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Effect of oxidised starch on high methoxy pectin–sucrose gels formed by rapid quenching

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Abstract

The effect of progressive replacement of sucrose by oxidised starch in mixtures with high methoxy pectin (1.0 wt%; degree of esterification 70.3%; pH 3) has been characterised by low amplitude oscillatory measurements (1% strain) of storage modulus (G'), loss modulus (G''), loss tangent (tan δ) and complex dynamic viscosity (η^*) across the frequency range 0.1–100 rad s^{-1}. Samples were prepared at 90°C, and measured 1 h after rapid quenching to 5°C. In the main series of experiments, the total concentration of cosolute (sucrose plus oxidised starch) was held constant at 65 wt%, and the starch concentration was varied between 0 and 50 wt% (i.e. 65–15 wt% sucrose). The observed moduli showed an initial sharp decrease (particularly evident at low frequency) and subsequent steady increase with increasing concentration of starch. Critical gel spectra were recorded at ~9 wt% starch (56 wt% sucrose) and ~41 wt% starch (24 wt% sucrose). The moduli of the mixed systems at low starch concentration, before the loss of gel structure, remained close to those for 1.0 wt% pectin at the same sucrose concentrations but in the absence of starch, and at high starch concentrations became close to those of the starch component in the absence of pectin. In the intermediate region, however, the overall moduli were substantially (up to ~10×) higher than those of the individual constituents. The origin of this behaviour was explored by quantitative analysis of the results from a subsidiary series of experiments in which the sucrose concentration was held fixed at 50 wt% and the concentration of oxidised starch (in mixtures with 1.0 wt% pectin at pH 3) was varied between 0 and 15 wt%. The observed values of G' and G'' at 0.1, 1.0, 10 and 100 rad s^{-1} could be matched, to within experimental error, by values calculated on the basis of excluded volume effects within a monophasic solution, with the volume occupied by 1 g of oxidised starch corresponding to ~1.8 g of solvent and the volume occupied by 1 g of (partially associated) pectin corresponding to ~20 g.

Keywords: Pectin–sucrose gels; Structuring food products; Biopolymer co-gels

1. Introduction

The work reported here, and in the three following papers (Evageliou, Richardson & Morris, 1999a,b,c), formed part of an industrial–academic LINK project "Behaviour of biopolymer mixtures in structuring food products", with public-sector funding from the UK Ministry of Agriculture, Fisheries and Food. In addition to the Cranfield group, the project consortium comprised of two research groups in the University of York (Institute for Applied Biology and Department of Chemistry), two of the largest users of industrial biopolymers (Unilever and Nestlé), and three major producers (Cerestar, Hercules and SKW Biosystems). The research was focused on binary mixtures of three different types of biopolymer: partially-depolymerised starch (oxidised starch and maltodextrins), pectin (high methoxy and low methoxy) and gelatin (type A and type B), with the central aim of gaining a better understanding of the effect of segregative interactions ("thermodynamic incompatibility") in biopolymer co-gels.

The most striking incompatibility effects were seen for the networks formed by low methoxy pectin on cooling in the presence of Ca^{2+}. Preparations of calcium pectinate in the absence of polymeric cosolute show "weak gel" rheology at high temperature, with a sigmoidal transition to a "true" gel network on cooling. On progressive incorporation of oxidised starch, this increase in gel strength on cooling was systematically reduced, and ultimately replaced by sharp decreases in modulus during cooling at high starch concentrations (Picout, Richardson, Rolin, Abeysekera & Morris, 2000a; Picout, Richardson & Morris 2000b), indicating progressive collapse of the developing network in response to segregative interactions between the two polymers. Closely similar effects were observed (Picout, Richardson & Morris, 2000c,d) for mixtures of calcium

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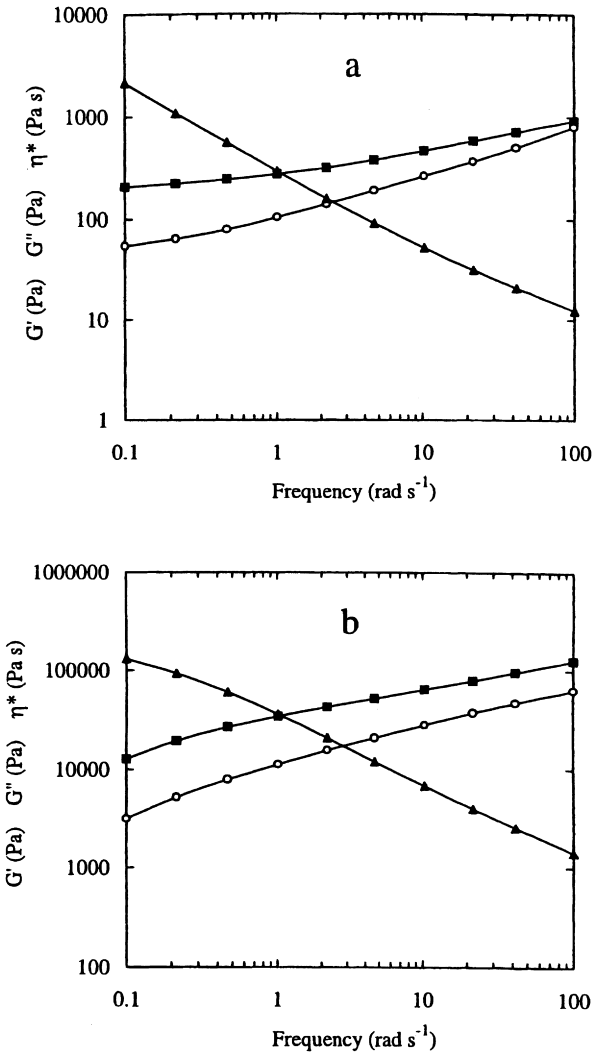

