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Glass transition-related or crystalline forms in the structural properties of gelatin/oxidised starch/glucose syrup mixtures

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Abstract

The structural properties of acid pigskin gelatin/oxidised waxy maize starch/glucose syrup mixtures at a 70% total level of solids were investigated. Depending on polymer composition and pH, mixtures form phase-separated gels with a gelatin or an oxidised starch continuous matrix. It was found that the continuous phase determined the mechanical behaviour of a mixture. Thus gelatin/glucose syrup systems or gelatin continuous mixtures show a three-fold transformation in viscoelasticity, namely: from solutions at high temperature (> 35°C) and rubbery solids at ambient temperature to high modulus viscoelastic liquids at subzero temperatures (e.g. $>10^6$ Pa at 10 Hz and −20°C). In the language of polymer physics a transition was observed from rubber-like to glass-like consistency. By contrast, oxidised starch/glucose syrup systems and oxidised starch continuous mixtures show a transformation from liquid-like to solid-like behaviour at subzero temperatures due to water crystallisation. Concentrated oxidised starch (≈31%) with glucose syrup (≈39%) systems are capable of forming intermolecular amylose-like structures at high temperatures (e.g. 80°C), which remain solid-like throughout the cooling run (down to −20°C). Therefore the development of crystallinity (water/polymeric aggregates) in starch-dominated systems prevents them from exhibiting glass-related viscoelasticity. The investigative approach in general, and this mixture in particular (with its multitude of structures) might provide guidance for development work in the confectionery industry. © 1998 Published by Elsevier Science Ltd. All rights reserved.

1. Introduction

High solids food systems—those with substantial concentrations of hydrocolloids and/or co-solutes—are of increasing academic and industrial interest (Carr, Sufferling, & Poppe, 1995; Izzo, Stahl, & Tuazon, 1995). Hydrocolloid (mainly gelatin) confectionery has typically 15–20% moisture in the final product. To make these products, a mixture with all the ingredients at a 30% moisture content is prepared (liquor), whose physicochemical characteristics determine largely the texture of the finished sweet. Liquors are deposited hot (e.g. 70°C) into dry powdered-starch moulds and the excess moisture is extracted by stoving the sweets in these moulds for several days at about 50°C. Enzymatically and chemically modified starches have been added to the basic gelatin formulation in an effort to create a range of textures (DeCock, 1995, 1996; Ring, 1995), but mechanistic understanding of the viscoelastic properties in these mixtures is lacking. Fundamental research, therefore, is required in gelatin/starch systems to characterise their structure and phase behaviour. Studies of this nature would confer considerable commercial benefit via improved product design and quality control.

In the last decade or so, advances in electronic instrumentation have made rheological techniques readily accessible, especially small deformation dynamic oscillation (Morgan, 1983; Nelson & Dealy, 1993). In the high solids field, oscillatory measurements should be used to define function–structure–texture relationships for solutions (e.g. glucose/corn syrups), rubbery materials (e.g. gummy bears), and biological glasses (e.g. fruit

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pastilles). This approach, of course, has been employed advantageously in studies of the linear viscoelastic behaviour of synthetic polymers producing shear moduli descriptions as a function of concentration (diluted polymer systems), molecular weight, temperature, and timescale of measurement (Arridge, 1975). For amorphous polymers the development of storage and loss moduli (G' and G'', respectively) falls within four discrete regions shown as a function of frequency of oscillation (ω) in Fig. 1 (parts I, II, III and IV). At long experimental times, i.e. low frequencies ($t = 1/\omega$), the G′ values rapidly approach zero, meaning that the applied energy is not stored but dissipates as heat in the medium. Shorter times see a rapid development of G′ and G″ proportional to ω^2 and ω, respectively (terminal zone; I). Further on the two traces flatten out and eventually cross over, thus delimiting the onset of the plateau region (Takahashi, Hase, Yamaguchi, & Noda, 1994). Throughout this region (II), the values of storage modulus remain relatively constant, whereas the trace of loss modulus shows a dip. Qualitatively, this behaviour is ascribed to more segments of a polymer contributing to the elastic component of a network which can be formed by topological entanglements or covalent cross-links (Ferry, 1980). At higher frequencies the G′ and G″ traces cross over for a second time and both moduli develop rapidly with the viscous component being predominant. This region is known as the glass transition where backbone adjustments (elastic contributions) are limited but short-range pendant group or molecular movements capable of dissipating energy are present (III). Finally, extremely high frequencies prevent configurational rearrangements and the system enters the glassy zone (IV). Only stretching and bending of chemical bonds is allowed here which develop rather an elastic than a viscous character, the moduli traces thus cross over for a third time and a hard-solid response is obtained (Frick & Richter, 1995).

Conventional rheometers can only probe a small window (0.001–10 Hz) of the frequency range (typically 20 decades) of Fig. 1, which represents the frequency dependence of viscoelastic functions at a constant (reference) temperature. In practical terms, therefore, frequency sweeps are taken every 10 degrees or so over a wide temperature range (typically from 130 to −50°C), and mechanical spectra are shifted along the frequency axis at the reference temperature using the time–temperature superposition principle (Tobolsky & Eyring, 1943; Dahlquist & Hatfield, 1952). Similarly, master curves can be obtained as a function of experimental temperature, or by changing systematically polymer concentration and molecular weight at a fixed temperature

