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Effect of sucrose, glucose and fructose on gelation of oxidised starch

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Abstract

The effect of sucrose on gelation of oxidised starch (partially depolymerised amylopectin with ~1 carboxyl group per 30 residues) has been studied by small-deformation oscillatory measurements (1 rad s^{-1}; 0.5 strain) of storage and loss moduli (G' and G''). Solutions were prepared at 95°C, cooled to 5°C at 1°C/min, and held at 5°C for 500 min. In the first series of experiments, the combined concentration of sucrose and starch was held fixed at 65 wt%. Sharp increases in moduli, attributable to double-helix formation, were observed during cooling, and moved to progressively higher temperature as the concentration of starch was increased (from 10 to 40 wt%). At all concentrations, however, G'' on reaching 5°C was higher than G', indicating that intermolecular interactions at this stage are largely topological. Formation of "true" gel networks ($G' \gg G''$) was observed during holding at 5°C, and is attributed to intermolecular helix–helix aggregation. The holding time at the onset of gelation decreased as the concentration (c) of starch was increased, and at concentrations in the range ~20 to ~30 wt% showed the c^{-2}-dependence expected for a simple dimerisation process. At higher concentrations the slope became steeper, consistent with some limited aggregation during cooling. In a second series of experiments, where c was held fixed at 40 wt% and sucrose concentration was varied (between 0 and 25 wt%), the time-course of gelation at 5°C showed little change with increasing sucrose content, but there were large increases in the moduli attained on completion of cooling. Measurement of the same samples by differential scanning calorimetry showed that the extent of ordering during cooling varied from ~2 to ~15% of the helix content in the ungelatinised granules as the sucrose concentration was increase from 0 to 25 wt%. Replacement of sucrose by glucose or fructose decreased the rate of conformational ordering during cooling, in the sequence: fructose < glucose < sucrose, but on holding at 5°C the order was reversed, with fructose causing rapid gelation and sucrose having least effect. A similar inverse correlation between ordering and aggregation has been observed for other biopolymer systems, and is tentatively ascribed to stability of individual helices inhibiting slight changes in conformation required for efficient packing.

Keywords: Gelation; Oxidised starch; Conformational ordering; Polysaccharide aggregation

1. Introduction

Conformational ordering and intermolecular association of biopolymers can often be promoted by introduction of sugars and related polyols as cosolute. This phenomenon can be explained, in part, by the associated reduction in water content. Water is a particularly effective solvent for biopolymers, because of its ability to form hydrogen bonds with hydroxyl groups and other polar substituents of the polymer chains. Partial replacement by a material with less capacity for hydrogen bonding would therefore be expected to promote polymer–polymer association by reducing competition from polymer–solvent interactions.

Different cosolutes, however, can differ substantially in the magnitude of the changes they produce, although their order of effectiveness with different biopolymers is often the same. For example, glucose, fructose and sucrose all enhance the stability of the conformationally ordered junctions in gels of gelatin (Oakenfull & Scott, 1986), carrageenan (Nishinari & Watase, 1992) and agarose (Watase, Kohyama & Nishinari, 1992), but to different extents, with the degree of stabilisation following the order: fructose < glucose < sucrose in each case. Generalities of this type are commonly attributed to modification of water structure, with the degree of modification induced by different polyols being determined by their steric compatibility with the pattern of hydrogen bonding between water molecules. In particular, the spacing of equatorial hydroxyl groups on sugar rings matches the "lattice" structure of liquid water (Tait, Suggett, Franks, Ablett & Quickenden, 1972), and the relative effectiveness of different sugars in stabilising intermolecular association of biopolymers often correlates well with their content of equatorial hydroxyls, calculated as a weighted-average for the configurations present in solution (e.g. Watase et al., 1992). As discussed

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in the preceding paper (Evageliou, Richardson & Morris, 2000c), however, such correlations may arise indirectly, through competition between water–cosolute and polymer–cosolute interactions (Nilsson, Piculell & Malmsten, 1990), rather than as a direct consequence of changes in water structure.

The present work centres on the effect of sucrose on gelation of oxidised starch, which, as outlined in an accompanying paper (Evageliou, Richardson & Morris, 2000b), can be regarded as partially depolymerised amylopectin, with a low content of carboxyl groups (~1 per 30 residues) formed during the depolymerisation (oxidation) reaction. The primary structure of amylopectin, like that of the essentially linear amylose component of starch, is based on a (1 → 4)-linked repeating sequence of α-D-glucosyl residues, but with extensive branching through (1 → 6) linkages. In the native starch granule, the linear sequences (of length ~15 residues) radiating from each branch point are associated into co-axial double helices, which can be packed in one, or both, of two polymorphic forms ("A-type" and "B-type"), depending on botanical source. In non-waxy starches, the granule also contains disordered amylose, but waxy varieties, such as the waxy maize starch used in commercial production of oxidised starch, consist almost entirely of (ordered) amylopectin. (For general background on structure and conformation of starch polysaccharides see, for example, Clark, Gidley, Richardson & Ross-Murphy, 1989; Gidley, 1987, 1989; Noel, Ring & Whittam, 1993; Ring, 1985).

On heating in excess water, the amylopectin structure melts, and the granules swell, in a process known as "gelatinisation", with (partial) release of disordered amylose, if present. On further heating and/or application of mechanical shear, the granule structure can be fully disrupted, giving a solution of starch polysaccharides. Oxidised starch is particularly susceptible to disintegration, and under the conditions of sample preparation used in this investigation (stirring for ~30 min at ~95°C) the granule structure is completely destroyed (Picout, Richardson, Rolin, Abeysekera & Morris, 2000).

On cooling, gelation of non-waxy starches occurs initially by association of amylose into double-helical junctions, with further association of helices into aggregated assemblies (of B-type morphology). Previous studies (e.g. Clark, 1995; Ring et al., 1987) have shown that re-ordering (retrogradation) of amylopectin also involves the same two processes (helix formation and development of B-type aggregates). In contrast to amylose, however, double-helix formation in amylopectin is likely to occur predominantly between adjacent strands within the same molecule, rather than between sequences on different molecules, with formation of a continuous network therefore arising largely from the aggregation process.

In the present work we have explored the effect of sucrose on the time-temperature course of structure formation from solutions of oxidised starch, using differential scanning calorimetry (DSC) to characterise (Cooke & Gidley, 1992) the extent of conformational ordering (double-helix formation) and small-deformation rheological measurements to follow development of network structure through helix–helix aggregation. We also report a brief comparison of the relative effectiveness of sucrose, glucose and fructose in promoting both stages of structuring.

2. Materials and methods

The oxidised starch was identical to the sample used by Evageliou, Richardson and Morris (2000a,b) for investigation of co-gelation with pectin (C * Set 06598, batch SH 1338, from Cerestar). It has an intrinsic viscosity (Giannouli, Richardson and Morris, unpublished) of $[\eta] = 0.35$ dl g^{-1} (measured in water at 20°C). Glucose and fructose (moisture content < 1%) were Reagent grade from BDH. The sucrose used was normal food grade, purchased locally. Citric acid was AnalaR grade from BDH. Distilled deionised water was used throughout.

Solutions were prepared by dispersing the starch in slightly more than the total amount of water required for the final solution, placing the sample in a water bath at ~95°C, and holding, with occasional stirring, until a clear solution was obtained (typically after 15–30 min, depending on concentration). The appropriate amount of sugar (sucrose, glucose or fructose) was then added, with continued stirring until the solution was again clear, and the sample was adjusted to the correct final weight by addition of water or continued evaporation, as appropriate. For consistency with our previous studies of co-gelation with high-methoxy pectin (Evageliou et al., 2000a,b), where acidic pH was required to induce gelation of the pectin component, all solutions included 0.6 wt% citric acid (added as the last stage of sample preparation).