Fig. 1. Mechanical spectra (1% strain; 5°C) showing the frequency-dependence of: G' (■); G'' (○); and η^* (▲) for 1.0 wt% high methoxy pectin (pH 3) in the presence of: (a) 65 wt% sucrose; and (b) 15 wt% sucrose plus 50 wt% oxidised starch.

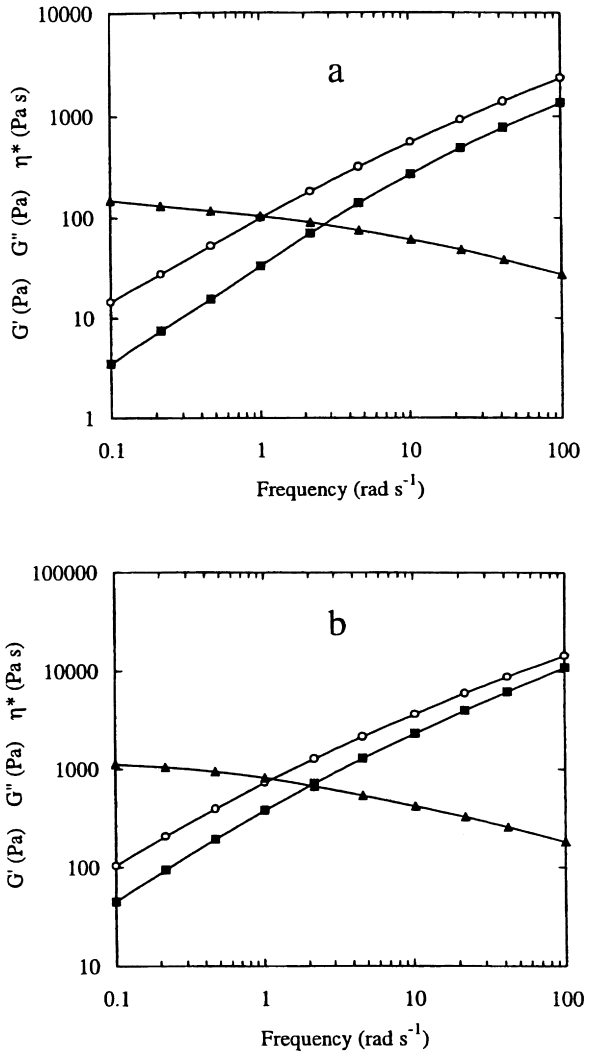

Fig. 2. Mechanical spectra (1% strain; 5°C) showing the frequency-dependence of G' (■); G'' (○); and η^* (▲) for 1.0 wt% high methoxy pectin (pH 3) in the presence of: (a) 15 wt% oxidised starch plus 50 wt% sucrose; and (b) 36 wt% oxidised starch plus 29 wt% sucrose.

pectinate with potato maltodextrin. In mixtures with gelatin, by contrast, the calcium pectinate network remained intact, giving a bicontinuous co-gel on gelation of the gelatin component (Gilsenan, Richardson & Morris, 2000a,b). Addition of salt to promote phase separation in the pre-gel state, however, gave co-gels with a continuous gelatin network and a dispersed phase of calcium pectinate. Mixtures of gelatin with potato maltodextrin also gave biphasic gels, with changes in phase continuity in response to changes in composition and/or cooling rate (Alevisopoulos, Kasapis & Abeysekera, 1996) and to application of shear during setting (Foster, Brown & Norton, 1996). Similar biphasic networks were obtained for gelatin in combination with oxidised starch (Roberts, Richardson & Morris, 2000), with analysis of co-gel moduli suggesting more complete segregation of the constituent polymers than in the pre-gel solution state.

The present investigation focuses on one of the remaining combinations, high methoxy pectin in mixtures with oxidised starch, holding pectin concentration fixed at 1.0 wt% and inducing gel formation by a rapid reduction in temperature (quenching). In the study reported in the following paper (Evageliou, Richardson & Morris, 2000a), a lower pectin concentration was used (0.5 wt%), samples were cooled at a fixed rate (1°C min^{-1}), and comparison was made between oxidised starch and potato maltodextrin as polymeric cosolute. During the course of these investigations of the mixed systems, we encountered some interesting and unexpected features of the gelation of the individual components, which are reported separately for high methoxy pectin (Evageliou, Richardson & Morris, 2000b), and oxidised starch (Evageliou, Richardson & Morris, 2000c).