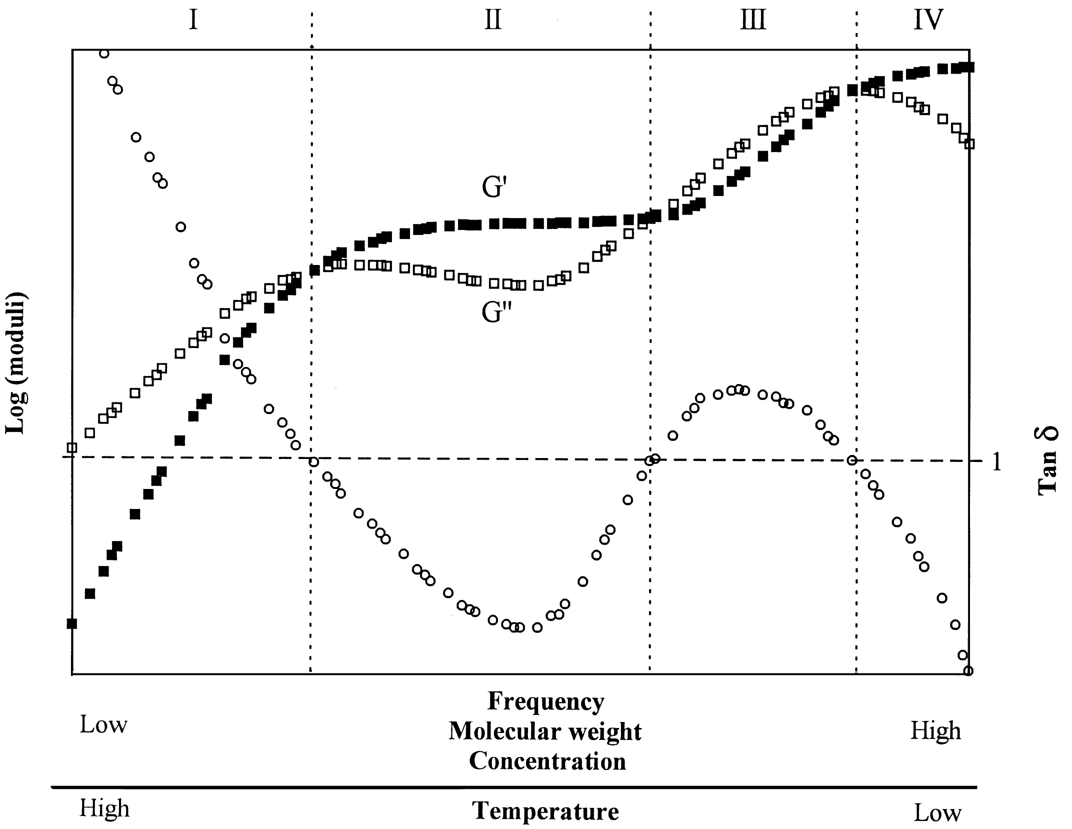

Fig. 1. Master curve of storage modulus, loss modulus, and their ratio (tan $\delta = G''/G'$) as a function of frequency of oscillation, polymer concentration, polymer molecular weight, and temperature showing the terminal zone (I), plateau (II), glass transition (III), and glassy region (IV).

(Fig. 1). The spectacular time–temperature dependence of viscoelastic parameters is also seen in their ratio (tan $\delta = G''/G'$) which fluctuates from liquid-like to solid-like behaviour and acquires the value of 1 at passing from one region to another ($G'' = G'$). According to Ferry (1950), the third point of equality should be considered as the glass transition frequency or temperature (Ω_g and T_g, respectively) at which the free volume per unit mass of a polymer collapses to about 3% of the total volume. The present investigation utilises the synthetic polymer approach in an effort to achieve a better understanding of the structure and phase behaviour of single and mixed gelatin/oxidised starch/glucose syrup systems.

2. Experimental section

2.1. Materials

The gelatin sample of this study was provided and characterised by Systems Bio-Industries, Research Centre, Baupte, F-50500 Carentan, France. It is the first extract of an acid process on pigskin (PS1) yielding a high quality material (the Bloom value is 305). The isoelectric point (pI) was obtained by completely deionising the sample on a bed of ion-exchange resin and measuring the pH of the eluent. The pI value was pH 8.7, thus producing a positively charged gelatin coil at the acidic pH of our investigation. The sample contains 80 ppm calcium, <0.1% sulphate, 0.16% chloride, 53 ppm phosphate, and 10% moisture. Gel permeation chromatography (GPC), calibrated with gelatin standards of known absolute mass distribution, yielded a number average molecular weight of 68,000 and a mass average molecular weight of 162,400. The following profile arises if the molecular weight spectrum is split into 10 characteristic mass classes:

$MW > 10^6$	1.17%
$10^6 > MW > 0.54\times10^6$	4.19%
Tetra + Penta	5.79%
Gamma	7.43%
Beta	21.58%
Alpha	24.38%
Subunits 1	12.85%
Subunits 2	12.06%
Subunits 3	4.98%
Subunits 4	5.60%

The oxidised waxy maize starch (C*06598) of this investigation was supplied by Cerestar, Havenstraat 84, B-1800 Vilvoorde, Belgium. The moisture content of the sample is 13%. It starts gelatinising at about 65°C. Molecular weight estimations were performed chromatographically on Shodex columns S805 and S804 in series. The system was calibrated with a set of pullulan standards. Starch solutions were made in a 90/10 mixture of dimethyl sulphoxide (DMSO) and water. Elution was with 0.05 M NaOH at a flow rate of 1 ml/min. The number average molecular weight was found to be 15,050 and the mass average molecular weight was 97,710. The GPC signal intensity versus retention times provides the following molecular weight distribution:

MW	5×10^3	5×10^3–25×10^3	25×10^3–2×10^5
Area (%)	1.81	20.98	60.19
MW	2×10^5–10^6	10^6–5×10^6	
Area (%)	16.76	0.26	

