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The influence of carrageenan on molecular mobility in low moisture amorphous sugars

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Abstract

The influence of less than 1% of κ-carrageenan on the mobility of glucose syrup was studied in the context of the glass–rubber transition using proton NMR relaxometry. Glass-transition temperatures, (T_g) were measured by differential scanning calorimetry (DSC) on glucose syrup samples containing 0 or 0.9% κ-carrageenan, between 0 and 1.4% KCl, and at water contents from 3.5 to 16% (wwb). Potassium chloride was added to vary the extent of gelation of the carrageenan in order to assess the effect of the biopolymer network on molecular mobility.

Contrary to the reported increase of the rheologically determined glass-transition temperature, in the presence of gelling agents, the addition of 0.9% κ-carrageenan to glucose syrup with and without KCl, had no effect on the DSC measured T_g. In addition, there was no effect on molecular mobility in the glassy region. The presence of carrageenan only significantly affected the mobile part of the NMR free induction decay at relatively high temperatures.

Keywords: Carrageenan; Sugar; Glass-transition; Molecular mobility

1. Introduction

Many foods contain polysaccharides such as starch, gums, pectin or carrageenan which even in small amounts contribute greatly to textural properties. For some polysaccharides, the presence of a specific cation promotes gelation and thus dramatically affects the rheological properties. There is a growing interest in the use of gelling agents in so-called high solids systems i.e. at water contents not exceeding 25% (wwb) for a range of food applications such as in confectionery products and flavour delivery systems. Of particular interest are the effects of network forming biopolymers on the glass–rubber transition temperature. In a series of studies, Kasapis and co-workers (Evageliou et al., 1998; Tsoga, Kasapis & Richardson, 1999; Kasapis and Al-Marhoobi, 2000; Kasapis, Al-Marhoobi & Khan, 2000) investigated in detail, using small deformation rheology, the glass-transition of high solids systems based on glucose syrup alone and glucose syrup/sucrose mixtures. These sugar mixtures contained a range of biopolymers including agarose, pectin, carrageenan and gellan. They found that, in some cases the glass-transition temperature was strongly influenced by the biopolymer. For example, these authors reported that the incorporation of 1% κ-carrageenan and 15 mM potassium in 80–85% solids glucose syrup — sucrose increased the rheologically measured T_g from −25 to −1°C (Kasapis et al., 2000). This is surprising since theories describing the T_g of mixtures (e.g. Couchman & Karasz, 1978) would predict a much smaller increase in T_g. The observation therefore raises the question as to whether the small amount of κ-carrageenan has immobilised the entire system including the sugars, or whether the rheological measurement is monitoring principally the behaviour of the polysaccharide network.

The aim of this study is to address this question by studying the effect of the addition of κ-carrageenan firstly on the T_g of high solids glucose syrup matrices using differential scanning calorimetry (DSC) and secondly on the molecular mobility of the various components using ^{1}H NMR relaxometry.

2. Materials and methods

2.1. Materials

κ-carrageenan was donated by SKW Biosystems (Newbury, UK) in the potassium form (Batch No. 9947).

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42 DE glucose syrup was obtained from ABR Foods Ltd (Corby, UK) (Sample No. 5429).

2.2. Sample preparation

κ-Carrageenan is known to form a gel in the presence of potassium but not with sodium (Morris, 1998). In order to investigate the role of gelation in controlling T_g and molecular mobility, it is of interest to vary the network strength, by adding different amounts of potassium to the sodium form of carrageenan. The latter was prepared by replacing potassium and other ions, by sodium, using extensive dialysis. 20 g of κ-carrageenan were dissolved in 1000 ml of water at 75°C, and the solution was dialysed against water at 70°C. This was followed by dialysis against 10 mM sodium chloride, at 60°C. The excess sodium chloride was then removed by dialysing against water at 50°C. The dialysis procedure took 5 days to complete. The dialysate was subsequently freeze-dried using an Edwards Pirani 1001 freeze dryer.

The potassium, sodium and calcium contents in κ-carrageenan, before and after dialysis were measured by inductively coupled plasma atomic emission spectroscopy (model M, SPECTRO Analytical Instruments, Kleve, Germany). Calibration curves for each mineral were obtained by using standard solutions (0–10 ppm) in 1% HCl.