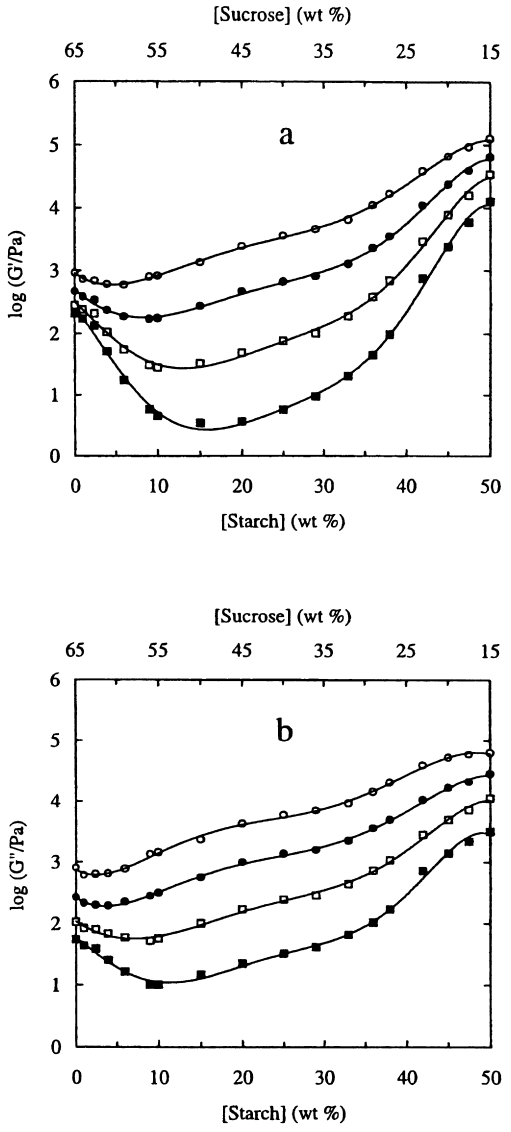

Fig. 3. Effect of progressive replacement of sucrose by oxidised starch in mixtures with 1.0 wt% high methoxy pectin (pH 3; 5°C; total concentration of cosolute 65 wt%). Values are shown for: (a) G'; and (b) G'' measured at frequencies (rad s^{-1}) of 0.1 (■); 1.0 (□); 10 (●); and 100 (○).

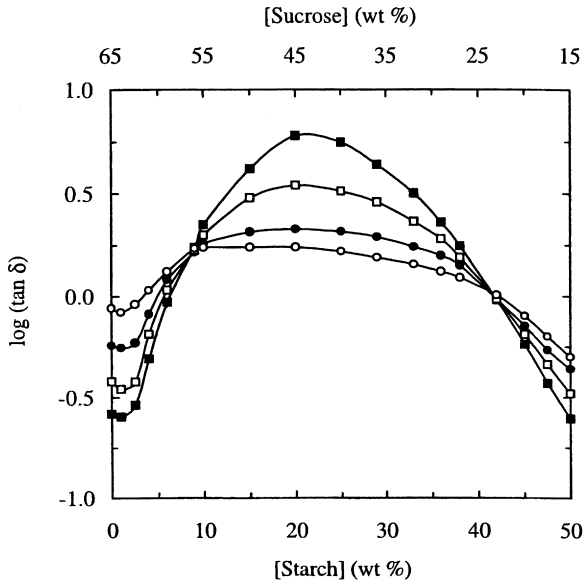

Fig. 4. Changes in tan δ, measured at frequencies (rad s^{-1}) of 0.1 (■); 1.0 (□); 10 (●); and 100 (○), on progressive replacement of sucrose by oxidised starch in mixtures with 1.0 wt% high methoxy pectin (pH 3; 5°C; total concentration of cosolute 65 wt%).

2. Materials and methods

Oxidised starch (modified maize starch C ∗ Set 06598 from Cerestar) was from the same batch (SH 1338) as used in the work on mixtures with gelatin (Roberts et al., 2000) and low methoxy pectin (Picout et al., 2000a,b). This material was developed primarily as an extender for gum arabic in the manufacture of confectionery gums and pastilles, and can be dissolved at concentrations up to ~50 wt%. It contains approximately one carboxyl group per 30 glucose residues. The high methoxy pectin sample used was a laboratory preparation from the Copenhagen Pectin Division of Hercules (sample number X-4938), and was supplied with the following analytical information: content of pure pectin = 97.7%, of which 85.1% is galacturonate with a degree of methyl esterification of 70.3%. Distilled deionised water was used throughout.