The glucose syrup of this investigation was a Cerestar product (01136; Batch NX8472). The dextrose equivalent (DE) of the sample is 42. The total level of solids is 81%. The water content of glucose syrup was taken into account in the preparation of samples. GPC analysis provides the following relationship between degree of polymerization (DP) and surface area (%) of the glucose syrup spectrum:

DP1	17.54
DP2	12.99
DP3	10.55
DP4	8.79
DP5	7.29
DP6	5.28
DP7	4.78
DP8	4.21
DP9	3.19
DP10	1.96
>DP10	23.40

2.2. Methods

Stock solutions of gelatin were prepared by dissolving the protein in tap water (70°C) and then mixing it with hot glucose syrup. Stock samples of oxidised starch were made by dispersing the polysaccharide in tap water and then mixing the slurry with glucose syrup. The mixture was then heated to 90°C until the starch was completely gelatinised, as documented by the disappearance of birefringence on a polarized light microscope. Liquors were produced by mixing appropriate amounts of high-solids gelatin and starch stocks at 70°C. A hydrochloric acid solution was used to adjust the pH of samples to 3.6 and 6.0. As can be seen in Table 1, the two pH investigations were carried out at a 70% (w/w) total level of solids.

Single stocks and liquors were loaded on the preheated plate (70°C) of a controlled stress Carri-Med CSL 500 rheometer, the bottom plate was raised and the exposed edges of the sample were covered with silicone

Table 1

Label	Starch (%)	Gelatin (%)	Glucose Syrup (%)	pH
SPS2	24.8	–	45.2	3.6
SPS4	19.6	1.9	48.5	3.6
SPS6	13.3	3.8	52.9	3.6
SPS8	7.1	6.1	56.8	3.6
SPS10	–	9.1	60.9	3.6
SPS12	31.2	–	38.8	6.0
SPS14	22.8	2.2	45.0	6.0
SPS16	15.5	4.4	50.1	6.0
SPS18	8.2	7.0	54.8	6.0
SPS20	–	9.7	60.3	6.0

fluid (50 cs) to prevent evaporation. Parallel plates with a diameter of 4, 2 and 0.8 cm were used thus allowing the recording of sample moduli from 10^1 to 10^7 Pa. The gap between the bottom and top plates was fixed at 1 mm. The operational strain was 1%, i.e. a level of deformation well within the linear viscoelastic region of our samples. Following loading, samples were first control cooled to −20°C and then heated to 70°C at a scan rate of 1°C/min (frequency of 1.6 Hz, which is about 10 rad/s). Periodically the temperature ramps were interrupted and mechanical spectra were obtained from 0.01 to 10 Hz. Thus readings of the rigidity/storage modulus (G′), viscous/loss modulus (G″), their viscoelastic ratio ($\tan\delta = G''/G'$), and complex dynamic viscosity (η^*) variation with temperature (T) and frequency (ω) were obtained, providing evidence of the relative liquid-like and solid-like character of the samples.

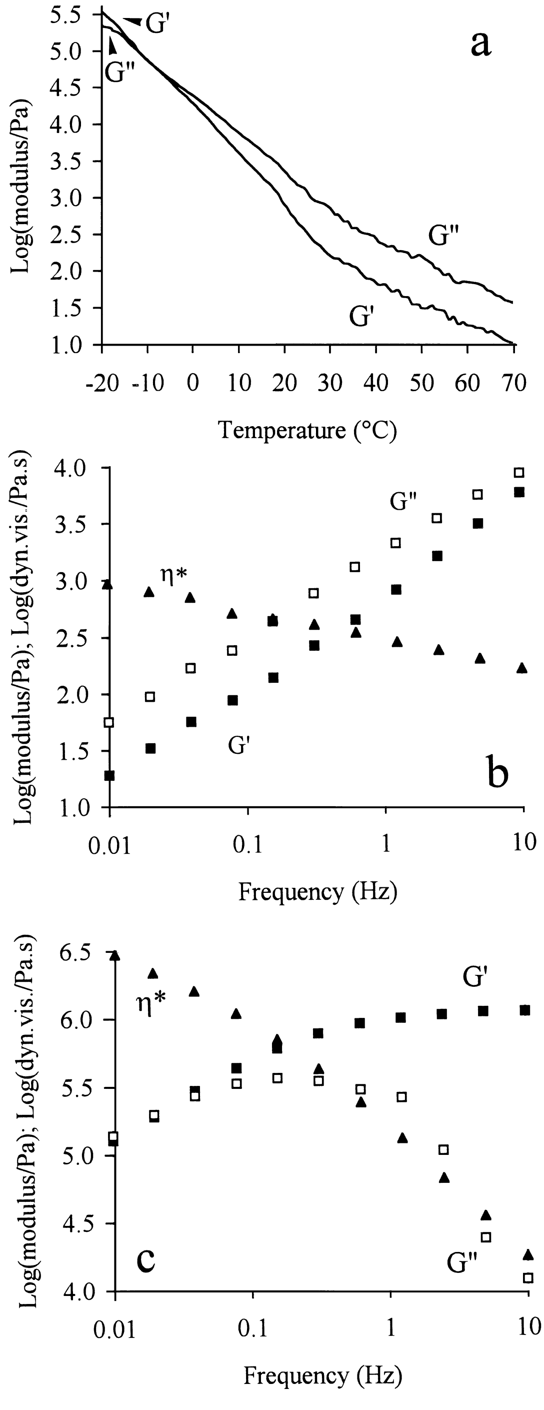

Fig. 2. Variation of storage modulus, loss modulus, and dynamic viscosity (a) as a function of temperature (sample SPS4), (b) frequency at 20°C (sample SPS14), and (c) frequency at −20°C (sample SPS18).