Rheological measurements were made using highly truncated cone-and-plate geometry (diameter 50 mm; cone angle 0.05 rad; minimum gap 1 mm) on a sensitive prototype rheometer designed and constructed by one of us (R.K.R.). Samples were loaded at 95°C, and their periphery was coated with light silicone oil to minimise evaporation. They were then cooled to 5°C at 1°C/min and held at 5°C for 500 min, with measurement of storage modulus (G') and loss modulus of (G'') at a fixed frequency of 1 rad s^{-1} and fixed strain of 0.5%. Temperature was controlled by a Haake circulating water bath and measured using a thermocouple in contact with the stationary element.

Differential scanning calorimetry (DSC) measurements were made on a Setaram microcalorimeter, using a heating rate of 0.5°C/min and sample mass of ~0.9 g. Sample and reference pans (balanced to within ±0.5 mg) were loaded at ambient temperature, cooled to 5°C, and held for appropriate times before scanning to 95°C. Baselines were interpolated by applying a polynomial fit to heat flow values

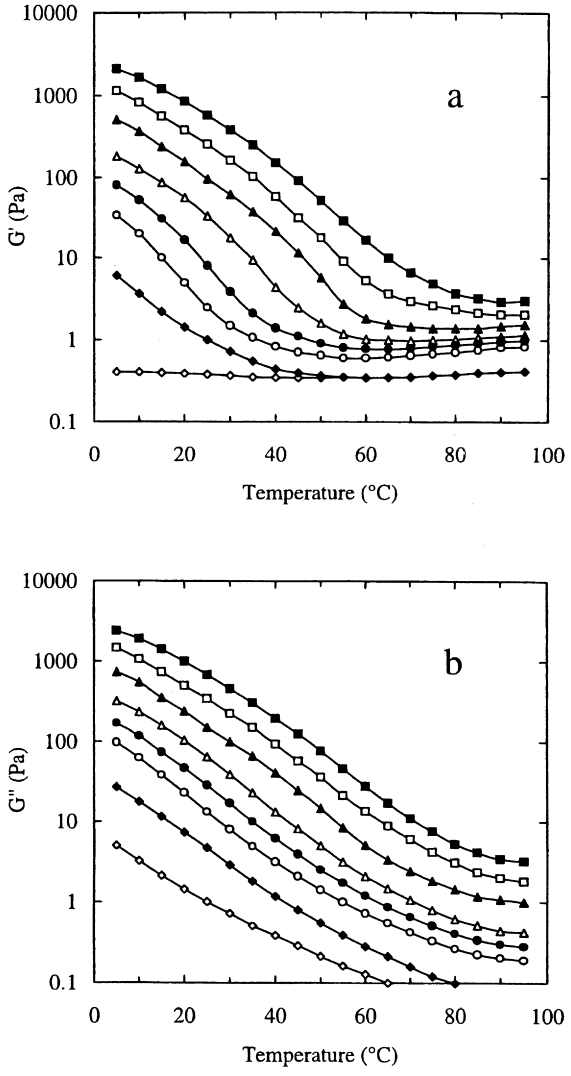


Fig. 1. Changes in (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain) during cooling from 95 to 5°C at 1°C/min for mixtures of oxidised starch with sucrose at a combined concentration of 65 wt%, illustrated for starch concentrations (wt%) of 10 (◇), 15 (◆), 20 (○), 22.5 (●), 25 (△), 30 (▲), 35 (□) and 40 (■).

recorded at temperatures above and below the range of the endothermic transition.

3. Results

3.1. Rheology at 65 wt% total solids

Fig. 1 shows the changes in G' (Fig. 1a) and G'' (Fig. 1b) observed during cooling from 95 to 5°C for mixtures of oxidised starch with sucrose at a combined concentration of 65 wt% (i.e. with water content held constant at 35 wt%). The starch concentrations used range from 10 to 40 wt% (with 55–25 wt% sucrose). The effect of starch concentration on the values of G' and G'' attained on completion of cooling is shown in Fig. 2a. Both figures have been presented previously in an accompanying paper on co-gelation with pectin (Evageliou et al., 2000b), where they were used for direct comparison with the corresponding mixed systems; Fig. 1, however, includes traces for two additional starch concentrations (22.5 and 35 wt%) which were not shown in the previous paper. The changes in moduli for the same preparations during holding for 500 min at 5°C are illustrated in Fig. 3, and the effect of starch concentration on the final values on G' and G'' recorded at the end of the holding period are shown in Fig. 2b.

As shown in Fig. 1a, the samples all have significant elastic character (G') at the loading temperature of 95°C, which, as will be demonstrated later by results from DSC, is well above the melting temperature for amylopectin double helices, even in the presence of high concentrations of sucrose. The elastic response must therefore come from physical entaglement (interpenetration) of neighbouring molecules. In the initial stages of cooling, most of the samples show a slight reduction in G', which can be explained by reduction in hydrodynamic volume as the temperature is decreased (Yamakawa, 1971), with consequent reduction in the degree of entanglement. At the highest starch concentrations, however, this initial reduction is swamped by a large increase in G', attributable to the onset of double-helix formation. The increase in G' is accompanied by a corresponding increase in G'' (Fig. 1b), and both are displaced to progressively lower temperature as the concentration of oxidised starch is decreased.

On completion of cooling to 5°C (at 1°/min), the viscoelastic response (Fig. 2a) at the lower end of the range of starch concentrations studied is predominantly solution-like ($G' \gg G''$). At higher concentrations the moduli converge, but G'' remains slightly higher than G' up to the highest concentration of oxidised starch used (40 wt%, in combination with 25 wt% sucrose). It seems likely, therefore, that the intermolecular interactions on completion of cooling are still largely topological, and that the increases in moduli (Fig. 1) result from association of flexible, disordered strands into stiff, extended helices, making it more difficult for individual molecules to move through the surrounding matrix of neighbouring chains. After holding for 500 min at 5°C (Fig. 2b), the samples prepared at starch concentrations below ~20 wt% ($\log c \approx 1.3$) remain predominantly viscous ($G'' > G'$), but at higher concentrations G' rises steeply above G'', demonstrating formation of an extensively crosslinked network during the holding period at 5°C.

As found during cooling (Fig. 1a), the changes in G' on holding at 5°C (Fig. 3a) occur in two stages: an initial slight reduction followed by a sharp increase, which is displaced to progressively longer times with decreasing concentration of oxidised starch and becomes undetectable at starch concentrations below ~15 wt%. As discussed above, the increase in elastic response can be attributed to intermolecular association by helix–helix aggregation. The initial reduction may also be due to aggregation of helical sequences, but within the same molecule, thus reducing the

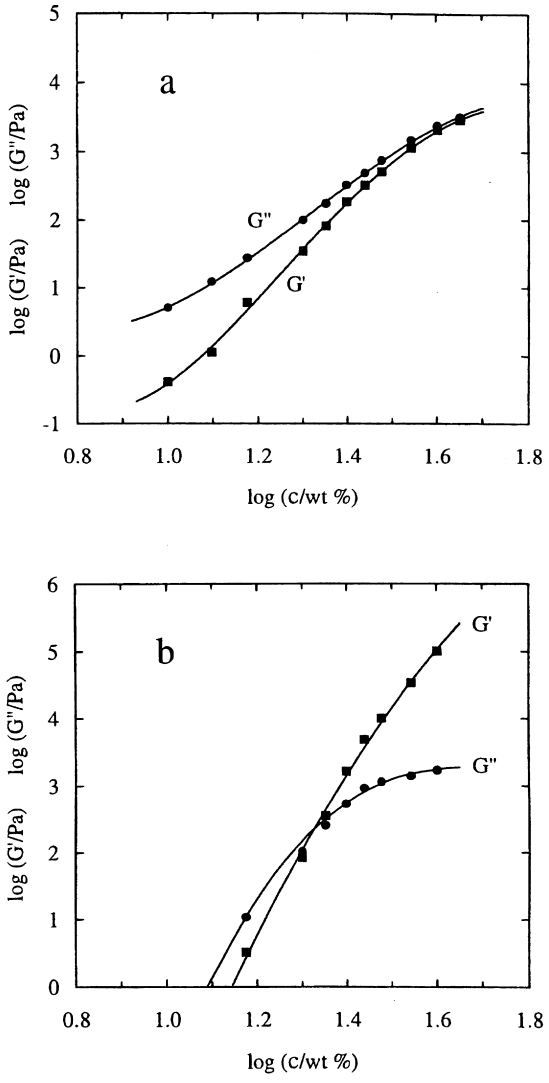


Fig. 2. Variation of G' (■) and G'' (●) with concentration (c) of oxidised starch in mixtures with sucrose at a combined concentration of 65 wt%: (a) on completion of cooling from 95 to 5°C at l°C/min; and (b) after holding at 5°C for a further 500 min.