In order to prepare low moisture systems, 0.35 g of κ-carrageenan was dissolved in 20 ml of water at 50°C, and 49.4 g of glucose syrup (water content: ca. 19%) was added followed, where appropriate, by 0–0.56 g KCl. After full dissolution of the materials, the samples were freeze-dried. The moisture contents of the samples were varied by hydration at 20°C over a range of relative humidities, achieved by saturated salt solutions. Water contents were measured gravimetrically by drying at 70°C, under vacuum. The water content values are reported on a total wet basis, while the carrageenan and KCl concentrations are presented on a dry basis.

2.3. DSC measurements

A Perkin Elmer Instruments DSC7 differential scanning calorimeter was used to measure the T_g. The instrument was calibrated with pure indium ($\Delta H_{fusion} = 28.4$ J/g and $T_m = 156.6$°C) and cyclohexane ($T_m = 6.7$°C). Samples (5–15 mg) were sealed in aluminum pans and heated at 10°C/min. An empty pan of the same type as the sample pan was used as a reference. The measurement was performed in the temperature range between −50 and 180°C. After the first heating, the sample was rapidly cooled down and reheated at the same rate of 10°C/min. Duplicate T_g (midpoint, Table 1) measurements were performed.

2.4. NMR measurements

Samples were sealed in 8 mm internal diameter NMR tubes and the proton free induction decays (FID) were measured between temperatures of −35 and 55°C using a Resonance Instruments MARAN 23 spectrometer operating at 23 MHz. Dwell times between 0.5 and 1 μs were used. The NMR signal was fitted to the following equation:

$$S(t) = A_1 \exp\left(\frac{-a^2t^2}{2}\right)\frac{\sin bt}{bt} + A_2\exp\left(\frac{-t}{T_{22}}\right)^{n_2} + A_3\exp\left(\frac{-t}{T_{23}}\right)^{n_3} \quad (1)$$

where A_i is the amplitude of component I and T_{2i} is the spin–spin relaxation time of the mobile component i. b is the half-width of a rigid proton spectrum (assumed to be rectangular) and a is the standard deviation of the gaussian lineshape which is convoluted with the rigid proton spectrum (McBrierty & Packer, 1995; Van den Dries, van Dusschoten & Hemminga, 1998a; Van den Dries, De Jager & Hemminga, 1998b). n_i is a lineshape exponent for the mobile components. A value of 1 describes an exponential while a value of 2 represents a gaussian. The FID results were satisfactorily described using values of $n_2 = 1$ and $n_3 = 2$. Component 3 was obtained only in conditions of high temperature and/or high water contents, typically well into the rubbery state.

In order to monitor the mobility of the immobile protons, the second moment was calculated (Buitink, van den Dries, Hoekstra, Alberda & Hemminga, 2000; Van den Dries et al., 1998a,b).

$$M_2 = a^2 + \frac{b^2}{3}. \quad (2)$$

3. Results and discussion

3.1. Dialysis and cation composition

During the dialysis, the potassium and calcium contents of the κ-carrageenan were decreased from 9.4 to 1.3% and from 0.6 to 0.1%, respectively, indicating that, more than 85% of these two cations had been removed. The sodium content increased from 2.3 to 3.8% after dialysis.

3.2. Glass transition

The DSC thermograms for all the samples analysed in this study showed a single glass-transition event which shifted to lower temperature with increasing water content (Fig. 1). The presence of κ-carrageenan had no significant effect on the appearance of the DSC thermograms and their dependence on water content. Similarly, the addition of KCl to allow the gelation of κ-carrageenan and the formation of network prior to reducing the water content by freeze-drying did not affect the DSC results. Fig. 1

