations in the refractive index of the system, and be due to other phenomena than associative phase separation. Hence, FG and κ-carrageenan may segregate when the temperature is lowered below the ordering temperature of CG leading to the formation of a bi-continuous network. This has previously been found for systems of gelatin and maltodextrin (Lorén, Langton, & Hermansson, 2002). The change in turbidity on changing concentrations of FG, ionic strength and temperature will be discussed below.

In a segregative phase separated system it is excepted that the biopolymer that first gels mainly determines the structure of the final gel (Doublier, Garnier, Renard, & Sanchez, 2000). This could also be the case in the mixed gels of CG and FG. At room temperature the CG has gone through ordering and formed a continuous network. The FG will still be in random coil conformation and probably distributed in the CG-network as dissolved molecules. At room temperature the mixed systems may therefore have a bi-continuous morphology.

A sample of 1% CG and 2% FG was centrifuged at 40 °C, and the associated phase consisting of FG and CG was collected. Preliminary results show that the associative phase consists of approximately 20 wt% of dry polymeric material, while the supernatant contains the rest.

This supernatant, consisting of both CG and FG, became transparent at temperatures above the ordering temperature of CG. After the ordering and gelling of CG, the turbidity in the supernatant re-appeared. This result supports the idea that the system undergoes a new and segregative phase separation, as CG goes from random coil to ordered conformation. One remaining question is, however, if the complexes are preserved in the gel state, or if they are dissolved following the ordering of CG. The onset and offset of the turbidity in the supernatant seem to be strictly related to the ordering/disordering temperature of CG.

In such a bi-continuous structure at room temperature, FG at low concentrations may perturb the carrageenan network when no extra salt is added to the system, and hence give gels with decreased gel rigidity. The addition of potassium may, however, strengthen the carrageenan network sufficiently to prevent disarrangement by the presence of FG. When the concentration of FG is increased, the excluded volume effect will probably be more dominant. This may increase the local concentration of CG (and FG) and thereby oppose the effect of the dearrangement by the presence of FG. It is worth noticing that as much as 10% FG can be added to the carrageenan without lowering the gel properties to any great extent. The system with the highest Young's modulus, 1% CG and 2% FG with 20 mM KCl, was chosen as the standard system for further examination.

3.3. Gel properties—effect of pH

The gel rigidity of the chosen system of 1% CG and 2% FG with 20 mM KCl was measured at different pH-values to reveal the importance of pH. The results are shown in Fig. 2, and it seems clear that the system is almost independent of pH in the vicinity of the isoelectric point, IEP ~ 8.7, of the FG. It can also be seen that the modulus decreases considerably at pH < 6. This drop in modulus at low pH may be due to a too strong associative phase separation leading to an overall reduction of the continuous elastically active CG network.

Increasing pH does not seem to have a major impact on the gel strength. This is probably due to the fact that net negatively charged FG contains 'patches' which are positively charged and this makes association possible also at pH > IEP (Michon et al., 2002). Such a behaviour at pH > IEP may also be expected if the system at room temperature is dominated by a bi-continuous network, and not by an associated phase of CG and FG.

3.4. Gel properties—effect of temperature

Fig. 3 shows the results from compression measurements for 1% CG, 1% CG with 20 mM KCl and 1% CG and 2% FG with 20 mM KCl. The moduli are given for the systems at both room temperature and at 4 °C, where FG in addition to CG also is in the ordered conformation (Haug et al., 2004; Norland, 1990). The modulus of 1% CG gels at room temperature and 4 °C is approximately identical and near 8 kPa (pole A). When 20 mM KCl is added to the system the Young's modulus increases to around 20 kPa. The increase in moduli for the gels at 4 °C is around 20% (pole B). Most biopolymer gels do not follow the theory of rubber elasticity, since the main assumptions of random-coil behaviour of the elastic chains and point-like cross-linking of the chains are not valid. Biopolymer gels usually have areas of rather extended junction zones, being different from the cross-linking points, and stiff elastic segments.