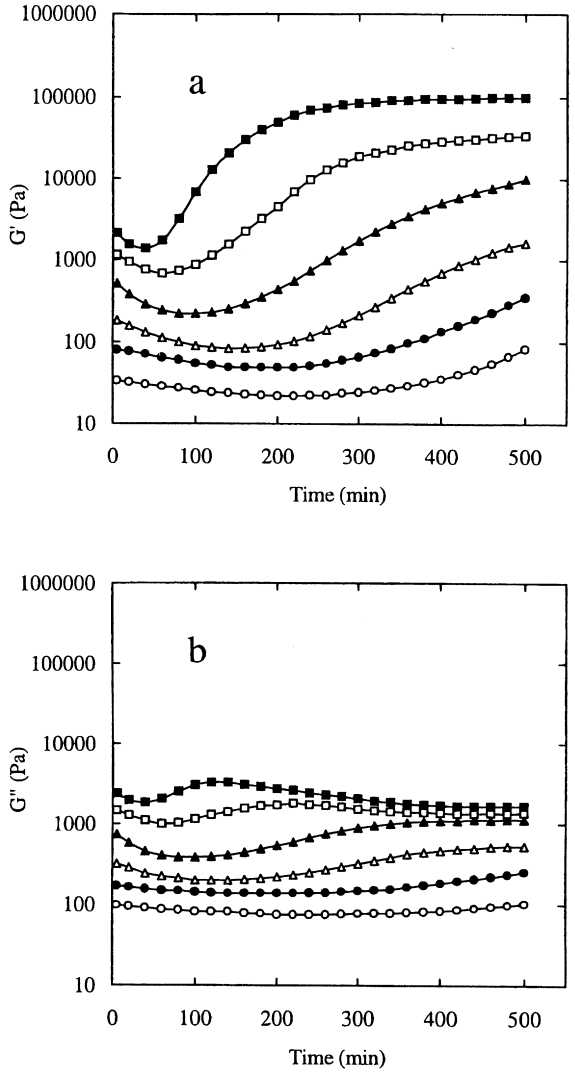


Fig. 3. Changes in (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain) during holding at 5°C after cooling from 95°C at 1°C/min for mixtures of oxidised starch with sucrose at a combined concentration of 65 wt%, illustrated for starch concentrations (wt%) of 20 (○), 22.5 (●), 25 (△), 30 (▲), 35 (□) and 40 (■).

number of individual strands forming "entanglements" with adjacent molecules (i.e. reducing the topological restrictions on network rearrangement).

At the highest concentrations of starch, the sharp increase in G' (Fig. 3a) is accompanied by a shallow maximum in G'' (Fig. 3b). An initial increase and subsequent decrease in G'' is often observed in the early stages of biopolymer gelation, and can be readily explained. Formation of a continuous network requires a minimum critical degree of crosslinking. Before this stage is reached, intermolecular association raises the effective molecular weight in solution, with consequent increase in viscous response (G''). At higher degrees of association, beyond the critical point, large cross-linked species in the solution state become incorporated in the gel network, thus raising G' but decreasing G''.

The time-dependent changes in the relative values of G' and G'' for the samples shown in Fig. 3 are illustrated directly in Fig. 4, which shows tan δ (G''/G') plotted against holding time at 5°C. In all cases, tan δ passes through a maximum, which occurs at progressively shorter times as the concentration of oxidised starch is increased. Thus, for the reasons discussed above, development of gel-like character is preceded by an initial increase in the relative proportion of viscous response.

The effect of starch concentration (c) on the final values of tan δ at the end of the 500 min holding period at 5°C is shown in Fig. 5, in direct comparison with the values attained on completion of cooling (i.e. at the start of the holding period). In both cases there is a smooth reduction in log tan δ with increasing log c. As mentioned previously, the values recorded on completion of cooling approach, but do not reach, tan $\delta = 1$ ($G' = G''$) at the upper end of the

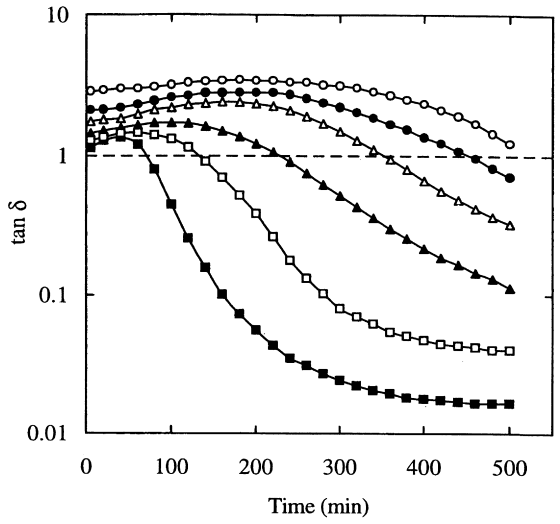


Fig. 4. Changes in tan δ (1 rad s^{-1}; 0.5% strain) during holding at 5°C after cooling from 95°C at 1°C/min for mixtures of oxidised starch with sucrose at a combined concentration of 65 wt%, illustrated for starch concentrations (wt%) of 20 (○), 22.5 (●), 25 (△), 30 (▲), 35 (□) and 40 (■).

concentration range studied. After holding for 500 min at 5°C, however, the separation of G' and G'' at high starch concentration approaches two orders of magnitude, which is typical of a strong polysaccharide gel (Ross-Murphy, 1984).

To quantify the relationship between sample composition and gelation rate, the point during the holding period at which the values of G' begin to rise after their initial decrease was determined by plotting the first differential of the holding curves in Fig. 3a ($d(\log G')/dt$) against time (t) at 5°C. The traces obtained are illustrated in Fig. 6 for starch concentrations of 20, 25, 30, 35 and 40 wt%; analogous curves were constructed for concentrations of 22.5 and

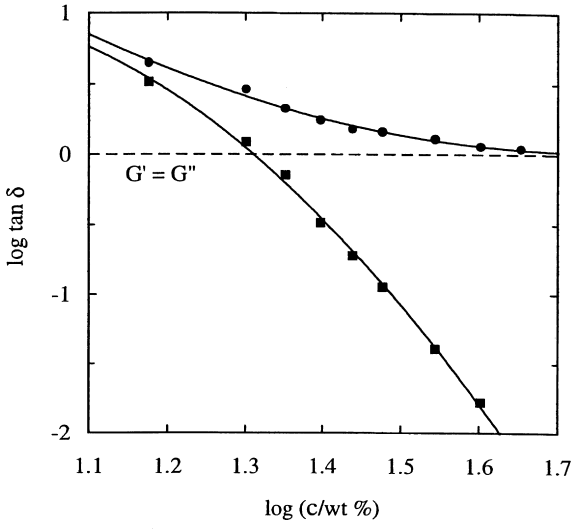


Fig. 5. Variation of tan δ with concentration (c) of oxidised starch in mixtures with sucrose at a combined concentration of 65 wt% on completion of cooling from 95 to 5°C at 1°C/min (●) and after holding at 5°C for a further 500 min (■).