Pectin was used at a fixed concentration of 1.0 wt% and fixed pH of 3.0 (controlled by addition of trisodium citrate dihydrate; AnalaR grade from BDH). In the main series of experiments, the total concentration of cosolute used was 65 wt%, comprising complementary amounts of sucrose and oxidised starch (ranging from 65 wt% sucrose, 0% starch to 15 wt% sucrose, 50 wt% starch). First, the starch was dispersed in slightly more than the total amount of water required for the mixed system, and dissolved by mechanical stirring in a water bath at 90°C. The required quantities of pectin, sucrose and trisodium citrate were then added, with continuous stirring until a clear solution was obtained, and the mixture was brought to the correct total weight by addition of water or continued evaporation, as appropriate. Comparative studies were made using 1.0 wt% pectin in the absence of starch, at sucrose concentrations between 65 and 45 wt%, and starch (20–50 wt%) in the absence of pectin, with incorporation of the sucrose required to maintain the total concentration at 65 wt% (i.e. 45–15 wt% sucrose). In a subsidiary series of experiments, the sucrose concentration was held fixed at 50 wt%; mixtures were again prepared at a pectin concentration of 1.0 wt%, with starch concentrations in the range 0–15 wt%, and comparisons were made with pectin (0.7–2.0 wt%) in the absence of starch, and with starch (7–20 wt%) in the absence of pectin.

Samples (at 90°C) were loaded onto an oscillatory

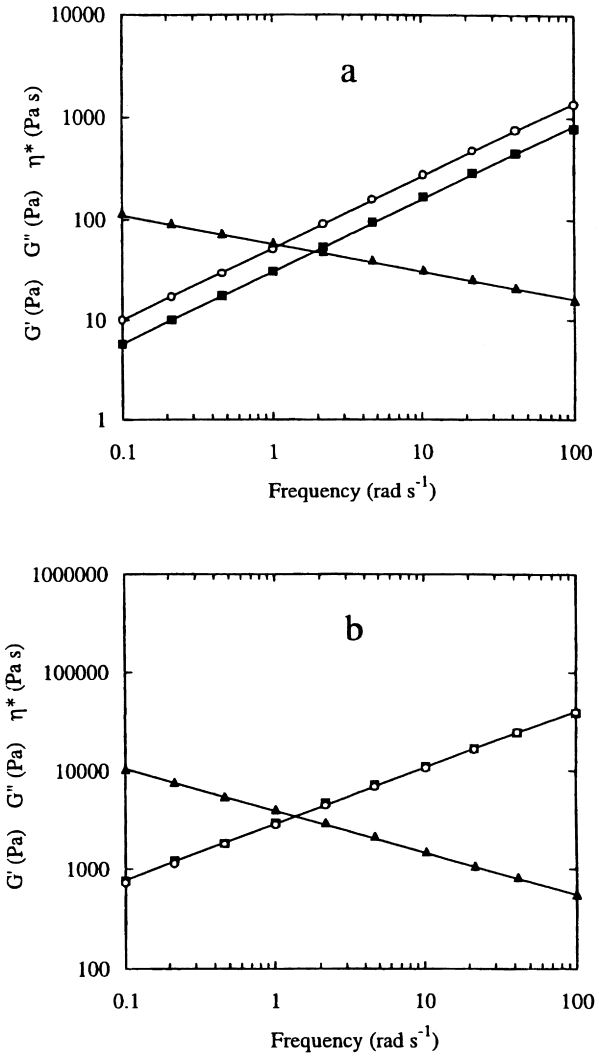

Fig. 5. Mechanical spectra (1% strain; 5°C) showing the frequency-dependence of G' (■); G'' (○); and η^* (▲) for 1.0 wt% high methoxy pectin (pH 3) in the presence of: (a) 9 wt% oxidised starch plus 56 wt% sucrose; and (b) 42 wt% oxidised starch plus 23 wt% sucrose.

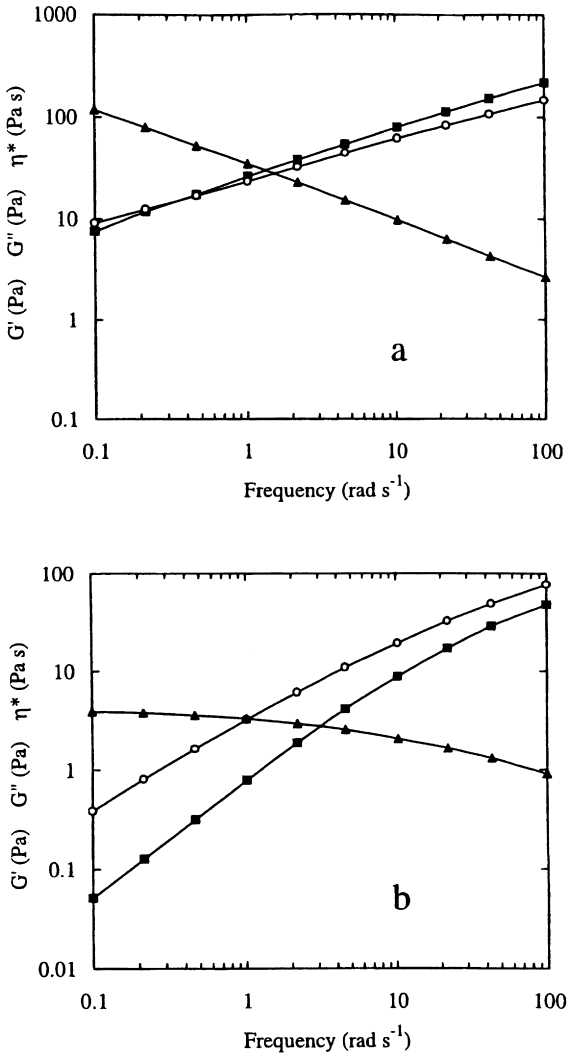

Fig. 6. Mechanical spectra (1% strain; 5°C) showing the frequency-dependence of G' (■); G'' (○); and η^* (▲) for 1.0 wt% high-methoxy pectin (pH 3) in the absence of starch at sucrose concentrations of: (a) 55 wt%; and (b) 45 wt%.