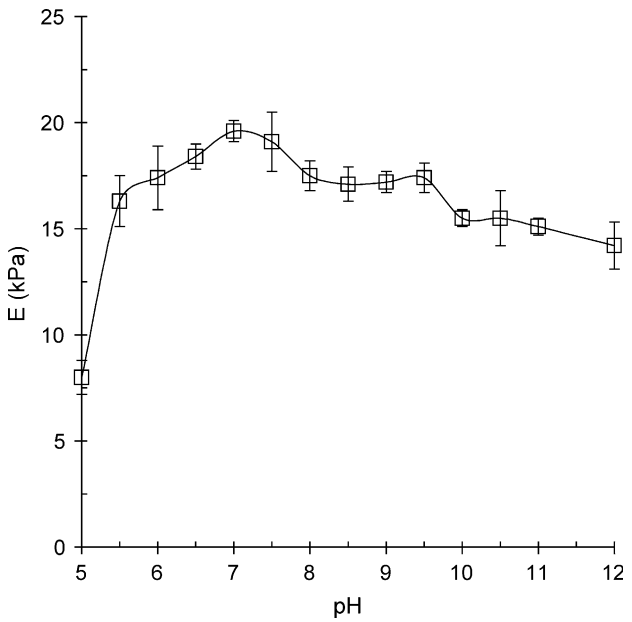

Fig. 2. Young's moduli for systems of 1% (w/v) CG and 2% (w/v) FG2 with 20 mM KCl at different pH-values at room temperature.

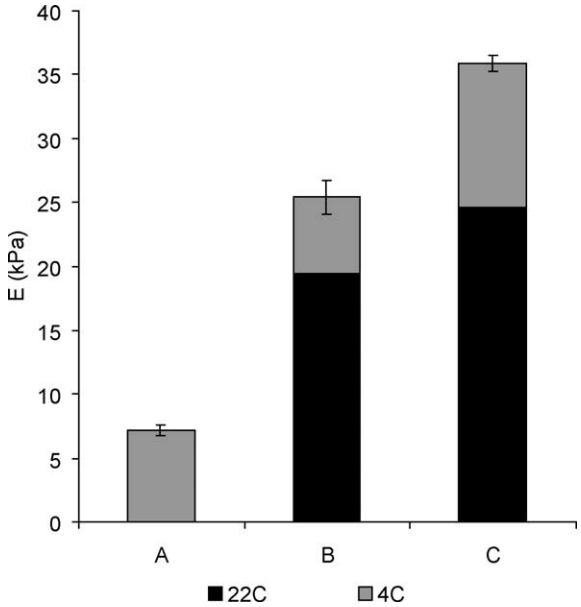

Fig. 3. Young's moduli for κ-carrageenan and carrageenan/fish gelatin (FG2) gels: A: 1% (w/v) CG at room temperature/4 °C, B: 1% (w/v) CG with 20 mM KCl and C: 1% (w/v) CG and 2% (w/v) FG2 with 20 mM KCl.

When the temperature is decreased the flexibility of the polymer chains in the network is also decreased due to less freedom of rotation and movement. This gives a stiff chain network where the elastic modulus is increasing with decreasing temperature (Moe, Draget, Skjåk-Bræk, & Smidsrød, 1992).

Fig. 3 also shows the Young's modulus for mixtures of 1% CG and 2% FG with 20 mM KCl. At room temperature CG is in an ordered conformation, whereas FG does not reach its ordered conformation until below 12 °C (Haug et al., 2004). This implies that both CG and FG are in the ordered state at 4 °C, but it would not be expected that 2% FG would give an increase in gel rigidity by forming a continuous network at this concentration since it is very close to c_0. Addition of 2% FG to the system increases the Young's modulus at room temperature, and E is approximately 25 kPa compared to around 20 kPa without FG.

At 4 °C the modulus is approximately 36 kPa. As outlined above, it is obvious that this increase in the gel strength could not be a result of the contribution from 2% FG alone, but perhaps rather be explained by an excluded volume effect. Mixing two polymers generally decreases the volume fraction available for the individual molecules, and hence increases the local concentrations of both polymers (Flory, 1953). If the local concentration of FG exceeds 2%, FG will gel at 4 °C and may contribute to the overall gel strength. Along the same line the main increase in gel strength at 4 °C can most probably be attributed to a local increase in CG concentration.

3.5. Gel properties—effect of CG concentration

Gels with increasing concentration of CG were analysed, and without any extra addition of potassium ions, a minimum concentration of 1% (w/v) was required to give measurable gels in compression analysis (data not included). This concentration was hence chosen as a standard for further experiments. As already shown in Fig. 3, 1% CG gel has a Young's modulus, E, of approximately 8 kPa in its pure form. It has previously been reported that the modulus is independent of the molecular weight of the κ-CG, as long as the molecular weight exceeds about 200 kDa (Rochas, Rinaudo, & Landry, 1990; Smidsrød & Grasdalen, 1984). The carrageenan used in this experiment was a high molecular weight sample with $M_w \sim 630$ kDa.