27.5 wt% starch (with 42.5 and 37.5 wt% sucrose, respectively), but are omitted for clarity. The intercepts with the axis at $d(\log G')/dt = 0$, which define the transition from decreasing to increasing values of G', could be determined with good precision (to within about ±5%). The time at 5°C required to reach this point will be denoted as t_0 (onset time for gel formation).

The variation of t_0 with starch concentration (c) is shown in Fig. 7a. There is a smooth, increasing progression to higher values of t_0 with decreasing concentration of starch. Fig. 7b shows the same data plotted on logarithmic axes. The decrease in $\log t_0$ with increasing $\log c$ is virtually linear across the concentration range from 20 to ~30 wt% starch, with a slope close to −2. At higher concentrations of starch (35 and 40 wt%), the decrease in $\log t_0$ becomes progressively steeper, which may reflect the increasing content of ordered structure already present in these samples at the start of the holding period (i.e. formed during the cooling process shown in Fig. 1).

3.2. Effect of sucrose on rheology of 40 wt% oxidised starch

The studies reported in the previous section originated from an investigation of the effect of cosolutes on gelation of high methoxy pectin (Evageliou et al., 2000b). The starting point was a preparation containing 65 wt% sucrose, which is a typical concentration for formation of strong pectin gels (Christensen 1986; Rolin 1993). One of the main aims of the investigation was to explore the effect of progressive replacement of sucrose by oxidised starch. To distinguish changes induced by introduction of starch from those caused by reduction in sucrose content, the effect of sucrose concentration on the rheology of the individual polymers (pectin and oxidised starch) was also characterised, yielding the results reproduced here in Figs. 1 and 2a for the starch component. In the present investigation, we also carried out an analogous series of experiments in which the concentration of starch was held fixed and only the sucrose concentration was varied. The starch concentration selected for study was 40 wt% (the highest concentration used in the experiments reported in the previous section) and the sucrose concentration was varied from 0 to 25 wt%, in increments of 5 wt%.

Fig. 8 shows the changes in G' (Fig. 8a), and G'' (Fig. 8b) observed for these samples on cooling from 95 to 5°C (at 1°C/min). The curves are broadly similar to those obtained (Fig. 1) on varying starch concentration at 65 wt% total solids, and the moduli at 5°C span approximately the same range of values. Thus reducing sucrose content from 25 to 0 wt% while holding starch concentration fixed at 40 wt% has roughly the same effect as reducing starch concentration from 40 to 10 wt% while holding the combined concentration of sucrose and starch fixed at 65 wt%. As the sucrose concentration is lowered, however, the initial reduction in G' becomes progressively more evident, which, as discussed later, is consistent with

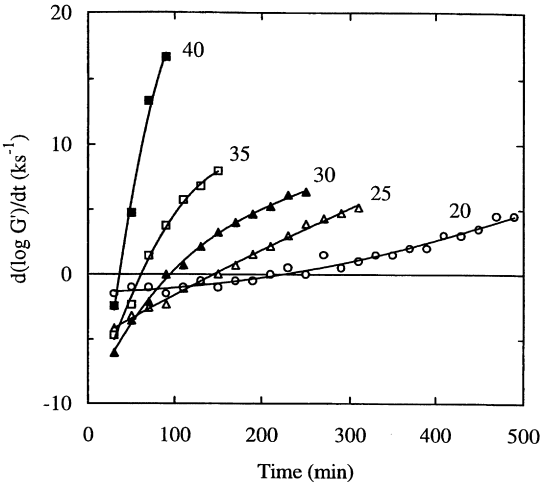


Fig. 6. Rate of change in log G' during holding at 5°C (Fig. 3a) for mixtures of oxidised starch with sucrose at a combined concentration of 65 wt%, illustrated for starch concentrations (wt%) of 20 (○), 25 (△), 30 (▲), 35 (□) and 40 (■). The points of intersection with the horizontal axis at $d(\log G')/dt = 0$ define the transition from decreasing to increasing values of G'.

"condensation" of sucrose along the individual strands of the branched amylopectin molecule, thus restricting the degree of contraction that can occur in response to reduction in temperature.

Fig. 9a shows the effect of sucrose concentration on the values of G' and G'' attained on completion of cooling to 5°C. In the absence of sucrose, G'' is about an order of magnitude greater than G', but the moduli converge as the concentration of sucrose is raised. The variation of log G' with sucrose concentration (wt%) is essentially linear, with a slope of ~0.145. Thus the value of G' for 40 wt% oxidised starch after cooling from 95 to 5°C at 1°C/min increases by about a factor of 10 for each 7 wt% of sucrose added to the starting solution. As shown in Fig. 10, however, the further increase in moduli during holding at 5°C becomes progressively smaller as the sucrose concentration is increased, with consequent convergence of the values of G' (Fig. 10a) and G'' (Fig. 10b) recorded at different concentrations of cosolute.

Fig. 9b shows the effect of sucrose concentration on the final values on G' and G'' after holding for 500 min at 5°C. In contrast to the massive variations observed (Fig. 9a) at the beginning of the holding period (i.e. on completion of cooling from 95°C), incorporation of 25 wt% sucrose changes the final moduli by less than an order of magnitude, and the separation of G' and G'' remains roughly constant (i.e. constant tan δ) across the full range of sucrose concentrations studied.

As described above, reduction in sucrose content at fixed concentration of starch (Fig. 8) has roughly the same effect on structure formation during cooling as reduction in starch concentration while holding the total solids content fixed (Fig. 1). The subsequent changes on holding at 5°C, however, are entirely different. One obvious difference is

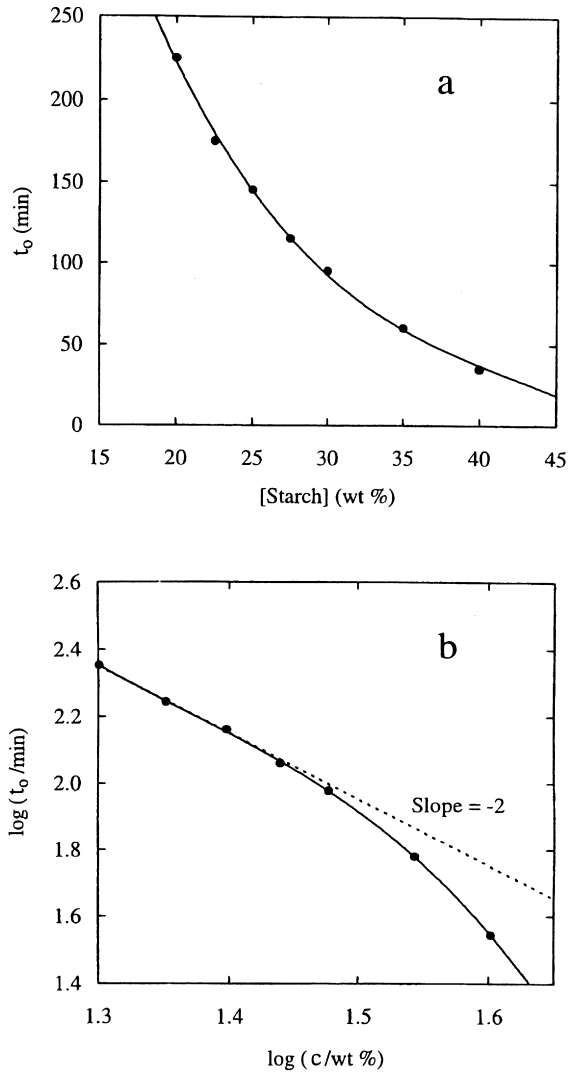


Fig. 7. Effect of starch concentration (c) in mixtures with sucrose at a combined concentration of 65 wt% on the onset time (t_o) for increase in G' (Fig. 3a) after initial reduction, plotted on: (a) linear; and (b) logarithmic axes.

that the values of G' for samples of different sucrose content converge during holding (Fig. 10a), whereas those observed at different starch concentrations diverge (Fig. 3a). A second major difference is that the sharp increases in moduli attributable to helix–helix aggregation are displaced to progressively longer times on decreasing starch concentration (Figs. 3, 6 and 7), whereas the corresponding increases for samples prepared with varying concentrations of sucrose but fixed content of oxidised starch occur (Fig. 10) over essentially the same time period.