3. Results and discussion

3.1. *Descriptive account of the viscoelastic characteristics of high solids gelatin/oxidized starch liquors*

In the course of this investigation it became apparent that the small deformation behaviour of the mixture SPS8 was different from the properties of the remaining five mixtures (SPS4, SPS6, SPS14, SPS16 and SPS18), which qualitatively fall into line with each other. We are going to describe first the time/temperature profile of the latter samples. Fig. 2(a) illustrates the development of viscoelasticity during cooling from 70 to −20°C at 1°C/min (sample SPS4). Clearly, at the high temperature end there is a predominant viscous element with G'' being half an order of magnitude higher than G' at 70°C. However, a reduction in the thermal energy/entropy of the system during cooling enhances the rigidity component so that the two traces converge and eventually cross over at sub zero temperatures. Thus at −20°C and the operational frequency (1.6 Hz) there is a transformation to a solid-like response with a high value of storage modulus (about 0.4×10^6 Pa). Nevertheless, the metamorphosis from a liquid-like to a solid-like consistency is unlike the sharp, sigmoidal disorder to order transition of the cold-setting biopolymer systems, which can result in the formation of gels (Norton, Goodall, Austen, Morris, & Rees, 1986; Piez & Sherman, 1970). Heating of those gels produces melting curves well displaced on the temperature axis from their cooling counterparts, a phenomenon known as thermal hysteresis (Dea, McKinnon, & Rees, 1972). By contrast the heating run of sample SPS4 follows back the cooling trace without signs of thermal hysteresis [not shown in Fig. 2(a) to avoid clutter]. Mechanical spectra of the aqueous biopolymer gels show G' values substantially above G'' (typically tan $\delta \leq 0.1$) both being almost independent of frequency so that η^* decreases linearly with a slope of -1 on a double logarithmic plot (Richardson & Kasapis, 1998). Mechanical spectra, therefore, were recorded to provide an accurate indication of the structural type of the five mixtures in our group.

Fig. 2(b) reproduces a standard frequency sweep at 20°C (e.g. sample SPS14), i.e. at a temperature where a liquid-like behaviour is indicated in Fig. 2(a). As frequency increases, the viscosity is reduced and the rigidity contribution converges upon the dominating loss component due to storage of energy by contortion of molecules into strained conformations. Overall this is a mechanical profile for a shear thinning viscous solution. In Fig. 2(c), the dependence of G' and G'' on frequency at -20°C is typically illustrated for the last sample of the group (sample SPS18 in the preceding paragraph). In accordance with the temperature sweep of Fig. 2(a), the value of storage modulus at 1.6 Hz (operational frequency of the temperature ramp) is higher than for the loss modulus. Unlike the flat frequency dependence of moduli in biopolymer gels (tan $\delta \leq 0.1$), however, $G'' > G'$ at 0.01 Hz (i.e. tan $\delta > 1$), soon after the two traces cross over and eventually G' becomes independent of frequency whereas G'' falls to quite low values at higher frequencies (at 10 Hz tan $\delta \approx 0.01$). A similar shape in the frequency profile of shear moduli has been reported for ice cream at subzero temperatures where formation of ice crystals generates a high G' value and a pronounced dip in the G'' trace (Dea, Richardson, & Ross-Murphy, 1984). In Fig. 2(c), therefore, a larger number of molecules are perceived at higher frequencies (i.e. at shorter timescales of measurement) as an integral part of the network by being able to support the imposed stress and contribute to the rigidity of the matrix. Obviously the frequency response illustrated here corresponds to a complex distribution of processes, but qualitatively, water and perhaps solute crystallisation should be behind the spectacular increase in rigidity and the reduction in the 'sol fraction' of the system (seen at higher frequencies).