3.6. Gel properties—effect of ionic strength

The system of 1% CG and 2% FG was investigated with respect to effects of changes in ionic strength and compared to the pure 1% CG system. The results are presented in Fig. 4(a)–(c). Fig. 4(b) and (c) compare the results from the compression measurements of 1% CG gels and mixed gels with increasing concentrations of KCl and NaCl. Potassium ions are specific bound to the CG molecules, while sodium

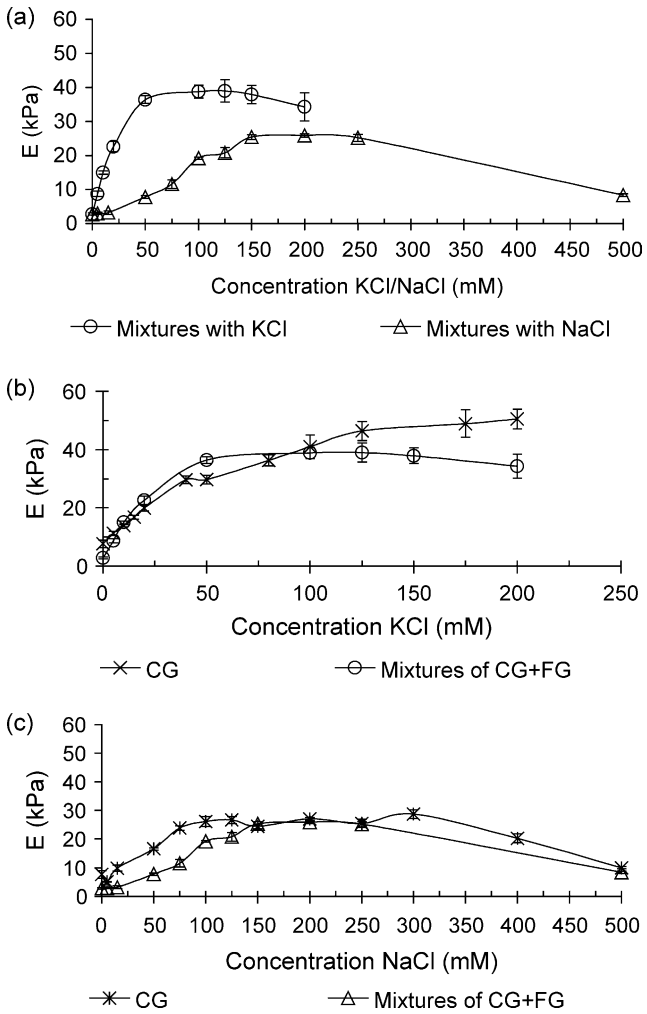

Fig. 4. The effect of adding KCl and NaCl on Young's modulus, E, for (a) the mixed system of 1% (w/v) κ-carrageenan and 2% (w/v) fish gelatin with KCl and NaCl (FG2), (b) mixtures compared to 1% CG on increasing KCl concentration and (c) mixtures compared to 1% CG on increasing NaCl concentration (room temperature; average ± SD).

ions influence the carrageenan network through general ionic effects (Piculell, 1995; Smidsrød & Grasdalen, 1984).

For the mixed system, increasing concentrations of KCl up to approximately 50 mM, give an increase in Young's moduli (Fig. 4(a) and (b)). At higher salt concentrations the gel rigidity levels off close to 40 kPa. The marked increase in moduli for both pure CG and the mixed gels at low ionic strengths may be explained by a combination of shielding off of long range repulsive electrostatic forces between CG molecules and between CG and FG molecules and specific binding of K^+ to CG. This may facilitate cross-linking, a shortening of elastic segments and give gels with higher moduli. Addition of low concentrations of sodium chloride to the mixed systems also increases the modulus, but relatively less compared to the potassium salt. The selectivity coefficient in the exchange reaction between potassium and sodium as counterion, is highly in favour of potassium ions in κ-carrageenan gel junction zones (Smidsrød & Grasdalen, 1984). The increase in gel rigidity

when sodium is added is probably solely due to screening off long-range electrostatic repulsion. At low ionic strengths the sodium ions are not able to compete with the potassium ions already bound in the junction zones, and no reduction in the gel strength is, therefore, observed.