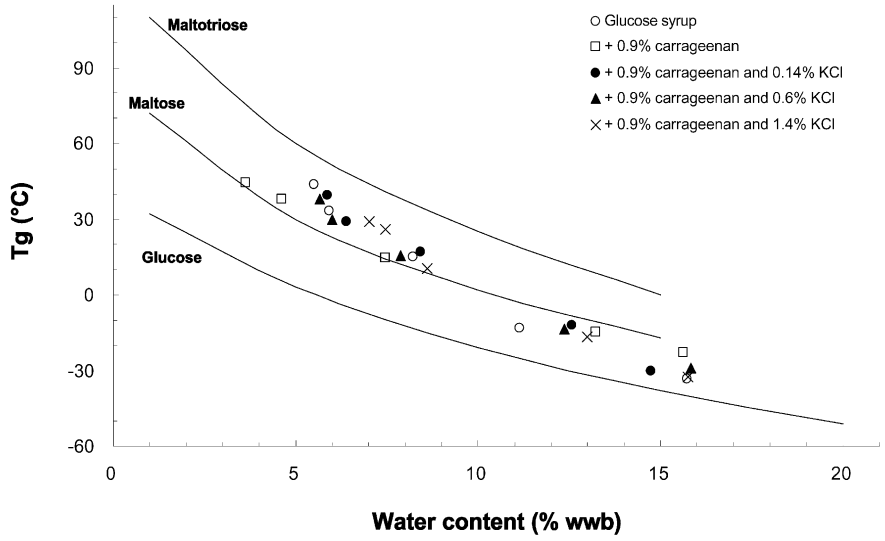

Fig. 1. The DSC measured T_gs (mid points) of glucose syrup with and without added κ-carrageenan (0.9%) and KCl, over a range of water contents. The lines for glucose, maltose and maltotriose are based on the values reported by Orford et al. (1989).

shows the dependence on water content of the T_g of glucose syrup with and without the addition of κ-carrageenan and KCl. As expected, the T_g decreased as water content increased. No differences were observed in the T_g values of samples containing κ-carrageenan compared with those for glucose syrup alone. The increase of the concentration of potassium up to 1.4% had no effect on T_g of the κ-carrageenan containing samples, despite a major effect on the textural properties of the freeze dried systems. The apparent hardness of the foam-like samples increased with increasing potassium levels. The comparison of the T_g versus water content data (Fig. 1) with those reported in the literature for glucose (Noel, Parker & Ring, 1996), maltose and maltotriose (Orford, Parker, Ring & Smith, 1989) shows that the values measured in this study compared closely with those of maltose regardless of the carrageenan and KCl contents. This is in agreement with the composition of the glucose syrup, since at a dextrose equivalent value of 42, it is expected to have similar properties to a disaccharide.

These results are not consistent with those obtained by Kasapis, (Kasapis et al., 2000), using small deformation rheology, where the T_g's of comparable glucose syrup/sucrose mixtures were significantly increased by the addition of network forming biopolymers. The water contents employed by Kasapis (15–20% wwb) were however higher

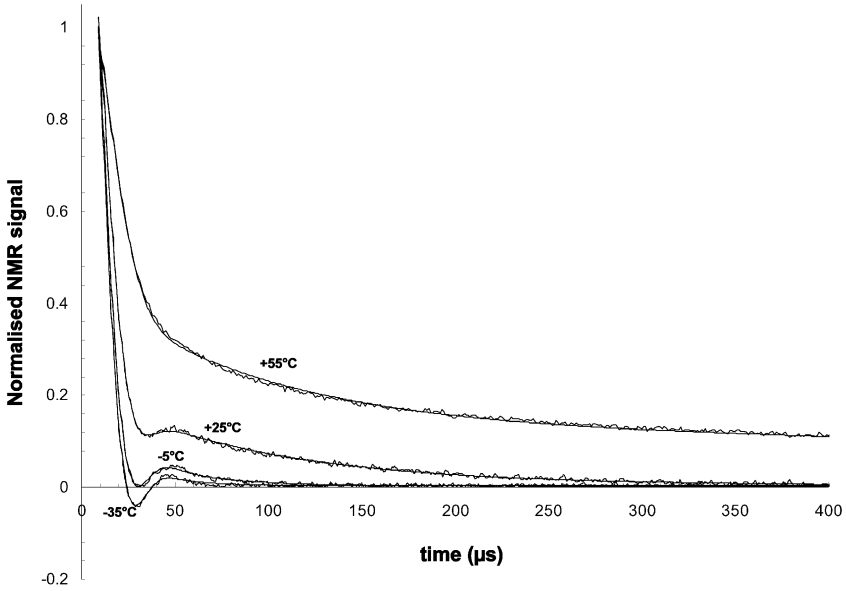

Fig. 2. The effect of temperature on the FID of glucose syrup containing 0.9% κ-carrageenan, 0.14% KCl and 8.4 ± 0.2% water (wwb). Both the measured data and the fitted decay curve are shown. For reasons of clarity, the diagram only includes the first 400 μs of the NMR signal.