rheometer pre-heated to ~40°C, and were then quenched to 5°C. After 1 h at 5°C, their rheology was characterised by small deformation oscillatory measurements (1% strain) of storage modulus (G'), loss modulus (G''), loss tangent ($\tan\delta = G''/G'$) and complex dynamic viscosity ($\eta^* = (G'^2 + G''^2)^{1/2}/\omega$) over the frequency range $\omega = 0.1$–100 rad s^{-1}. Most of the 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.), with temperature control by a Haake circulating water bath. At the highest starch concentrations (45–50 wt%), however, the moduli exceeded the (6-decade) dynamic range of this instrument, and measurements were made using parallel-plate geometry (40 mm diameter; 1 mm separation) on a Carri-Med CSL500 rheometer.

3. Results

In normal commercial practice, the concentration of sucrose used to induce gelation of high methoxy pectin at acid pH is typically ~65 wt% (Christiansen, 1986; Rolin, 1993). In formulating the mixed systems studied in the present work, however, it was obviously impossible to introduce high concentrations of oxidised starch (in combination with 1.0 wt% pectin at pH 3) while still maintaining the sucrose concentration at this normal value. Instead, the procedure adopted in the main series of experiments was to hold the total concentration of cosolute fixed at 65 wt% by reducing the sucrose concentration from 65 to 15 wt% as the starch concentration was raised from 0 to 50 wt%.

Fig. 1 shows the mechanical spectra recorded (1 h after rapid quenching to 5°C) for compositions at the extremes of this range (i.e. 1.0 wt% pectin with 65 wt% sucrose, or with

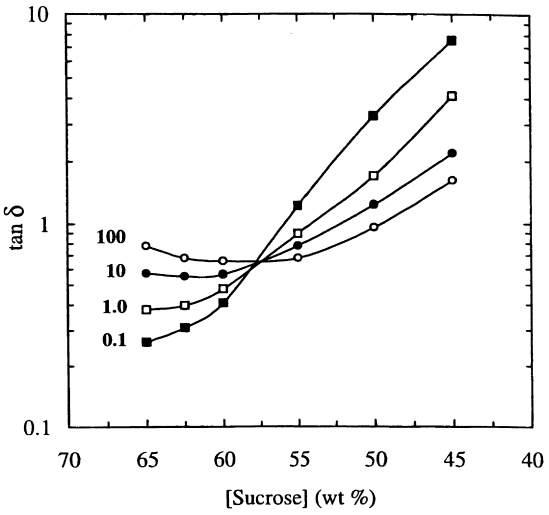

Fig. 7. Effect of sucrose concentration on observed values of tan δ (1% strain; 5°C), measured at frequencies (rad s^{-1}) of 0.1 (■); 1.0 (□); 10 (●); and 100 (○), for 1.0 wt% high methoxy pectin (pH 3) in the absence of starch.

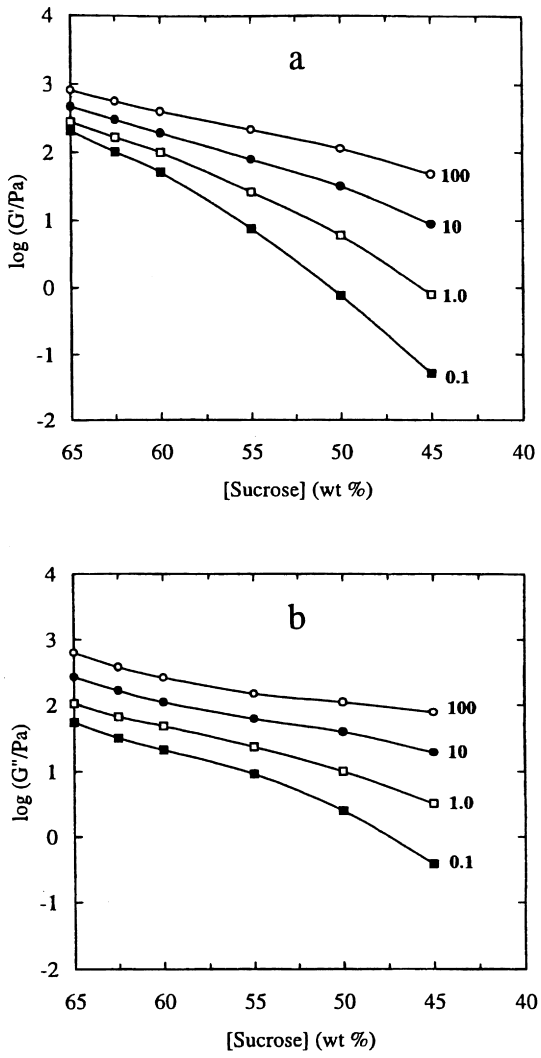

Fig. 8. Effect of sucrose concentration on observed values (1% strain; 5°C) of (a) G' and (b) G'', measured at frequencies (rad s^{-1}) of 0.1 (■); 1.0 (□); 10 (●); and 100 (○), for 1.0 wt% high methoxy pectin (pH 3) in the absence of starch.