When the ionic strength is between 50 and 150 mM the moduli for the pure 1% CG continue to increase for both NaCl and KCl. The systems with potassium give the strongest gels, and this is, again, probably due to the specificity by which the CG binds up potassium ions in junction zones. Above 100 mM NaCl the gel strength seems to level off at $E \sim 25$ kPa for both the mixed gels and the pure CG gels. When the concentration of sodium increases to above 200 mM, the rigidity decreases, probably due to screening off of short range electrostatic attractions between FG and CG, and ion exchange of potassium ions for sodium ions in the κ-carrageenan junction zones.

For the potassium systems, the elastic moduli increase further until the ionic strength reaches 150 mM, and E is close to 40 kPa for the mixed gels. The gel rigidity in the pure CG gels continues to increase when the concentration of KCl is increased further. At ionic strengths >150 mM, the mixed gels with extra potassium ions seem to give gels with higher elastic moduli compared to the systems with extra sodium chloride (Fig. 4(a)).

The solutions of CG with 500 mM KCl did not form a gel. This can be attributed to a precipitation of CG caused by the high concentrations of this specific ion. This salting out effect is more pronounced with potassium than sodium salts for sulphated polysaccharides (Smidsrød & Grasdalen, 1984) and this may be the reason why solutions with 500 mM NaCl, unlike KCl, still form gels.

3.7. Turbidity—effect of fish gelatin concentration

Fig. 5 shows the variation in the turbidity when increasing concentrations of FG are added to 1% κ-CG or 1% κ-CG with 20 mM KCl or NaCl. All three series exhibit a maximum turbidity at around 2% FG. The gels with 20 mM KCl, however, show a considerably lower turbidity compared to the other two systems. This may be due to CG's high selectivity for potassium ions (Rochas & Rinaudo, 1984; Smidsrød & Grasdalen, 1984) and that addition of these ions leads to the formation of a coarser and tighter structure in the mixture at higher potassium concentrations (Hermansson, 1989). This may result in gels of less turbidity. Fig. 1 shows the changes in gel modulus when the concentration of FG is increased. By comparing Figs. 1 and 5 it can be seen that the system with potassium has a maximum in gel strength when the turbidity is at its highest, while the other two systems have a minimum in gel strength in this range. The maximum in elastic modulus can be explained by the addition of 20 mM KCl. Strong electrostatic interactions, in the absence of extra potassium ions, may reduce the formation of the CG gel network due to a reduced degree of segregative phase separation, and hence

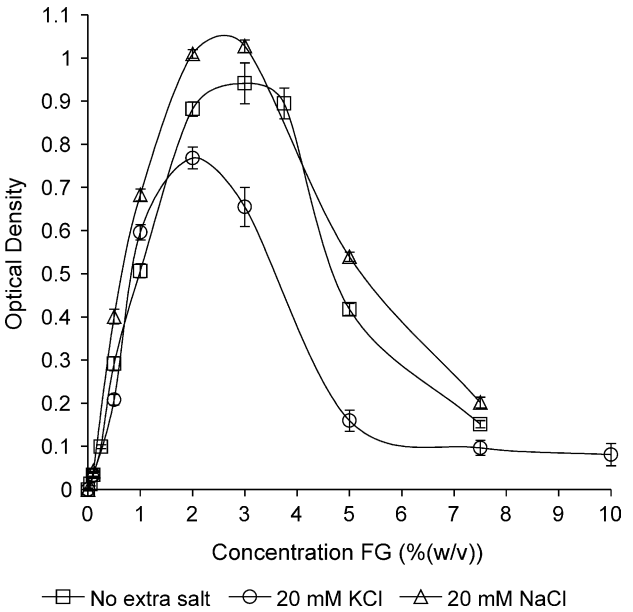

Fig. 5. Turbidity measurements at 405 nm in gels made of 1% κ-carrageenan with increasing concentration of fish gelatin (FG2), with no extra salt and with 20 mM KCl or NaCl (room temperature; average value ± SD).

result in systems with lower elastic moduli. The turbidity decreases at higher concentrations of FG, suggesting that the degree of segregative phase separation also decreases. At concentrations of FG higher than 7.5% the systems are almost transparent, and this could be due to more compatible systems or the formation of soluble complexes (Tolstoguzov, 1986).