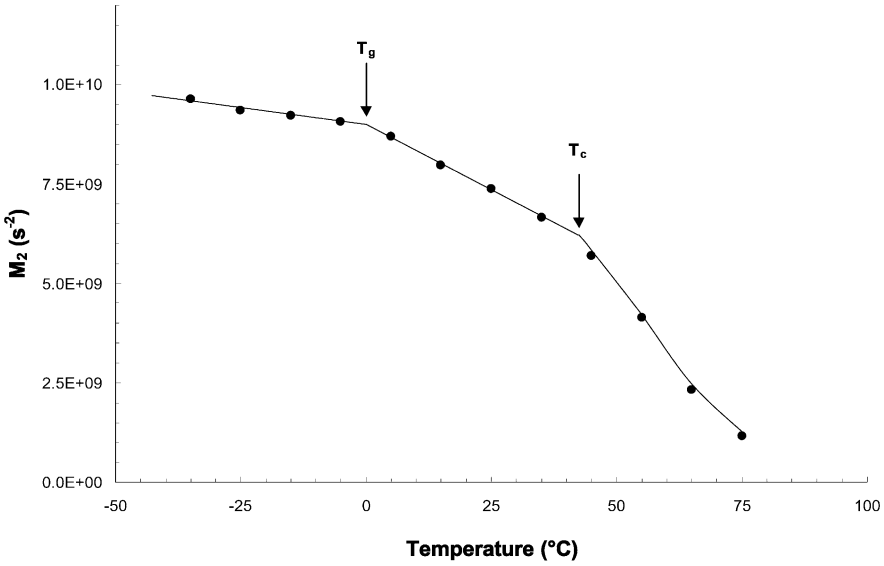

Fig. 3. The second moment shows the increase in the mobility of the 'rigid' component of glucose syrup containing 0.9% κ-carrageenan, 0.14% KCl and 8.4% water as temperature is increased. The arrows correspond to the glass transition temperature (T_g) and the critical temperature (T_c) as defined by Buitink et al. (2000).

than used here (4–16%wwb). The difference in the behaviour of calorimetrically and rheologically determined glass-transition temperatures could imply that the small deformation rheological tests are sensitive mainly to the polysaccharide network, while calorimetry is monitoring the most abundant phase, i.e. the aqueous sugar. The DSC results represent the anticipated behaviour since in terms of glass–rubber properties, the T_g of a homogeneous mixed system is determined by its number average molecular weight, in turn determined by the weight average of the components and is unaffected by small amounts of biopolymer.

3.3. Molecular mobility

Spin–spin relaxation NMR was used to assess the effect of the incorporation of κ-carrageenan on the molecular mobility of the various components in glucose syrup. The extent of the network was varied by changing the KCl concentration.

3.3.1. Molecular mobility in relation to the glass–rubber transition

The free-induction decays acquired over a range of sample composition and temperatures were fitted as described above. Fig. 2 shows typical FIDs for a sample containing 0.9% κ-carrageenan, 99.0% glucose syrup, 0.14% KCl and 8.4% moisture, over a range of temperatures spanning the DSC measured T_g. The T_g for this system is 17°C (midpoint). It is clear that the FIDs acquired on the system well into the glassy state, show a very pronounced beat pattern, characteristic of low molecular mobility and strong dipolar interaction in the solid component. As the temperature increased, the sinc pattern became less pronounced and had disappeared in conditions where the material was well into the rubbery state, typically above the temperature of the endpoint of the DSC glass-transition. For data acquired at high water contents and/or temperature, the FIDs were successfully described using Eq. (1). A small value of b was obtained leading to the sinc part of Eq. (1) approaching unity, reflecting the disappearance of the beat pattern. In these systems with considerable mobility, an additional mobile component, with a gaussian lineshape, was obtained. A biexponential lineshape was suggested for the NMR signal of mobile systems, i.e. in the rubbery state, by Van den Dries et al. (1998b) in their studies on carbohydrate–water systems of comparable water contents, to those investigated in this work. However, for the data obtained here, fitting based on Eq. (1) gave a more satisfactory description. The exact form of the slow decaying component (component 3) reflects to a large extent the nature of the magnetic field inhomogeneity.