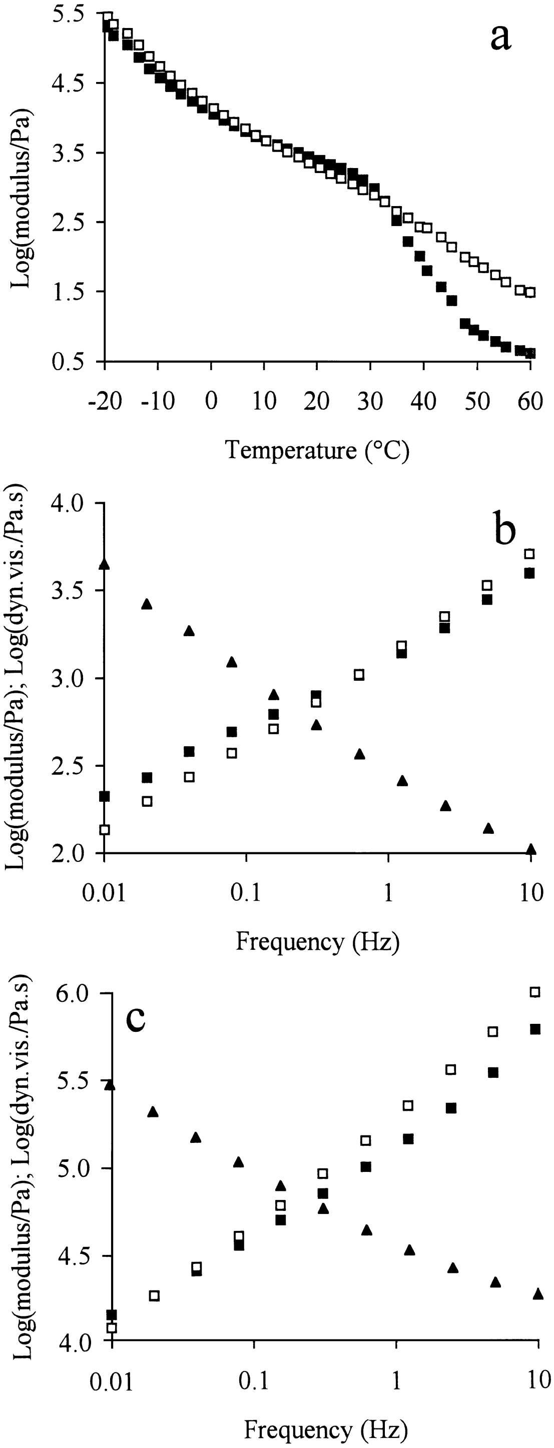

Fig. 3. Development of G′ (■), G″ (□), and η^* (▲) as a function of (a) temperature, (b) frequency at 20°C, and (c) frequency at −20°C for sample SPS8

As mentioned at the beginning of this section, an entirely different pattern of rheological response has been recorded for the liquor SPS8 [Fig. 3(a)]. This time cooling from 70 to -20°C at 1°C/min results in a 3-fold variation in viscoelasticity (experimental frequency of 1.6 Hz). Thus at high temperatures the system appears as a solution ($G'' > G'$), with the modulus traces crossing over at about 35°C (leading to viscoelastic solids; $G' > G''$) and once more at about 10°C yielding again viscoelastic liquids ($G'' > G'$). The dominant viscous consistency at subzero temperatures is in direct contrast with the response in Fig. 2(a), where elastic properties are mainly observed in this temperature range. The unusual temperature-course of shear moduli in Fig. 3(a) is reproduced in the mechanical spectra of Fig. 3(b) and (c). Both at 20 and −20°C increasing the frequency of oscillation results in a transformation from an apparently solid-like ($G' > G''$ at 0.01 Hz) to a liquid-like ($G'' > G'$ at 10 Hz) state. Clearly, lower temperatures induce earlier crossing of the traces (≈ 1 Hz and 0.02 Hz at 20 and −20°C, respectively). As pointed out for the temperature runs of Figs. 2(a) and 3(a), there is a strong antithesis between the solid-to-liquid change in Fig. 3(b) and (c), and the frequency sweeps of the grouped samples which at 20°C correspond to viscoelastic solutions [Fig. 2(b)] or show dominant rigidities at −20°C [$G' > G''$ at frequencies greater than 0.04 Hz in Fig. 2(c)].

3.2. Characterisation of the phase behaviour in high solids gelatin/oxidised starch liquors using the rheology of single components

Besides the scientific interest, the transformation from a solution to a crystalline solid in Fig. 2 or from a low-viscosity liquid and a solid-like body to finally a high-viscosity liquid in Fig. 3 is of practical importance. In the following, gelatin/glucose syrup and oxidised starch/glucose syrup samples are used to provide a guide to macromolecular arrangements in the liquors. The high solids systems of gelatin and oxidised starch were analysed at pH 3.6 (SPS10 and SPS2, respectively) and pH 6.0 (SPS20 and SPS12, respectively). There is no contrariety in viscoelasticity between the two pH groups. At each experimental pH, however, the protein and polysaccharide samples reproduce the observed differences between SPS8 and the remaining liquors.

In Fig. 4(a) gelatin/glucose syrup preparations (e.g. SPS20) depict the type of behaviour documented for the liquor SPS8. As expected (Michon, Cuvelier, & Launay, 1993), at temperatures higher than 35°C the gelatin chains exist as disordered coils and samples flow. The triple helix of gelatin is thermally stable at lower temperatures, thus allowing structure formation which is accompanied by a characteristic drop in the tan δ values (< 1). Fig. 4(b) shows the frequency response of our sample at 20°C, which is distinct from the almost frequency-independent, well separated (tan $\delta \approx 0.05$) traces of moduli for aqueous (no co-solute) gelatin gels (Clark, Richardson, Ross-Murphy & Stubbs, 1983; Richardson & Kasapis, 1998). Instead the mechanical spectrum obtained shows a pronounced dependence of loss modulus on frequency with the two traces converging at the high frequency end. Further cooling induces a third transformation in Fig. 4(a) (tan δ becomes > 1), which is also shown in the form of a solid-like to liquid-like development in the mechanical spectrum of Fig. 4(c) (−20°C).