3.3. Differential scanning calorimetry

In the first series of DSC experiments, the samples used were identical to those described in the previous section (40 wt% oxidised starch; 0–25 wt% sucrose). The solutions were filled into the DSC pan at ~90°C, cooled to ambient

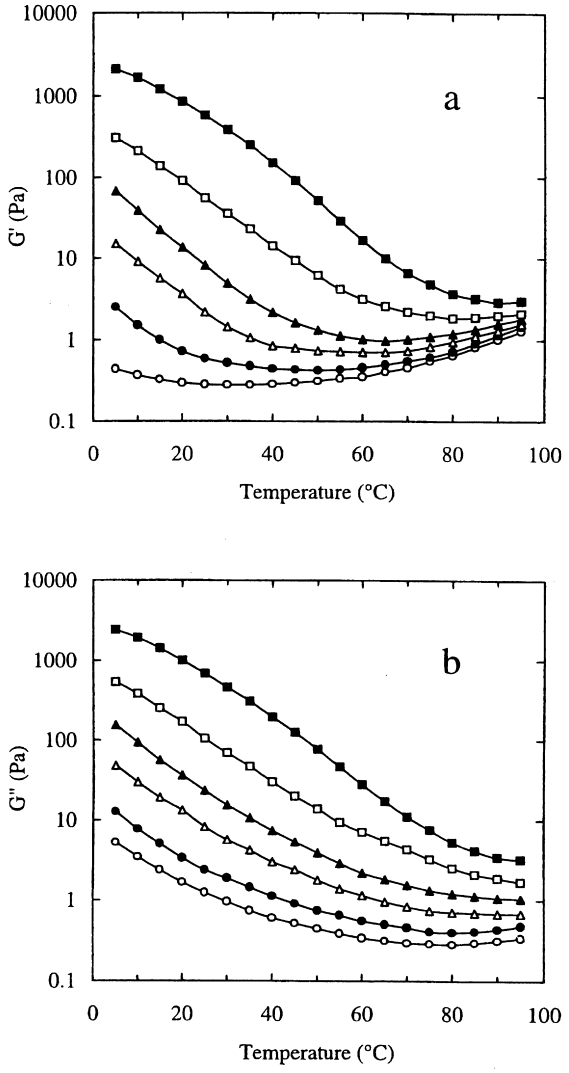


Fig. 8. Changes in (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain) during cooling from 95 to 5°C at 1°C/min for 40 wt% oxidised starch in the presence of sucrose at concentrations (wt%) of 0 (○), 5 (●), 10 (△), 15 (▲), 20 (□) and 25 (■).

temperature over a period of ~25 min, weighed for balancing with the reference pan, loaded into the calorimeter, cooled to 5°C at 1°C/min, and held at 5°C for ~25 min to allow the instrument to equilibrate. These conditions were chosen to give roughly the same extent of structure formation as in the early stages of the holding period in the rheological experiments (Fig. 10). The samples were then scanned to 98°C at a heating rate of 0.5°C/min, which represents a compromise between the conflicting aims of avoiding excessive "thermal overshoot" while minimising further structure formation in the early stages of heating (where the sample is at low temperature). The resulting traces are shown in Fig. 11. All samples show a single, well-defined endotherm, which increases in magnitude and extends to progressively higher temperature as the concentration of sucrose is raised. The variation of transition enthalpy (ΔH) per gram of starch with increasing concentration of sucrose is shown in Fig. 12. There is a smooth upward curvature from $\Delta H \approx 0.25$ J/g for 40 wt% oxidised starch in water to $\Delta H \approx 2.0$ J/g for the sample incorporating 25 wt% sucrose.

To give an indication of the extent of conformational ordering implied by these enthalpy values, Fig. 13 shows the endotherm obtained on heating a slurry of ungelatinised oxidised starch (30 wt% in water). The transition enthalpy is 13.0 J/g, which is close to the value of $\Delta H = 13.2$ J/g obtained (Abdulmola, Hember, Richardson & Morris, 1996) on the same instrument for unmodified waxy maize starch, indicating that the oxidation process does not cause any significant loss of conformational order within the ungelatinised granules. Comparison with the values of ΔH shown in Fig. 12 suggests that the moduli observed for the same samples on completion of cooling to 5°C (Fig. 9a) correspond to levels of structural ordering ranging from ~2% to ~15% of the order present intitially in the starch granules prior to gelatinisation.

A second series of DSC experiments was carried out to explore the extent of additional conformational ordering on holding at low temperature, by recording heating endotherms for the same preparation of oxidised starch after storage for increasing lengths of time at 5°C. The sample used was 30 wt% starch in combination with 35 wt% sucrose. The reason for choosing this composition was that the initial reduction and subsequent increase in G' and G'' (Fig. 3) during holding are particularly well defined, and occur on a convenient timescale for duplication in DSC studies. Solutions were loaded and cooled as described above, and heating scans were recorded for samples held for 0.5, 1.5, 4.0 and 6.0 h at 5°C. The endotherms obtained are shown in Fig. 14.

There is a massive increase in peak area (i.e. transition enthalpy) with increasing storage time. As shown in Fig. 15, the variation of ΔH with holding time is essentially linear, confirming that the initial decrease and subsequent increase in moduli observed over the same time period (Fig. 3) are both associated with a progressive increase in helix content. The value of ΔH recorded at the longest holding time used (6 h) is ~10 J/g, which, by comparison with the gelatinisation endotherm (Fig. 13), corresponds to more than 75% of the content of ordered structure present intitially in the ungelatinised starch granule. The small positive intercept at zero time in Fig. 15 can, of course, be attributed to the (constant) fraction of ordered structure formed during cooling to 5°C and balancing of the calorimeter.

3.4. Relative effectiveness of sucrose, glucose and fructose

The composition studied in the second series of DSC experiments described above (30 wt% oxidised starch with 35 wt% sucrose) was also used as the starting point for a brief investigation of the effect of replacing sucrose by glucose or fructose (the other most common sugars encountered in food applications of biopolymers). Since the

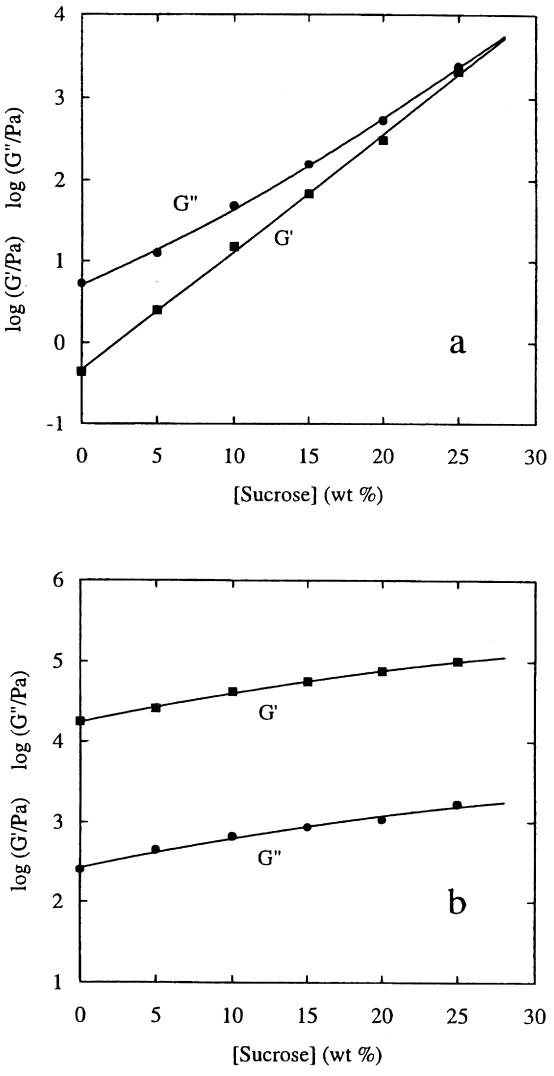


Fig. 9. Variation of G' (■) and G'' (●) with concentration of sucrose in mixtures with 40 wt% oxidised starch: (a) on completion of cooling from 95 to 5°C at 1°C/min; and (b) after holding at 5°C for a further 500 min.