Table 1
Comparison of the DSC and NMR (M_2) break points for glucose syrup containing 0.9% κ-carrageenan, 0.14% KCl and 8.4% water (w/w wb)

T (°C)	Onset	Midpoint	End
DSC	−5	17	30
NMR	0	–	42

Typical results for the effects of temperature on molecular mobility are shown on Figs. 3–5 for the glucose syrup — κ-carrageenan (0.9%) system containing 0.14% KCl and 8.4% water. As the temperature increased, the second moment (M_2) decreased (Fig. 6). The dependency of M_2 on temperature presented typically two distinct transition

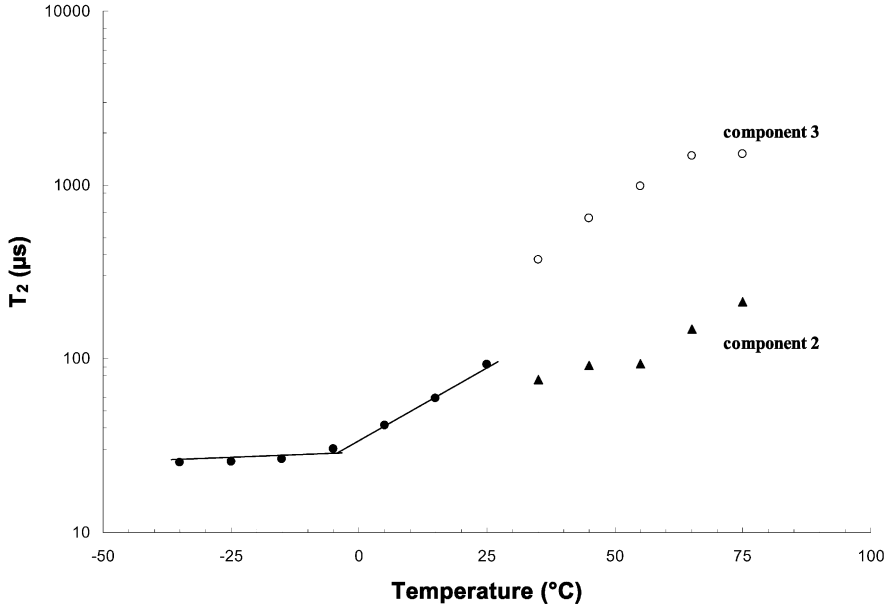

Fig. 4. The T_2 of the 'mobile' component of the FID as a function of temperature for the same glucose syrup system as in Fig. 3. At a temperature of ≥35°C, 2 mobile components were resolved.

temperatures where, the M_2 decrease became steeper, reflecting motional changes in the rigid component (Fig. 3). The lower of these 2 transitions was assigned by Hemminga and co-workers (Buitink et al., 2000) to the onset of significant molecular mobility associated with the glass-transition temperature (T_g). The second transition temperature referred to by the same authors as the critical temperature (T_c) was associated with the crossover from solid-like to liquid-like dynamics, where the material first begins to show properties of a viscous liquid (Rössler, Tauchert & Eiermann, 1994).

It is interesting to speculate as to the origin of the multiple breaks. The first and most obvious point to consider is whether the breaks correspond with the onset and end temperatures of T_g as determined by DSC (Table 1). The determination of these parameters by DSC (Fig. 1) is subject to an error of the order of ±5°C, principally due to the error in measurement of the water content, because of drying or