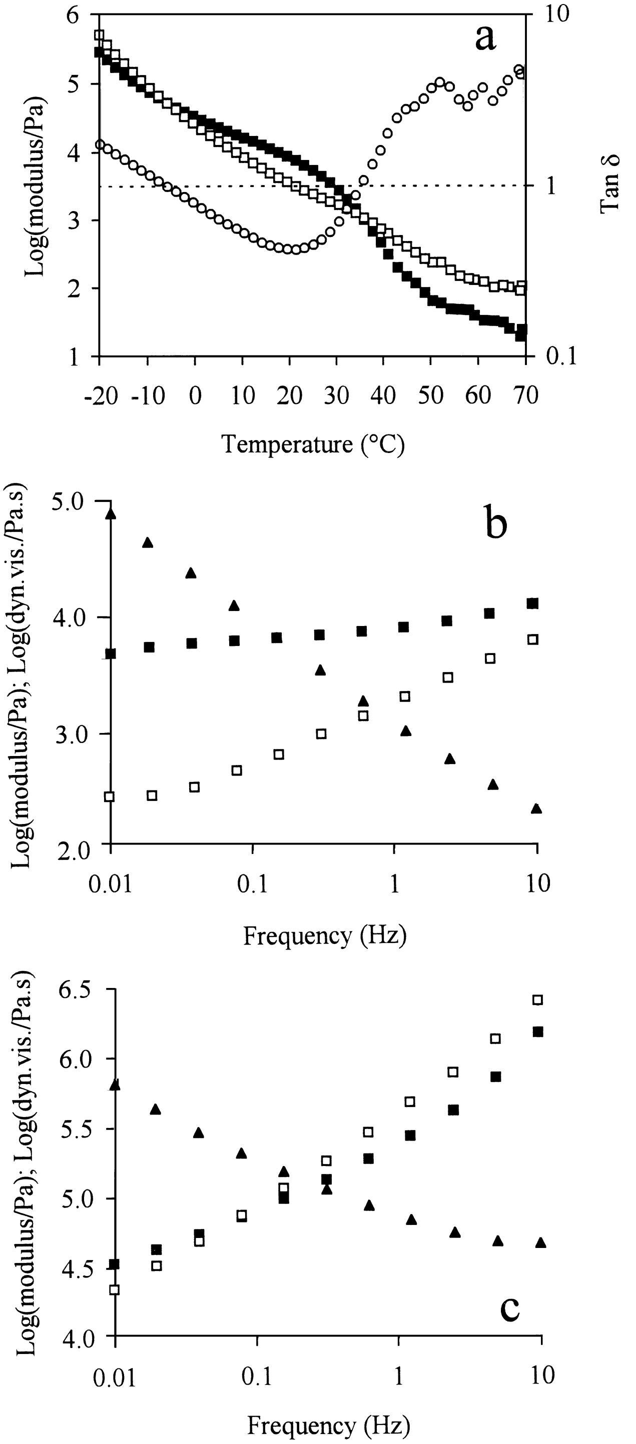

Fig. 4. Changes in G′ (■), G″ (□), tan δ (○), and η^* (▲) as a function of (a) temperature, (b) frequency at 20°C, and (c) frequency at −20°C for sample SPS20.

To rationalise the mechanical behaviour of gelatin/glucose syrup samples, and therefore that of liquor SPS8, one should follow the teachings of the master curve in Fig. 1. As the temperature is reduced, the first crossing step reflects the transformation from a gelatin solution to a rubbery gel. Diminishing long-range configurational motions due to immobilisation (freezing out) of large segments in the gelatin should account for the second crossing step at lower temperatures. This process allows the short configurational motions to dominate (freezing in) and develop a viscous rather than an elastic identity in the system. As a result a transition zone is observed between a rubber-like and a glass-like consistency and the values of both moduli start soaring well beyond 10^6 Pa [Fig. 4(c)]. Cooling of the samples at a temperature below −20°C should eventually lead to the glassy state where modulus values of 10^9 Pa have been reported for amorphous synthetic polymers (Ferry, 1950). Alternatively the onset of the glass transition regime [second crossing point in Fig. 4(a)], and therefore the process of vitrification, can be seen earlier during a cooling scan if a higher amount of co-solute is used. Thus increasing the concentration of co-solute from 70 to 85% in a 0.5% gellan sample has shifted the temperature for the onset of glass transition from about 5 to 38°C (Papageorgiou, Kasapis, & Richardson, 1994). In the absence of gellan, the co-solute remains in the form of a Newtonian solution with a flat dependence of viscosity on frequency and a dominating loss component. The antiplasticizing effect of co-solute on polymer vitrification has been also documented for high methoxy pectin, in both cases the co-solute being a blend of 50% sucrose topped up with glucose syrup to avoid crystallisation (Al-Ruqaie, Kasapis, Richardson, & Mitchell, 1997). Addition of the sucrose/glucose syrup sample at a level of 70% (i.e. 50% sucrose plus 20% glucose syrup) to gelatin (1.5, 3, 5 and 10% series), however, has reproduced frequency sweeps intermediate between those of a ‘good’ biopolymer gel (flat moduli with tan $\delta \approx 0.05$) and the rubbery plateau of Fig. 4(b) (tan $\delta = 0.283$ at 1.6 Hz). Thus mechanical spectra of gelatin/sugar/glucose syrup at 5°C showed some frequency dependence of modulus with a tan δ value of 0.151 at 1.6 Hz (Papageorgiou & Kasapis, 1995, and unpublished results). In the present study, glucose syrup was used as the only co-solute which at a level of 60.3% induces a rubber-like frequency sweep at 20°C [Fig. 4(b)] and a tan δ value of 0.657 at 5°C [frequency of 1.6 Hz in Fig. 4(a)], thus leading soon after to a glass transition. Obviously the above temperature lag in the glass transition of gelatin invites further research in the effect of type of co-solute on biopolymer vitrification over this range of high solids (60–85%).

Gratifyingly the viscoelastic properties of oxidized starch/glucose syrup preparations reflect those seen for the remaining liquors (SPS4, SPS6, SPS14, SPS16 and SPS18). As shown in Fig. 5(a), cooling down of the sample SPS2 is accompanied by a liquid-like profile at high temperatures and a solid-like response at sub zero temperatures in the way discussed earlier for Fig. 2(a). Similarly mechanical spectra of sample SPS2 reproduce the shear thinning solution behaviour [20°C in Fig. 5(b)] and the rapid development of storage modulus [−20°C in Fig. 5(c)] noted for the above group of liquors in Fig. 2(b) and (c), respectively.