3.8. Turbidity—effect of ionic strength

The addition of salt to the system of 1% κ-CG and 2% FG also changes the turbidity, as seen in Fig. 6. Both addition of NaCl and KCl give a maximum turbidity at ionic strengths of approximately 10–20 mM. The turbidity decreases when the ionic strength is above 20 mM, both for NaCl and KCl, but the decline in turbidity for gels containing KCl are more distinct. For an associatively phase separated system it is expected that the separation should decrease when the ionic strength increases, while the trend should be opposite for a segregated system (Piculell et al., 1995). As explained earlier, it is not likely that gels of CG and FG are merely associatively phase separated. The gels may have a bi-continuos morphology where CG is gelled and FG is in a solubilised state. The turbidity drops when the concentration of potassium ions increases. If the system is both associated (complexes) and segregated (bi-continuous structure), this means that the associative phase separation may dominate at low ionic strengths, giving high turbidity, while segregation may be the major phase behaviour at higher ionic strengths. The drop in turbidity in Fig. 6 could, therefore, be due to a more segregated system, leading to a more dominant CG network, giving less turbid gels (due to

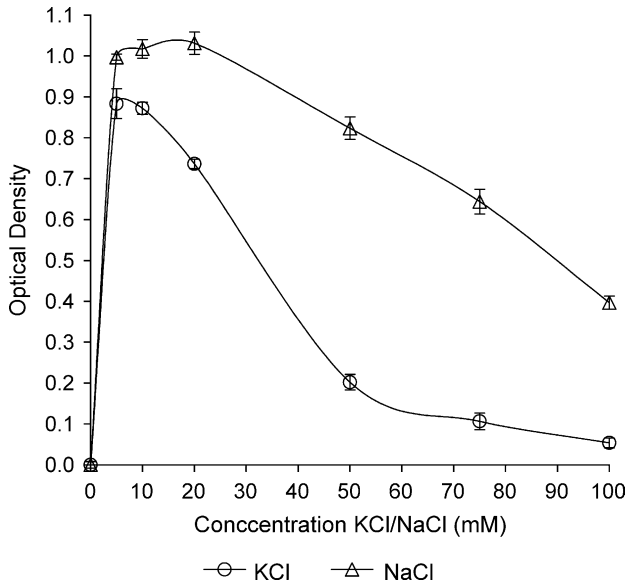

Fig. 6. Turbidity measurements at 405 nm in gels made of 1% κ-carrageenan and 2% fish gelatin (FG2) with extra salt added (room temperature; average ± SD).

changes in, e.g. refractive index caused by changed gel morphology). Comparing the Young's moduli in Fig. 4(a) with the turbidity in Fig. 6, it seems like a high degree of associative phase separation gives gels with lower moduli. This seems to agree with the explanation of a more dominant and stronger CG network at high concentrations of KCl.

3.9. Small-strain oscillatory measurements

The carrageenan sample was also investigated by small-strain oscillatory measurements, and the gelling kinetics of the carrageenan (data not included) was found to be similar to previous experiments performed by Hermansson (1989). The gelling temperature for the pure 1% κ-CG solution, which contains 0.0138 M K^+-ions, was found to be approximately 28 °C and the gel melted at 48 °C. Addition of 20 mM KCl to a 1% κ-CG solution increased the gelling and melting temperature to 36–37 and 55–57 °C, respectively.

Fig. 7 gives the results from the oscillatory measurements of the samples of 1% κ-CG and 2% FG in 20 mM KCl. In Fig. 7(a) the temperature was changed from 60 °C to room temperature (22 °C), kept at room temperature for 2 hours and then heated to 60 °C again. The sample gels at approximately 37 °C, and melts at 56–57 °C, and after 2 h at 22 °C the storage modulus equals approximately 9 kPa. In Fig. 7(b) the samples were cooled from 60 to 4 °C, kept at 4 °C for two hours and re-heated to 60 °C. The setting curve shows that the gelation of FG, in fact, seems to give an extra contribution to the gel strength, through an extra setting which can be seen from the G'-curve. This is rather unexpected since 2% FG does not gel alone (Haug et al., 2002b). The gelling and melting temperature for CG are