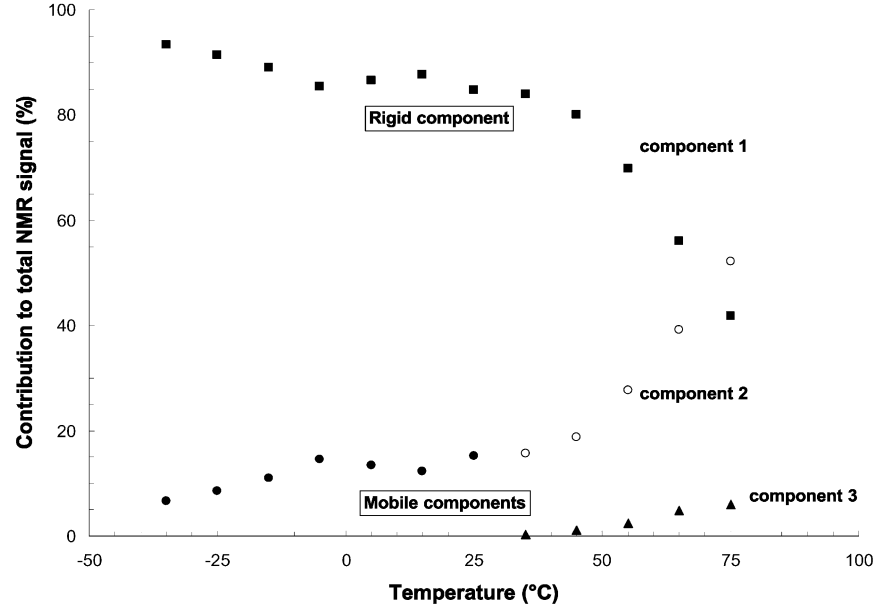

Fig. 5. The relative contribution of the amplitudes of the rigid and the mobile components of the FID. At a temperature of ≥35°C, 2 mobile components were resolved. The material was the same as for Fig. 3.

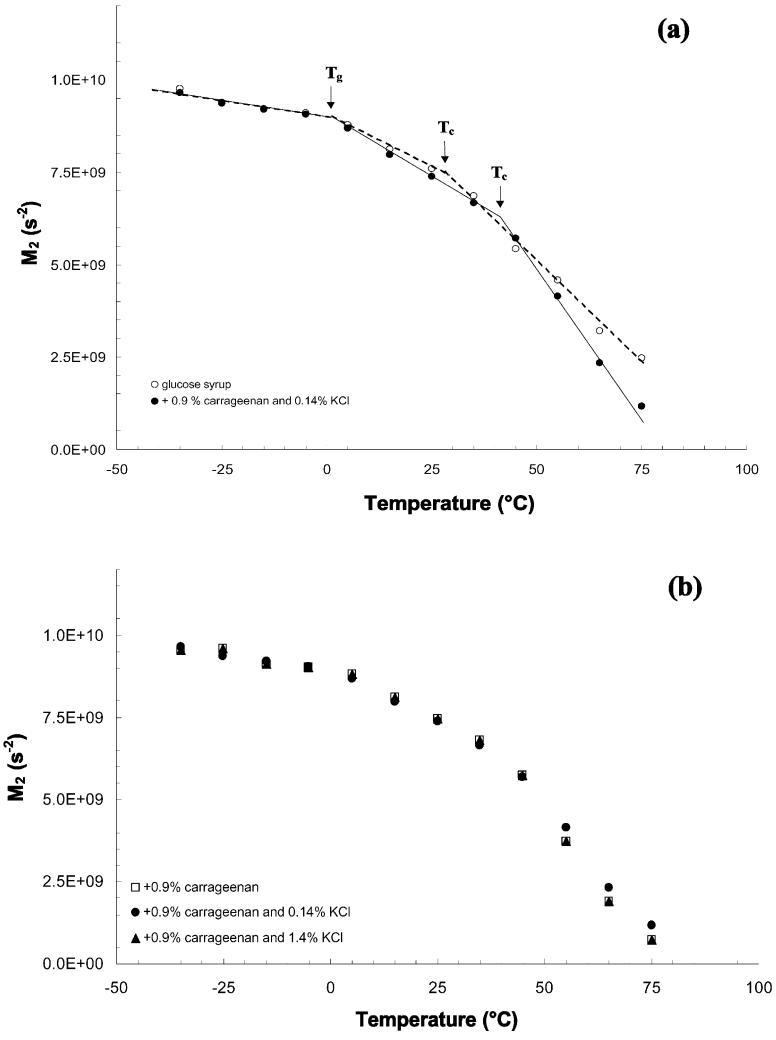

Fig. 6. The effect of κ-carrageenan and KCl on the second moment versus temperature of glucose syrup systems at constant water content (8.4 ± 0.2% wwb).

absorption of water when loading small amounts of material into a DSC pan at ambient temperature and humidity.

The first transition in M_2, which for the glucose syrup — κ-carrageenan (0.9%) system containing 0.14% KCl at 8.4% moisture occurred at ca. 0°C was below the DSC measured T_g (midpoint at ca. 17°C). However, this M_2 transition temperature correlated approximately with the first signs of the departure of the heat capacity (c_p) from that of the extrapolated glass value (∼ − 5°C). The second M_2 transition, T_c (ca. 42°C) correlated only approximately with the temperature at which the c_p of the liquid state was reached (ca. 30°C), even allowing for the errors in measurement. It can be seen therefore that, these correlations are at best, only approximate.