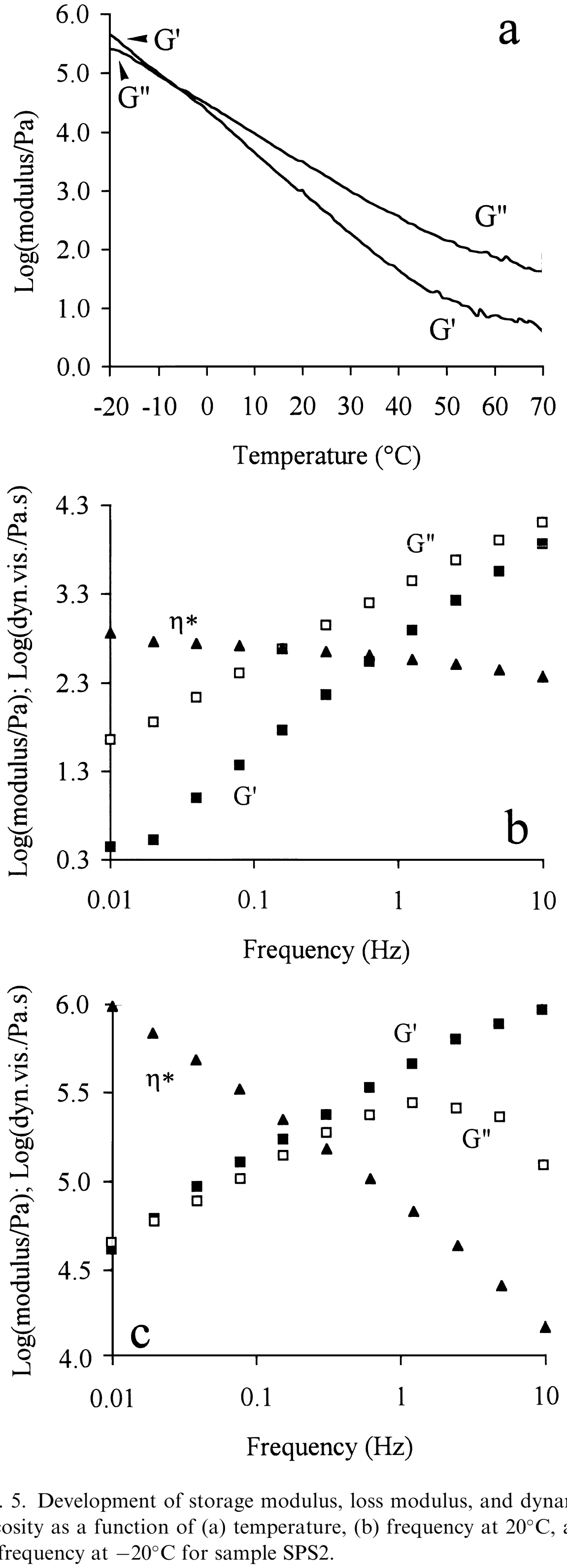

Fig. 5. Development of storage modulus, loss modulus, and dynamic viscosity as a function of (a) temperature, (b) frequency at 20°C, and (c) frequency at −20°C for sample SPS2.

It is now well documented that aqueous mixtures of gelatin with starch or starch hydrolysate products form phase-separated composite systems where gelation of the gelatin and starch hydrolysate phases or the gelatinisation of starch creates structures with physical properties identical to the individual components in isolation (Kasapis, Morris, Norton, & Brown, 1993; Abdulmola, Hember, Richardson, & Morris, 1996). In view of this, the present study argues for phase-separated high solids liquors whose viscoelasticity is determined by the component that forms the continuous matrix. Thus the liquor SPS8 should develop a gelatin continuous phase, which undertakes a disorder–order transformation during cooling. For the first time, however, further cooling induces a second change in viscoelasticity and the onset of gelatin's vitrification is observed. It seems therefore that solvent, co-solute and gelatin combine intimately in an arrangement that promotes a transition from rubber-like to glass-like consistency instead of the usual development of crystallinity observed for aqueous preparations of the protein at sub zero temperatures.

On the other hand, the remaining five liquors should form an oxidised starch continuous phase, which remains liquid-like for most of the temperature ramp but develops a dominant rigidity at sub zero temperatures. Fig. 6 indicates that the rigidity of starch-dominated rheology is due to crystallisation in these systems. Thus heating of the SPS2 sample using differential scanning calorimetry shows an endothermic spectrum which starts at −17°C and ends at −6°C with the temperature at maximum heat flow being about −9°C. As noted in Fig. 2(c), the broad thermal feature is unlikely to correspond to the melting of a single species but it should combine complex endothermic events from the water and perhaps the glucose syrup.

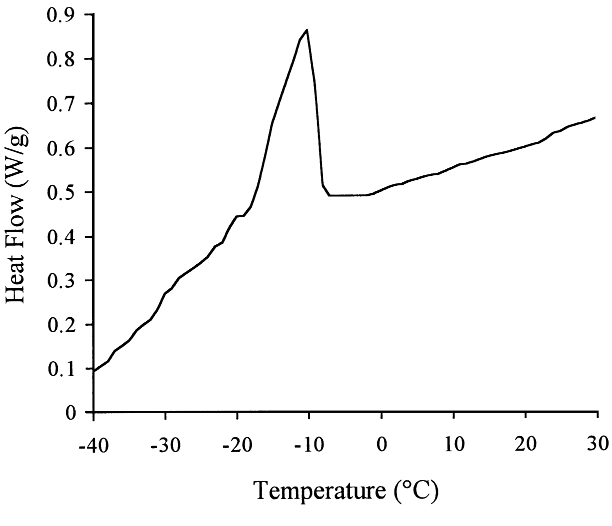

Fig. 6. Heating of the sample SPS2 in a Perkin–Elmer II calorimeter at a rate of 10°C/min.