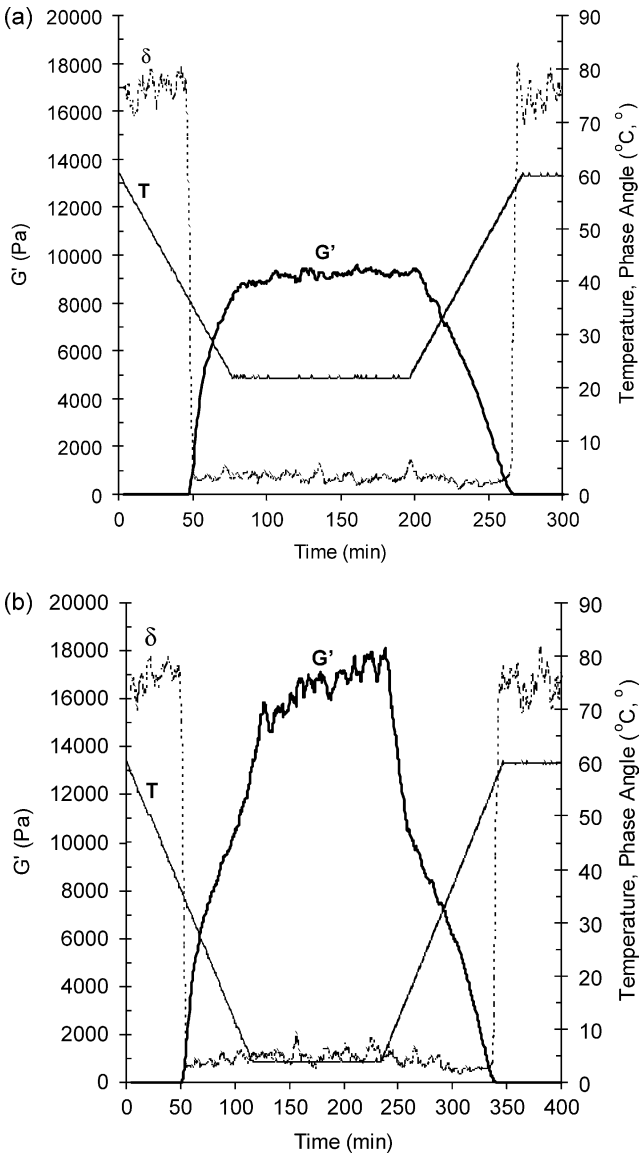

Fig. 7. Small-strain oscillatory measurements for 1% (w/v) CG and 2% (w/v) FG3 with 20 mM KCl. The system was matured at (a) room temperature and (b) 4 °C.

identical to those in Fig. 7(a). After 2 h at 4 °C, G' equals about 17 kPa, and the G'-curve has the characteristic increasing slope of a non-equilibrium gel, typical for, e.g. a gelatin gel (Busnel, Morris, & Ross-Murphy, 1989). Small-strain oscillatory measurements of the mixed gels reveal that the gelling and melting temperature of the mixtures almost follow the gelling and melting for pure CG. This is likely to be due to the network in the mixtures being dominated by the CG, since this biopolymer gels at the highest temperature and probably determines the structure of the final gel.

Fig. 8 shows the small-strain oscillatory measurements for 1% CG and 10% FG with 20 mM KCl. The double setting at around 4 °C is more pronounced, and it is possible to detect changes in the phase angle when FG sets and melts. Comparing Figs. 7(b) and 8 it can be seen that the storage

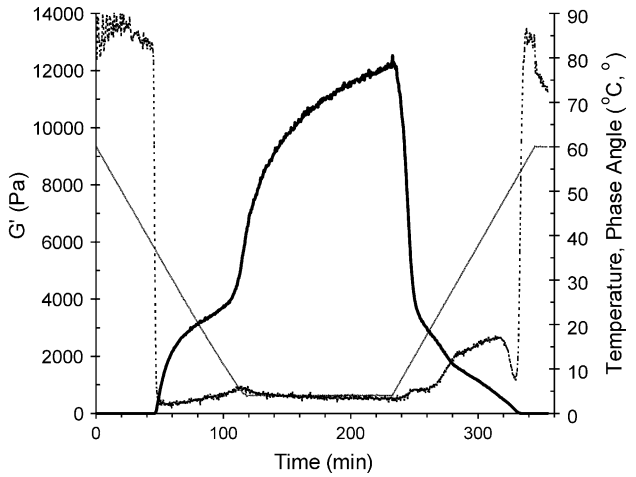

Fig. 8. Small-strain oscillatory measurements of 1% (w/v) CG and 10% (w/v) FG3 with 20 mM KCl.

modulus is approximately 20% lower compared to the system with 2% FG, which is in good accordance with the results from the compression measurements (Fig. 1).

3.10. Gelling and melting temperatures of the mixed systems

The phase angles at 1 Hz and a temperature gradient of 0.5 °C/min for the two mixed systems and 1% CG with 20 mM KCl are given in Fig. 9. The system with 1% CG and 2% FG gels at a slightly higher temperature than 1% CG with 20 mM KCl, and it also melts at a temperature slightly higher. When 10% FG is added to 1% CG with 20 mM KCl the system gels at a temperature higher than 1% CG, and 1% CG and 2% FG, but it melts at approximately the same temperature as carrageenan alone. The phase angle also starts to increase at around the temperature where FG is known to melt, and is higher (around 15°) than expected for pure 1% CG gels, until the CG melts at around 54 °C.