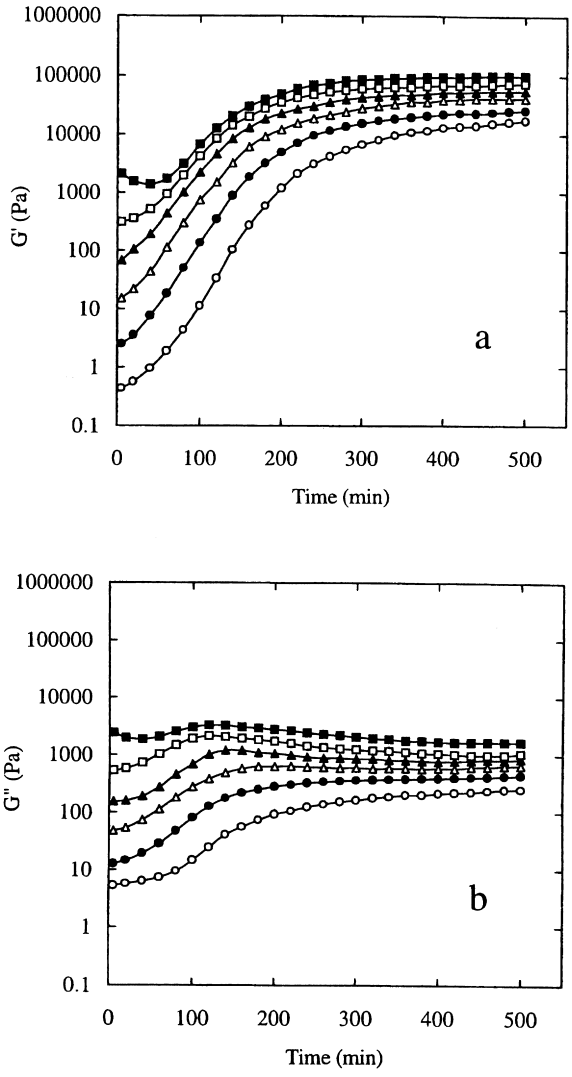


Fig. 10. Changes in (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain) during holding at 5°C after cooling from 95 at 1°C/min for 40 wt% oxidised starch in the presence of sucrose at concentrations (wt%) of: 0 (○), 5 (●), 10 (△), 15 (▲), 20 (□) and 25 (■).

molecular weight of sucrose is 342, in comparison with 180 for glucose or fructose, the concentration of either monosaccharide required to give a solution with the same molar concentration of individual sugar rings as in a 35 wt% solution of the sucrose disaccharide is ~36.84 wt% ($35 \times 2 \times 180/342$), which was therefore the concentration used in preparation of mixtures with 30 wt% starch for comparison, on a molar basis, with the same concentration of starch in combination with 35 wt% sucrose. To allow comparisons of the relative effectiveness of the three different sugars to also be made on a weight basis, the behaviour of 30 wt% starch in the presence of 36.84 wt% sucrose was also studied. Fig. 16 shows the changes in G' (Fig. 16a) and G'' (Fig. 16b) seen during cooling from 95 to 5°C for samples prepared using 36.84 wt% of all three sugars. There is an obvious systematic increase in the extent of structure formation in the order: fructose < glucose < sucrose, which, as outlined in the Section 1, is the sequence anticipated from steric compatibility with water structure, and observed experimentally for conformational ordering and gelation of other biopolymers.

During holding at 5°C (Fig. 17), however, there is a sharp reversal in order, with glucose and, particularly, fructose causing a much greater, and more rapid, increase in moduli than the same weight concentration of sucrose. Since the cooling and holding traces shown in Figs. 16 and 17 for 30 wt% starch with 36.84 wt% sucrose are only marginally higher than the corresponding traces for the same concentration of starch in combination with 35 wt% sucrose (shown by filled triangles in Figs. 1 and 3), comparison on the basis of molar concentration of sugar rings gives the same qualitative pattern as comparison on a weight basis. It would therefore appear that the relative effectiveness of the different sugars in promoting initial conformational

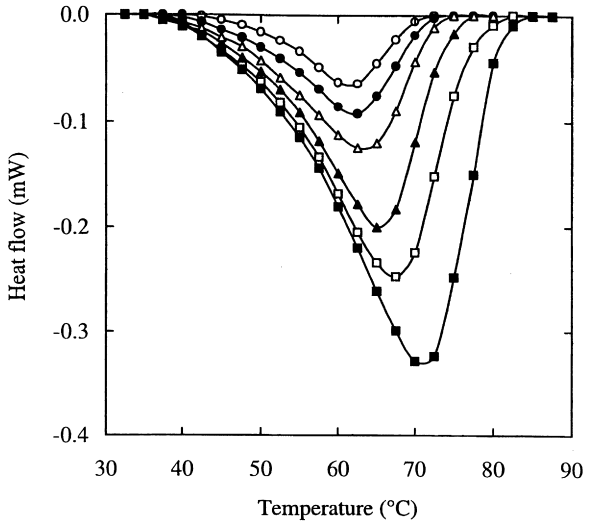


Fig. 11. DSC endotherms, recorded at a heating rate of 0.5°C/min after thermal equilibration (~25 min) at 5°C, for 40 wt% oxidised starch in the presence of sucrose at concentrations (wt%) of: 0 (○), 5 (●), 10 (△), 15 (▲), 20 (□) and 25 (■).

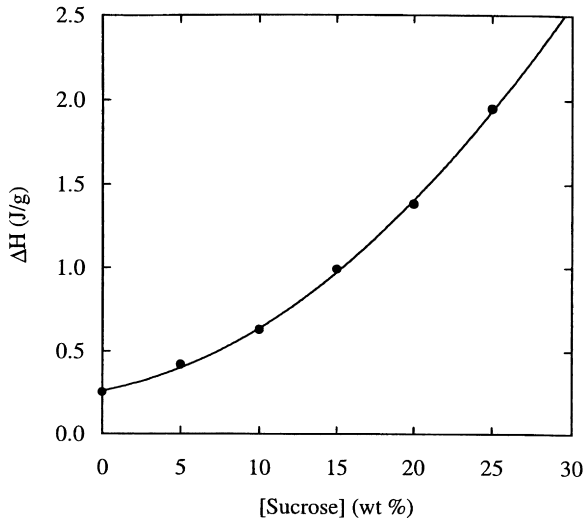


Fig. 12. Effect of sucrose concentration on the enthalpy (ΔH) of the endothermic transitions obtained (Fig. 11) for 40 wt% oxidised starch after thermal equilibration (~25 min) at 5°C.

ordering (double-helix formation) during cooling follows the normal progression (fructose < glucose < sucrose), but that their order of effectiveness in inducing helix–helix association (formation of B-type aggregates) is reversed (sucrose < glucose < fructose).