15 wt% sucrose plus 50 wt% oxidised starch). In both cases, the overall response is gel-like ($G' > G''$), although the moduli show greater frequency-dependence than is usual for biopolymer gels (see, for example, Ross-Murphy, 1984), and the separation between G' and G'' decreases with increasing frequency, indicating a substantial "sol fraction" within the gel network. At intermediate compositions of cosolute, however, the spectra become typically solution-like, as illustrated in Fig. 2 for starch concentrations of 15 and 36 wt% (in combination with, respectively, 50 and 29 wt% sucrose). Viscous response predominates ($G'' > G'$) and the frequency-dependence of η^* is far smaller than in the gel spectra shown in Fig. 1.

The variation of G' and G'' with increasing concentration of starch (and decreasing concentration of sucrose) is shown in Fig. 3 for four frequencies of oscillation spanning the range used (at $\omega = 0.1$, 1.0, 10 and 100 rad s^{-1}). At high frequency, where transient entanglements between branched, disordered chains of oxidised starch would be expected to make a substantial contribution to the overall rheological response, there is an essentially monotonic increase in both moduli with increasing starch concentration. The gross changes in network character evident in Figs. 1 and 2, however, become increasingly apparent at lower frequencies, where there is sufficient time for entanglements to come apart within the period of oscillation. At the lowest accessible frequency (0.1 rad s^{-1}) there is a drastic (approximately 100-fold) decrease in G' (Fig. 3a) as the starch concentration is raised from 0 to ~10 wt%, with a smaller (~10-fold) accompanying decrease in G'' (Fig. 3b). On further increase in starch concentration, there is then a smooth rise in both moduli, but with a substantial increase in slope above ~35 wt%.

The corresponding variations in tan δ (G''/G') are shown in Fig. 4. As found for the individual moduli (Fig. 3), the composition-dependent changes become more apparent at lower frequencies. At two points in the composition range the plots of tan δ versus starch concentration for different frequencies of oscillation converge and cross (at ~9 and ~42 wt% starch). The mechanical spectra at these values of cosolute composition (where tan δ is independent of frequency) are shown in Fig. 5. In both cases, log G' and log G'' vary linearly with log ω across the full three decades of frequency accessible, and have a common slope (~0.65 at 9 wt% starch and ~0.60 at 42 wt%). Such behaviour is characteristic of a critically crosslinked network (Durrand, Delsanti, Adam & Luck, 1987; te Nijenhuis & Winter, 1989). At 42 wt% starch (Fig. 5), the absolute values of G' and G'' are also closely coincident (i.e. tan$\delta \approx 1$).

Thus, on progressive replacement of sucrose by oxidised starch, network continuity is lost when the starch content

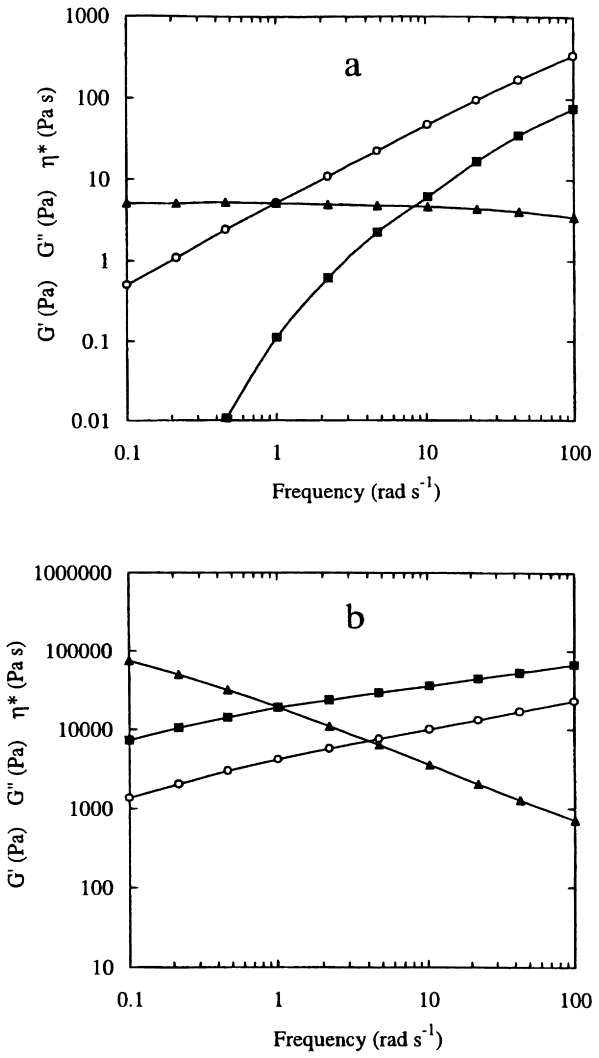

Fig. 9. Mechanical spectra (1% strain; 5°C) showing the frequency-dependence of G' (■); G'' (○); and η^* (▲) for: (a) 10 wt% oxidised starch with 55 wt% sucrose and (b) 50 wt% oxidised starch with 15 wt% sucrose, in the absence of pectin.