Alternative explanations for these features can be found in the literature (Sokolov, 1996). It would appear that, there is a qualitative change in behaviour about 10–20% above T_g, where the material on cooling first shows signs of a transition from a viscous liquid to solid-like behaviour with spatial and dynamic heterogeneities. There appears to be a transition in several dynamic properties at this temperature, such as isotropic correlation times of introduced or 'guest' molecules approaching those of the glass former, and thus probing the dynamics of the glass. There are also changes in the diffusion mechanism and the breakdown of translational rotational coupling. Part of this behaviour can be attributed to the presence of aggregates, which have been likened to a cage surrounding a molecule. This has been observed using deuterium NMR to measure rotational and translational correlation times (Rossler et al., 1994). It is interesting to speculate as to whether rheological methods will be more appropriate for monitoring changes from liquid like to solid like behaviour and so may measure a temperature of transition closer to T_c than T_g, giving apparently anomalously high glass-transition values. These changes, while at first appearing over complicated, are perhaps not too surprising, when it is considered that glass transitions can occur over a wide temperature range and therefore, must

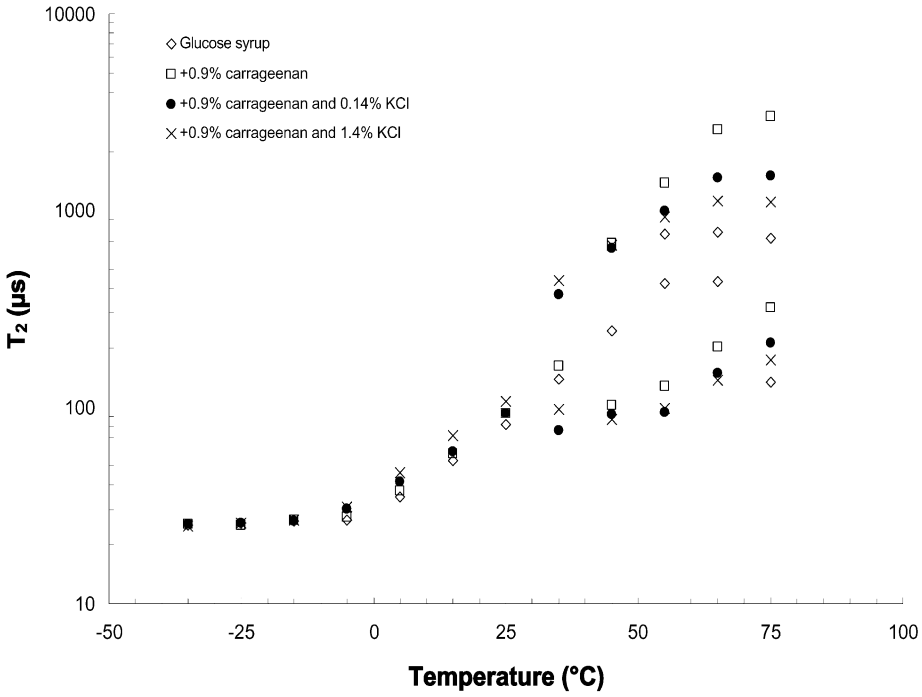

Fig. 7. The effect of κ-carrageenan and KCl on the T_2 of the mobile components of 8.4% moisture glucose syrup.

encompass some dramatic and multifarious changes in properties. The range (T_c–T_g) may represent a region of increased stability, and in particular, resistance to recrystallization (Sokolov, 1996). Being especially wide in biological materials, (T_c–T_g) may be of particular relevance to the investigation of food systems (Buitink et al., 2000).

The measurement of a lower T_g by NMR than by DSC could be considered counterintuitive in the context of the timescales probed by the two techniques. This is of the order of seconds to minutes for DSC compared with tens of μs to ms for spin–spin relaxometry NMR. It is therefore tempting to assign this behaviour to the sensitivity of NMR to molecular mobility allowing the technique to monitor the early onset of mobility during the glass–rubber transition. It has been suggested previously by Ablett, Izzard and Lillford (1992), based on the results of annealing experiments on sucrose glasses, that the onset of the DSC transition is a more reliable indication of T_g.

The effect of increasing temperature on the mobility of the less rigid components, was an increase in the T_2 of the exponential component of the FID (Fig. 4), and, at temperatures of between 25 and 35°C the appearance of a Gaussian third component. The T_2 showed a clear onset of mobility, departing from close to the rigid-lattice limit at a temperature of ca. −5°C, which is in agreement with the M_2 and the initial departure of the heat capacity from the c_p of the glass.