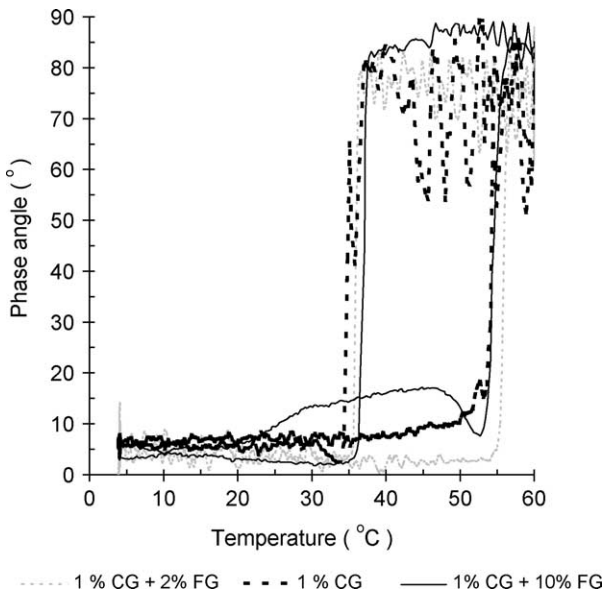

Fig. 9. Changes in phase angle for 1% CG with 20 mM KCl and mixtures of 1% CG and 2% FG3 or 10% FG3, where both systems are added 20 mM KCl.

4. Conclusions

The results from the compression measurements clearly show that the mixed gels of CG and FG are strongest at 4 °C. The system undergoes associative phase separation at 60 °C, giving opaque solutions, but the system also give turbid gels. The turbidity in the gels is strictly related to the ordering of CG, and could be caused by segregation. The segregation may lead to the formation of a bi-continuos structure. One of the remaining questions is weather the associated phase of FG and CG at 60 °C is still present in the gel state, or weather these complexes are dissolved upon the ordering of CG. High concentrations of KCl give gels with higher elastic moduli and this is probably due to the specific binding of potassium to CG. These systems are also less turbid, which may be caused by a more dominating carrageenan network in the bi-continuos structure. The changes in gel modulus and turbidity in systems with sodium are probably due to general electrostatic effects. The gelling and melting temperature of the systems with 1% CG and 2 or 10% FG are only slightly different from pure CG.

Acknowledgements

The authors would like to thank engineers Ann-Sissel Ulset and Ingrid Aune, and siv. ing. Hanne Devle for skilful technical assistance.

References

Antonov, Yu. A., & Goncalves, M. P. (1999). Phase separation in aqueous gelatin-κ-carrageenan systems. *Food Hydrocolloids*, *13*, 517–524.

Busnel, J. P., Morris, E. R., & Ross-Murphy, S. B. (1989). Interpretation of the renaturation kinetics of gelatin solutions. *International Journal of Biological Macromolecules*, *11*, 119–125.

Doublier, J.-L., Garnier, C., Renard, D., & Sanchez, C. (2000). Protein-polysaccharide interactions. *Current Opinion in Colloid and Interface Science*, *5*, 202–214.

Flory, P. J. (1953). *Principles of polymer chemistry*. London: Cornell University Press, pp. 523–530.

Haug, I. J., Devle, H., Draget, K. I., & Smidsrød, O. (2002). Mixed systems of fish gelatin and κ-carrageenan. In P. A. Williams, & G. O. Phillips (Eds.), *Gums and stabilisers for the food industry 11* (pp. 172–177). Cambridge: The Royal Society of Chemistry.

Haug, I., Draget, K. I., & Smidsrød, O. (2004). Physical and rheological properties of fish gelatin compared to mammalian gelatin. *Food Hydrocolloids*, *18*, 203–213.

Hermansson, A.-M. (1989). Rheological and microstructural evidence for transient states during gelation of kappa-carrageenan in the presence of potassium. *Carbohydrate Polymers*, *10*, 163–181.

Lorén, N., Langton, M., & Hermansson, A. M. (2002). Determination of temperature dependent structure evolution by fast-Fourier transform at late stage spinodal decomposition in bicontinuous biopolymer mixtures. *Journal of Chemical Physics*, *116*(23), 10536–10546.