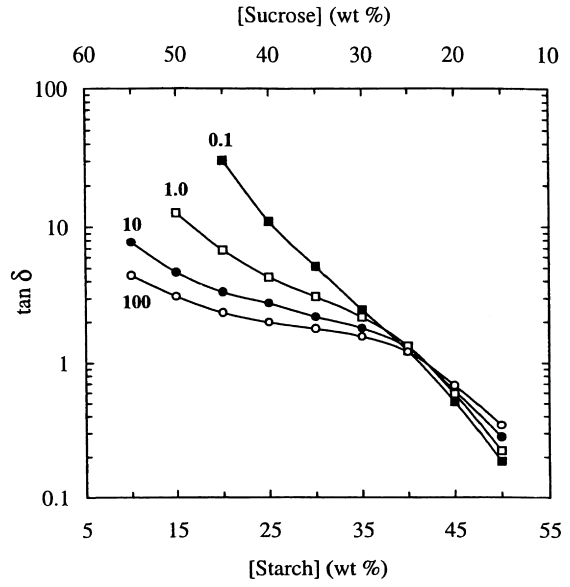

Fig. 10. Composition-dependence of tan δ (1% strain; 5°C), measured at frequencies (rad s^{-1}) of 0.1 (■); 1.0 (□); 10 (●); and 100 (○), for mixtures of surcrose and oxidised starch at a total concentrations of 65 wt%, in the absence of pectin.

reaches ~9 wt% (with 56 wt% sucrose), and re-established at ~42 wt% (with 23 wt% sucrose). Formation of a continuous network at high concentrations of oxidised starch can obviously be attributed to gelation of the starch component. Loss of the initial high methoxy pectin network, however, could in principle be due either to the increasing content of oxidised starch or to the decreasing content of sucrose. This was tested by examining the effect of changes in sucrose concentration in the absence of starch.

The rheological response of 1.0 wt% high methoxy pectin (pH 3) in the presence of 65 wt% sucrose (Fig. 1a) is predominantly elastic ($G' > G''$). On reduction in sucrose concentration to 55 wt%, some gel-like character is preserved (Fig. 6a), but the mechanical spectrum obtained on further decrease to 45 wt% sucrose (Fig. 6b) is similar to that of a normal polysaccharide solution. As shown in Fig. 7, the critical gel point (characterised as the composition at which tan δ is independent of frequency) comes at ~57 wt% sucrose, which is close to the sucrose concentration of ~56 wt% at which gel-like character is lost in the mixed systems with oxidised starch (Fig. 4). The variation of G' and G'' with sucrose concentration (65–45 wt%) is shown in Fig. 8. As found for the mixed systems (Fig. 3), the decrease in modulus with decreasing concentration of sucrose increases in magnitude with decreasing frequency of oscillation, and is greater for G' (Fig. 8a) than for G'' (Fig. 8b).

As a further comparison between the mixed systems and the individual constituents, the rheology of oxidised starch (10–50 wt%) was characterised for mixtures with sucrose at a total concentration of 65 wt% (i.e. 55–15 wt% sucrose), but in the absence of pectin. The mechanical spectra recorded at the extremes of this range (Fig. 9) show the expected change from solution-like response at low starch concentration (10 wt%; Fig. 9a) to gel-like character at high concentration (50 wt%; Fig. 9b). As shown in Fig. 10, the critical gel point (tan δ independent of frequency) is reached at ~40 wt% starch (plus 25 wt% sucrose), which is again in reasonable agreement with the starch concentration of ~42 wt% at which gel-like character is re-established in the mixed systems with pectin (Fig. 4). The concentration-dependence of G' and G'' at 0.1, 1.0, 10 and 100 rad s^{-1} is shown in Fig. 11. As found for the mixed systems (Fig. 3) and for pectin in the absence of starch (Fig. 8), the composition-dependent changes in moduli become progressively more pronounced with decreasing frequency.

Fig. 12 shows a direct comparison of G' (at 0.1, 1.0, 10 and 100 rad s^{-1}) for the mixed systems (Fig. 3a) with the corresponding values for the starch–sucrose preparations