3.3.2. Effect of the κ-carrageenan network on molecular mobility

The only noticeable effects of the incorporation of 0.9% κ-carrageenan on the NMR results were observed at high temperatures: (i) the slightly lower M_2 values and (ii) the ability to resolve 2 liquid-like components in the carrageenan containing systems.

The addition of KCl to enable the gelation of κ-carrageenan, prior to the preparation of the glass through freeze-drying was carried out to assess whether the biopolymer network affects the rotational mobility in the system. The presence of KCl had no noticeable effect on M_2 (Fig. 6) and only a small effect on the T_2 values of the mobile components reflecting a slight decrease in the mobility of the components of the glucose syrup–water phase. This is not consistent with its considerable effect on mechanical properties and the rheologically measured glass–rubber transition temperature as demonstrated by Kasapis and co-workers (Kasapis et al., 2000).

We can attempt to interpret the relatively small decrease in the T_2s of the mobile components in the samples where a carrageenan network existed, if we assume a model of exchange and spin diffusion transfer similar to that presented by Hills (Hills, 1992). A Goldman–Shen (Goldman & Shen, 1966) experiment would, by measuring the spin diffusion component directly, place upper limits on values for chemical exchange coefficients in a similar way to that carried out by Van den Dries (Van den Dries et al., 1998a). In the absence of this information, as in the present work, we can assume sensible values for these parameters. As the chemical exchange process involves an alignment of water molecules, the value tends to decrease at low moisture contents. The highest values observed by Hills (Hills, 1992)

were of the order of $k_b = 5 \times 10^3\ s^{-1}$, therefore only in the case of T_2 for the second component (up to 3 ms) could this be significantly influenced by chemical exchange. We therefore interpret the appearance of multiple components (Figs. 4, 5 and 7) as a manifestation of multiple uniform phases in the carrageenan containing systems rather than exchange or averaging between a mobile fraction water and immobile carrageenan fractions. We propose that, as the material is heated, we move from a uniform glassy phase, where all the sample is coupled and behaves in the same solid like fashion, to a situation where we have a carrageenan/sugar rich water poor phase (low mobility/low T_2) and an additional phase where the water content is higher than the glucose syrup alone. The T_2 then has a value higher than for glucose syrup alone; 3 ms as opposed to ~0.7 ms (Fig. 7). It is also possible that there is a contribution from chemical exchange i.e. exchange between the fairly immobile carrageenan and much higher mobility water rich phases leading to a T_2 somewhere between the two. As the temperature is increased still further, the tendency appears to be towards one value of T_2 for the single mobile phase.

The effect of the addition of potassium chloride was to reduce the T_2 value for both mobile phases particularly where the T_2s are already fairly high. The fact that the gelation of the κ-carrageenan through the addition of KCl, leads to a reduction of the T_2 of the second component by less than a factor of 2 (from 0.32 ms for carrageenan/glucose syrup to 0.17 ms for the systems containing KCl) and that of the third component by a factor of three (from 3 to 1.1 ms), may be a reflection of different concentrations of carrageenan, in the phases. In the absence of further experimental evidence, it is difficult to conclusively identify any mechanisms by which the T_2 of the mobile components are affected by the presence of carrageenan and KCl.

4. Conclusion

This work showed no evidence of any increase in the glass-transition temperature measured by calorimetry, as a result of the incorporation of 0.9% κ-carrageenan in low water materials regardless of whether the biopolymer formed a gelation network or not. Furthermore the rotational mobility of the small molecules of the glucose syrup system was for the most part unaffected by the biopolymer network. These results represent the expected behaviour in view of the small amount of κ-carrageenan added but are not consistent with the reported increase of the rheologically determined T_g on similar κ-carrageenan–glucose syrup systems. In this study, the samples were prepared by rehydrating freeze-dried preparations, while the rheological studies of Kasapis and co-workers used the concentration of semi-dilute preparation through boiling, to achieve the desired water content. It could therefore be argued that, part of the differences between the results of this study and the findings based on small deformation rheology may be due to the difference in the sample preparation methodologies which changes the integrity of the κ-carrageenan network. However, we have performed DSC experiments on samples containing 15–25% water prepared using the method described by these authors (unpublished results) and found that the T_g values were still unaffected by the presence of the biopolymer network.

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