4. Discussion

The comparisons shown in Figs. 2 and 9 of the values of G' and G'' recorded on completion of cooling to 5°C, and after holding at 5°C for a further 500 min, strongly suggest that the increases in moduli observed during cooling (Figs. 1 and 8) arise predominantly from expansion and stiffening of individual molecules (with consequent enhancement of topological interactions), and that formation of a "true" crosslinked gel network by intermolecular helix–helix aggregation occurs largely during the holding period at 5°C.

For the samples prepared at 65 wt% total solids, the holding time (t_o) at the onset of gelation for starch concentrations between ~20 and ~30 wt% follows (Fig. 7b) the c^{-2}-dependence expected for a simple dimerisation process. At higher concentrations the slope becomes steeper, but, as suggested previously, this probably reflects an increase in the extent of intermolecular association that occurs during cooling, with consequent reduction in gel times at 5°C.

A much steeper concentration-dependence of gel time ($\sim c^{-5.2}$) was observed in an investigation by Clark (1995), using partially depolymerised amylopectin prepared by acid hydrolysis and dissolved in water. The small content of carboxyl groups in the oxidised starch sample used in the present work is unlikely to be a significant factor. Introduction of increasing concentrations of sucrose with decreasing starch concentration might be expected to have a greater effect on gelation properties, but, as shown in Fig. 10, the changes in moduli observed for samples prepared with the same concentration of starch but varying concentrations of sucrose (0–25 wt%) occur over essentially the same time period (which, in itself, is indicative of a process controlled predominantly by simple mass-action).

The most likely explanation for the large difference in concentration-dependence between the two investigations is the different criteria used to define gel time. In the investigation by Clark (1995), the samples were prepared in narrow tubes, which were periodically tipped a few degrees from the vertical. The gel time was taken as the point at which the surface of the sample first remained perpendicular to the walls of the tube, rather than flowing towards the horizontal. The criterion used in the present work, by contrast, was the onset of the increase in G' leading to gel formation, rather than the actual development of a cohesive network, which probably requires the elastic modulus to reach a finite threshold value. It is obvious from Fig. 3a that the times required for the samples of low starch concentration to reach even the minimum value of G' observed at the highest concentration (40 wt%) would be very much greater than the t_o values plotted in Fig. 7b, thus giving a much steeper concentration-dependence, as observed by Clark (1995).

Another major difference is that in Clark's investigation the samples were quenched to 5°C, whereas in the present work they were cooled from 95°C over a period of ~90 min. It is possible that the weak, topological network formed during this comparatively slow cooling process may have biased subsequent aggregation towards intermolecular association, whereas rapid quenching might be expected to favour intramolecular ordering, with association into a

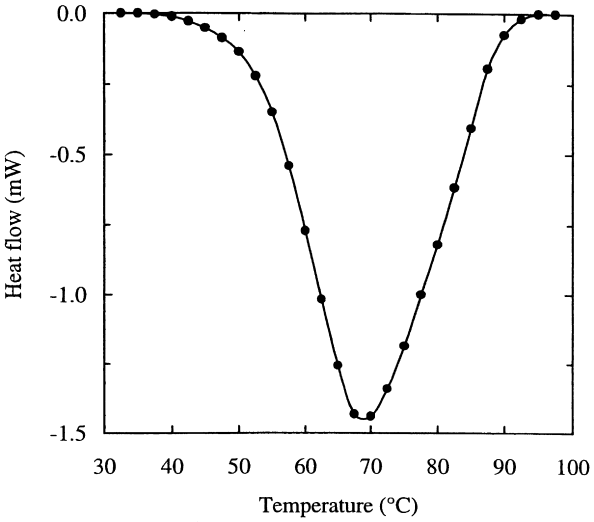


Fig. 13. Gelatinisation endotherm (0.5°C/min) for oxidised starch (30 wt% in water).

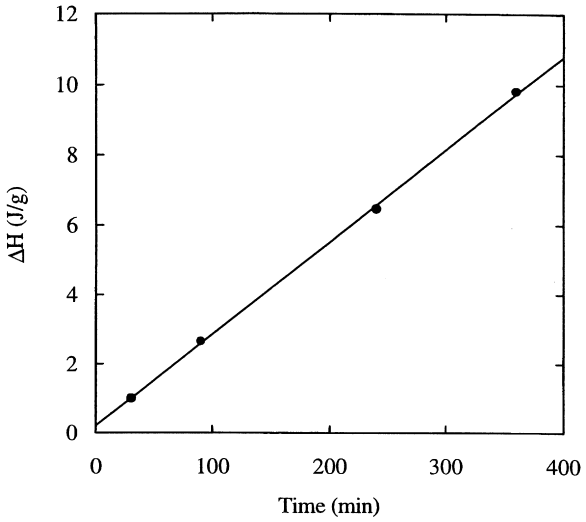


Fig. 15. Effect of holding time at 5°C on the enthalpy (ΔH) of the endothermic transitions obtained (Fig. 14) for 30 wt% oxidised starch in combination with 35 wt% sucrose.

continuous network then occurring by a process more akin to crystallisation than to simple helix–helix aggregation.

The main aim of the present investigation, however, was to explore the effect of cosolute. As shown in Fig. 12, increasing concentration of sucrose (0–25 wt%) at constant concentration of starch (40 wt%) causes a large (~8-fold) increase in the values of ΔH for samples measured shortly after completion of cooling to 5°C. Previous studies (Cooke & Gidley, 1992) have shown that the changes in enthalpy from disordering of amylopectin crystallites during gelatinisation of starch come predominantly from melting of double helices, rather than from the accompanying disaggregation process. It seems evident, therefore, that incorporation of sucrose causes a large increase in the rate of helix formation, as is also indicated by the changes in moduli during cooling observed (Fig. 8) for the same samples.

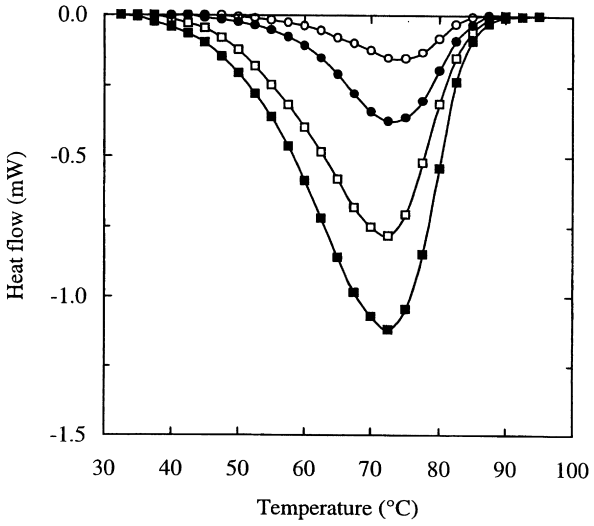
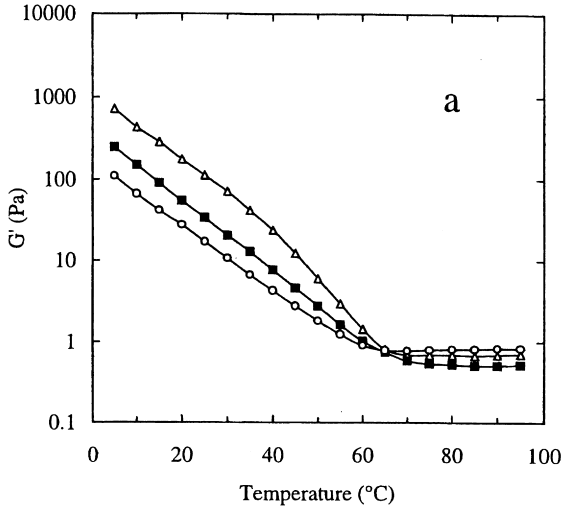


Fig. 14. DSC endotherms (0.5°C/min) for 30 wt% oxidised starch in combination with 35 wt% sucrose after holding at 5°C for 30 min (○), 1.5 h (●), 4 h (□) and 6 h (■).