At high enough concentrations of the oxidised starch, gelatinisation results in structure formation at the highest temperatures used in this investigation. As shown in Fig. 7(a), high G' and G'' values, with the solid-like response being dominant, are recorded at 80°C for a formulation of 31.2% polymer and 38.8% co-solute (SPS12). Both moduli develop substantially during subsequent cooling with the G' maintaining higher values than G'' throughout the accessible experimental range. Mechanical spectra at −20°C show frequency dependence but remain solid-like even at the highest experimental frequency [10 Hz in Fig. 7(b)]. The evidence in Fig. 7 strongly suggests that the starch-related rheology can be further consolidated by crystallinity in the form of amylose-like intermolecular sequences in a concentrated starch network. The aggregates of the oxidised starch network stabilise a rigid structure at the high temperature end and subdue the glassy element of the system at the low end of the temperature run, in

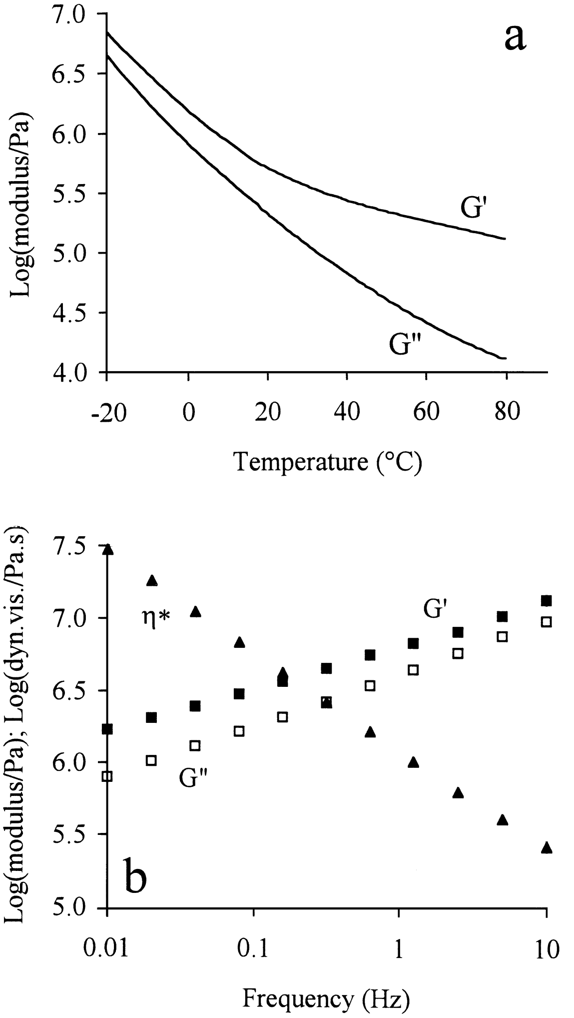

Fig. 7. Variation in storage modulus, loss modulus, and dynamic viscosity as a function of (a) temperature, and (b) frequency at −20°C for sample SPS12.

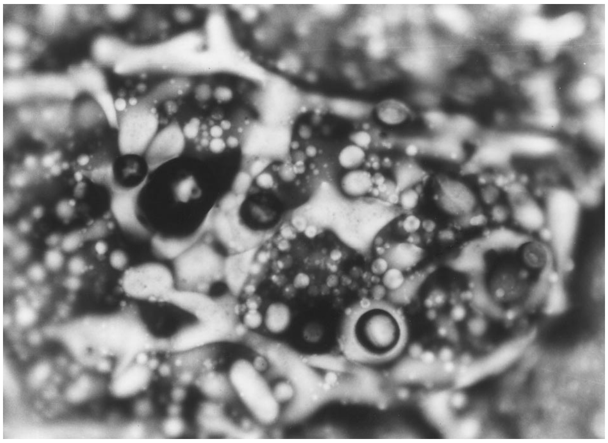

Fig. 8. Micrograph of sample SPS18, which reflects its phase behaviour at 70°C. Magnification is 80×. Samples were stained with iodine vapour. The microscope was a Diaphot-TMD with bright field optics.

accordance with the thermal behaviour of highly cross-linked crystalline synthetics (Ferry, 1980).

Finally, we would like to address the difference in viscoelasticity between two samples of similar composition, i.e. SPS8 with 7.1% oxidised starch and 6.1% gelatin, and SPS18 with 8.2% oxidised starch and 7.0% gelatin (in both cases the weight ratio of starch to gelatin is about 1.17). Rheological evidence for these two liquors indicates gelatin and starch-dominated structures, respectively. To solve the conundrum confocal laser scanning microscopy was employed to capture the macromolecular organisation of the two liquors upon mixing at 70°C. In doing so, mixtures were frozen rapidly in liquid nitrogen thus retaining the phase arrangement at 70°C. We have been unable to detect phase separation in the SPS8 sample at 70°C. By contrast, micrographs of the SPS18 sample at 70°C show a starch continuous phase surrounding the spheroidal gelatin inclusions; this appears in Fig. 8 as a grey/dark background (iodine-stained starch phase) encompassing white globes and clusters of gelatin. It seems, therefore, that at pH 3.6 the positively charged gelatin coils in solution repel themselves strongly, thus spreading uniformly throughout the system. Then, upon cooling they are able to form the continuous network which imparts high-solids gelatin properties to the liquor SPS8. Lower net charge density at pH 6, on the other hand, allows closer proximity and hence segregation of gelatin coils from the starch phase in solution. Subsequent cooling results in phase separated systems with oxidised starch forming the continuous phase, thus determining the overall rheology in liquor SPS18.

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