McKinnon, A. A., Rees, D. A., & Williamson, F. B. (1969). Coil to double helix transition for a polysaccaride. *Journal of the Chemical Society D-Chemical Communications*, 701.

Michon, C., Cuvelier, G., Launay, B., Parker, A., & Takerkart, G. (1995). Study of the compatibility/incompatibitility of gelatin/iota-carrageenan/water mixtures. *Carbohydrate Polymers*, *28*, 333–336.

Michon, C., Konaté, K., Cuvelier, G., & Launay, B. (2002). Gelatin/carrageenan interactions in coil and ordered conformations followed by a methylene blue spectrophotometric method. *Food Hydrocolloids*, *16*, 613–618.

Moe, S. T., Draget, K. I., Skjåk-Bræk, G., & Smidsrød, O. (1992). Temperature dependence of the elastic modulus of alginate gels. *Carbohydrate Polymers*, *19*, 279–284.

Morris, V. J. (1986). Multicomponent gels. In G. O. Phillips, D. J. Wedlock, & P. A. Williams (Eds.), *Gums and stabilisers for the food industry 3* (pp. 87–99). New York, London: Elsevier.

Morris, V. J., & Chilvers, G. R. (1983). Rheological studies of specific cation forms of κ carrageenan gels. *Carbohydrate polymers*, *3*(2), 129–141.

Norland, R. E. (1990). Fish gelatin. In M. N. Voigt, & J. K. Botta (Eds.), *Advances in fisheries and biotechnology for increased profability* (pp. 325–333). Lancaster: Technomic Publishing Company Inc.

Piculell, L. (1995). Gelling carrageenans. In A. M. Stephen (Ed.), *Food polysaccharides and their applications* (pp. 205–244). New York: Marcel Dekker.

Piculell, L., Bergfeldt, K., & Nilsson, S. (1995). Factors detemining phase behaviour of multi component polymer systems. In S. E. Harding, S. E. Hill, & J. R. Mitchell (Eds.), *Biopolymer mixtures* (pp. 13–35). Nottingham: Nottingham University Press.

Rees, D. A., Steele, W., & Williamson, F. B. (1969). Conformational analysis of polysaccharides. III. The relation between stereochemistry and properties of some natural polysaccharides sulfates (1). *Journal of Polymer Science: Part C*, *28*, 261–276.

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

Rochas, C., & Rinaudo, M. (1984). Mechanism of gel formation in kappa-carrageenan. *Biopolymers*, *23*(4), 735–745.

Rochas, C., Rinaudo, M., & Landry, S. (1990). Role of the molecular weight on the mechanical properties of kappa carrageenan gels. *Carbohydrate Polymers*, *12*, 255–266.

Smidsrød, O., & Grasdalen, H. (1982). Some physical properties of carrageenan in solution and gel state. *Carbohydrate Polymers*, *2*, 270–272.

Smidsrød, O., & Grasdalen, H. (1984). Polyelectrolytes from seaweeds. *Hydrobiologica*, *117/116*, 19–28.

Smidsrød, O., Haug, A., & Lian, B. (1972). Properties of Poly (1,4-hexuronates) in the gel state I. Evoluation of a methode for determination of stiffness. *Acta Chemica Scandinavia*, *26*, 71.

Tolstoguzov, V. B. (1986). Functional properties of protein-polysaccharide mixtures. In J. R. Mitchell, & D. A. Ledward (Eds.), *Functional properties of food macromolecules* (pp. 385–415). London: Elsevier.

Tolstoguzov, V. B., & Braudo, E. E. (1983). Fabricated foodstuffs as multicomponent gels. *Journal of Texture Studies*, *14*, 183–212.

Tromp, R. H., van de Velde, F., van Riel, J., & Paques, M. (2001). Confocal scanning light microscopy (CSLM) on mixtures of gelatin and polysaccharides. *Food Research International*, *34*, 931–938.

Veis, A. (1964). *The macromolecular chemistry of gelatin*. New York: Academic Press.

Ward, A. G., & Courts, A. (1977). *The science and technology of gelatin*. New York: Academic Pres.

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

Welti, D. (1977). Carrageeenans. Part 12. The 300 MHz proton magnetic resonance spectra of methyl β-D-galactopyranoside, methyl 3,6,-anhydro-α-D-galactopyranoside, agarose, kappa-carrageenan, and segments og iota-carrageenan and agarose sulphate. *Journal of Chemical Research*, *6*, 3566–3587.