As outlined in Section 1, cosolutes can promote self-association of biopolymers by: (i) replacing part of the water; and (ii) associating with the water that remains. Both of these will, of course, reduce the effectivenes of polymer–water interactions in competing with polymer–polymer association. Theoretical and experimental studies by Nilsson et al. (1990), however, have shown that conformational equilibria of biopolymers can also be displaced by direct interactions between the polymer and the cosolute, leading to enhancement or depletion of the concentration of cosolute at the surface of the polymer chains.

In the investigation reported in the preceding paper (Evageliou et al., 2000c), we compared the relative effectiveness of sucrose, glucose and fructose in promoting gelation of high methoxy pectin at acidic pH, and found that glucose is much more effective than fructose, with sucrose also having a much greater effect than fructose, but substantially less than glucose. This departure from the normal order of effectiveness (fructose < glucose < sucrose) anticipated from water–cosolute interactions, and observed experimentally for gelation of other biopolymers (e.g. Nishinari & Watase, 1992; Oakenfull & Scott, 1986; Watase et al., 1992) was attributed to unusually strong polymer–cosolute interactions induced by hydrogen bonding between the carbonyl moiety in the methyl ester and carboxylic acid groups of pectin and the primary alcohol groups of the cosolute molecules (2 in fructofuranose, 1.5 per residue in sucrose and 1 in glucopyranose). Since attachment of sugars to the polymer chains would, of course, inhibit association into intermolecular junctions, and thus partially offset the effect of reduction in water content, the anticipated order of effectiveness follows the sequence observed experimentally (fructose < sucrose < glucose). Evidence of similar



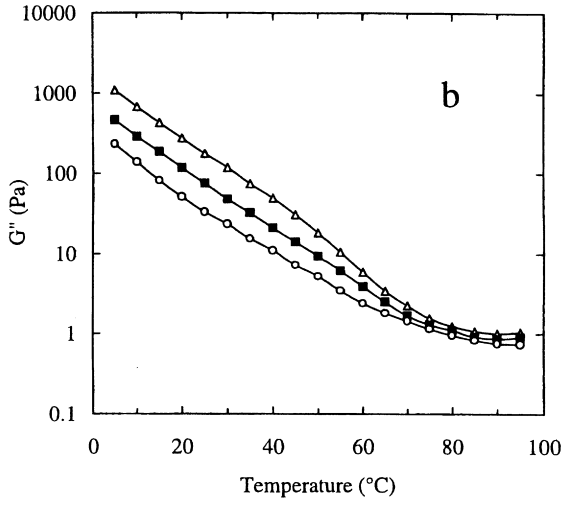
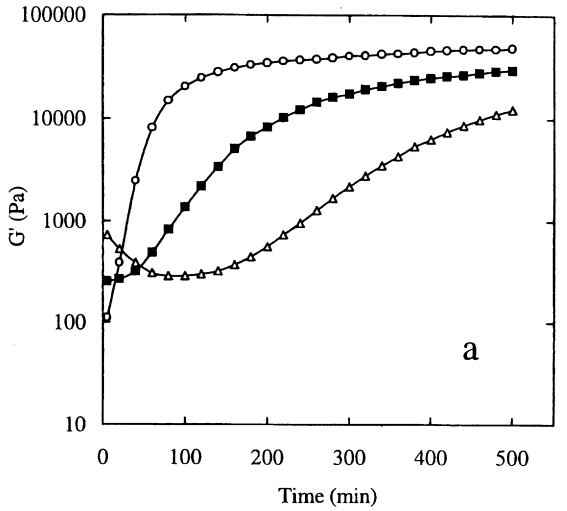


Fig. 16. Changes in (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain) during cooling from 95 to 5°C at 1°C/min for 30 wt% oxidised starch in mixtures with sucrose (△), glucose (■) or fructose (○) at a concentration of 36.84 wt%.



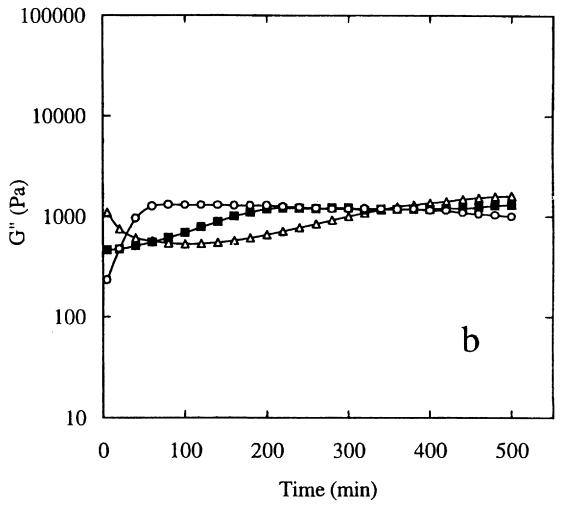


Fig. 17. Changes in (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain) during holding at 5°C after cooling from 95°C at 1°C/min for 30 wt% oxidised starch in mixtures with sucrose (△), glucose (■) or fructose (○) at a concentration of 36.84 wt%.

behaviour has been seen for hyaluronate (Nakamura, 2000) and high acyl gellan (Tsoga, Richardson and Morris, unpublished), both of which have a high content of substituents in which the polar carbonyl moiety is also present as an acceptor for hydrogen bonding.

As shown in Fig. 16, however, the rate of ordering of oxidised starch during cooling in the presence of equal concentrations of the same three sugars follows the normal sequence (fructose < glucose < sucrose). Thus, as might be anticipated from the very low content of carboxyl groups in oxidised starch (~1 per 30 residues), hydrogen bonding of these groups to the primary hydroxyl groups of the cosolute molecules appears to be swamped by water–cosolute interactions.

During subsequent holding at 5°C the order is reversed, with fructose causing a very rapid increase in G' (Fig. 17a) and sucrose having least effect. Thus, while sucrose seems very effective in accelerating conformational ordering (Figs. 8, 9a and 12), it appears to be far less effective in promoting the helix–helix aggregation step required for development of a continuous network. This could explain why increasing sucrose concentration at fixed concentration of starch causes little change in the time-course of gelation (Fig. 10), or in the moduli attained after holding for 500 min at 5°C (Fig. 9b).

The reversal of sequence for sucrose, glucose and fructose between the ordering and aggregation steps is puzzling. Similar behaviour has, however, been observed for other biopolymer systems, including xanthan (Frangou, Morris, Rees, Richardson & Ross-Murphy, 1982), where urea abolishes "weak gel" properties but displaces the order–disorder transition to higher temperature; *Rhizobium trifolii* capsular polysaccharide (Gidley, Eggleston & Morris, 1992), where partial debranching increases the stability of

the ordered structure but progressively eliminates gel formation; deacylated gellan (Morris, Richardson & Whittaker, 1999), where high concentrations of NaCl decrease the stability of individual double helices but enhance the stability of the helix–helix aggregates which crosslink the gel network; and κ-carrageenan (Norton, Morris & Rees, 1984), where the lyotropic effect of co-anions produces large changes in stability of the double helix, with correspondingly large, but converse, changes in the extent of helix–helix aggregation and associated thermal hysteresis.

It would therefore appear that in these systems, and in the oxidised starch–sugar systems studied in the present work, changes that enhance the stability of the individual ordered structures decrease their tendency to associate further into aggregated assemblies. Mechanistically, it is possible that aggregation requires subtle changes in helix geometry for optimum packing, and that these changes become more difficult as the stability of the isolated helices is increased. Assessment of the validity of this concept could be a worthwhile target for future research.

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