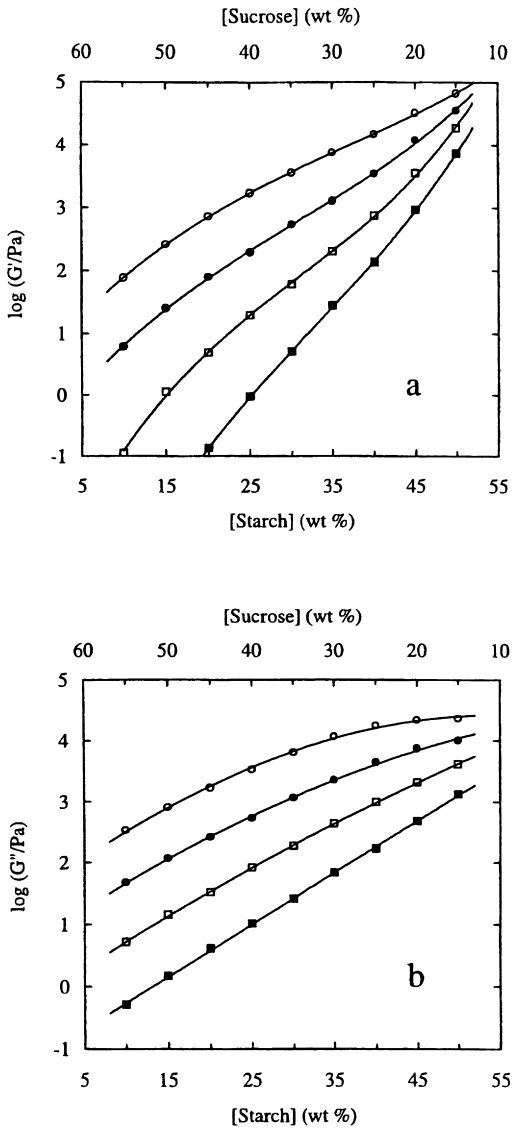

Fig. 11. Composition-dependence of (a) G' and (b) G'' (1% strain; 5°C), measured at frequencies (rad s^{-1}) of 0.1 (■); 1.0 (□); 10 (●); and 100 (○), for mixtures of sucrose and oxidised starch at a total concentrations of 65 wt%, in the absence of pectin.

(with no pectin) and for the pectin–sucrose samples (with no starch). The steep reduction in G' at 0.1 rad s^{-1} (Fig. 12a) as the starch concentration in the mixed systems is increased from 0 to ~10 wt% is matched closely by the corresponding reduction in G' for pectin in the absence of starch as the sucrose concentration is reduced from 65 to 55 wt%. At higher starch (lower sucrose) concentrations the G' values for the mixed systems then rise above the corresponding values for pectin alone, with the divergence occurring at progressively lower starch concentrations as the frequency is raised from 0.1 rad s^{-1} (Fig. 12a) to 100 rad s^{-1} (Fig. 12d). At high starch concentrations (above ~30 wt%), the values of G' for the mixed systems (at all frequencies) run parallel to those for starch in the absence of pectin, but are systematically higher (by ~50%). A qualitatively similar pattern was observed for G'' (and, therefore, for η^*).

It seems evident from Fig. 12 that the initial reduction in moduli on partial replacement of sucrose by oxidised starch arises (at least predominantly) from the reduction in sucrose concentration rather than from the presence of starch, and that the subsequent increase is due predominantly to the increase in starch concentration, but enhanced by the presence of pectin. The origin of the overall rheology at intermediate starch concentrations, where the moduli of the mixed systems exceed those of the individual components by at least an order of magnitude, however, is less obvious.

Detailed analysis of the mixed systems discussed so far, where the concentrations of starch and sucrose were varied simultaneously, would be virtually impossible. Instead, we carried out a subsidiary series of experiments in which the concentration of sucrose was held fixed and only the polymer concentrations were varied. As shown in Fig. 12, the maximum deviation between the observed moduli for the mixed systems and the individual constituents occurs at ~46 wt% sucrose (19 wt% starch) for measurements made at 0.1 rad s^{-1} (Fig. 12a), increasing to ~53 wt% (12 wt% starch) at 100 rad s^{-1} (Fig. 12b). As a compromise, the sucrose concentration chosen for quantitative comparison of the rheology of the mixed systems and the individual constituents was 50 wt%. Data analysis was carried out using the in-built curve fitting and minimisation routines on a Microsoft Excel spreadsheet package (Version 5.0).

The first step in the analysis was to obtain calibration curves of modulus versus concentration (c) for the individual polymers in 50 wt% sucrose. The pectin concentrations used were 0.7, 1.0, 1.4 and 2.0 wt%. Fig. 13 shows the mechanical spectra recorded at the extremes of this range. The spectrum obtained for 0.7 wt% high methoxy pectin (pH 3) in 50 wt% sucrose (Fig. 13a) is similar in form to that of a normal polysaccharide solution. At 2.0 wt% pectin, the overall response (Fig. 13b) is predominantly elastic ($G' > G''$), but with much greater frequency-dependence and far smaller separation of the individual moduli (i.e. greater $\tan\delta$) than for an extensively crosslinked gel network.

Fig. 14 shows the concentration-dependence of G' (Fig. 14a) and G'' (Fig. 14b) at 0.1, 1.0, 10 and 100 rad s^{-1}, plotted on logarithmic axes. All eight curves could be fitted, with good precision, by a second-order polynomial relationship of the general form:

$$A = p(2)B^2 + p(1)B + p(0) \quad (1)$$

where $A = \log(G'/\text{Pa})$ or $\log(G''/\text{Pa})$ and $B = \log(c/\text{wt\%})$. The polynomial parameters used are listed in Table 1 and the fitted curves are shown by the solid lines in Fig. 14.

The concentrations of oxidised starch used for calibration were 7, 10, 15, and 20 wt%. The mechanical spectra

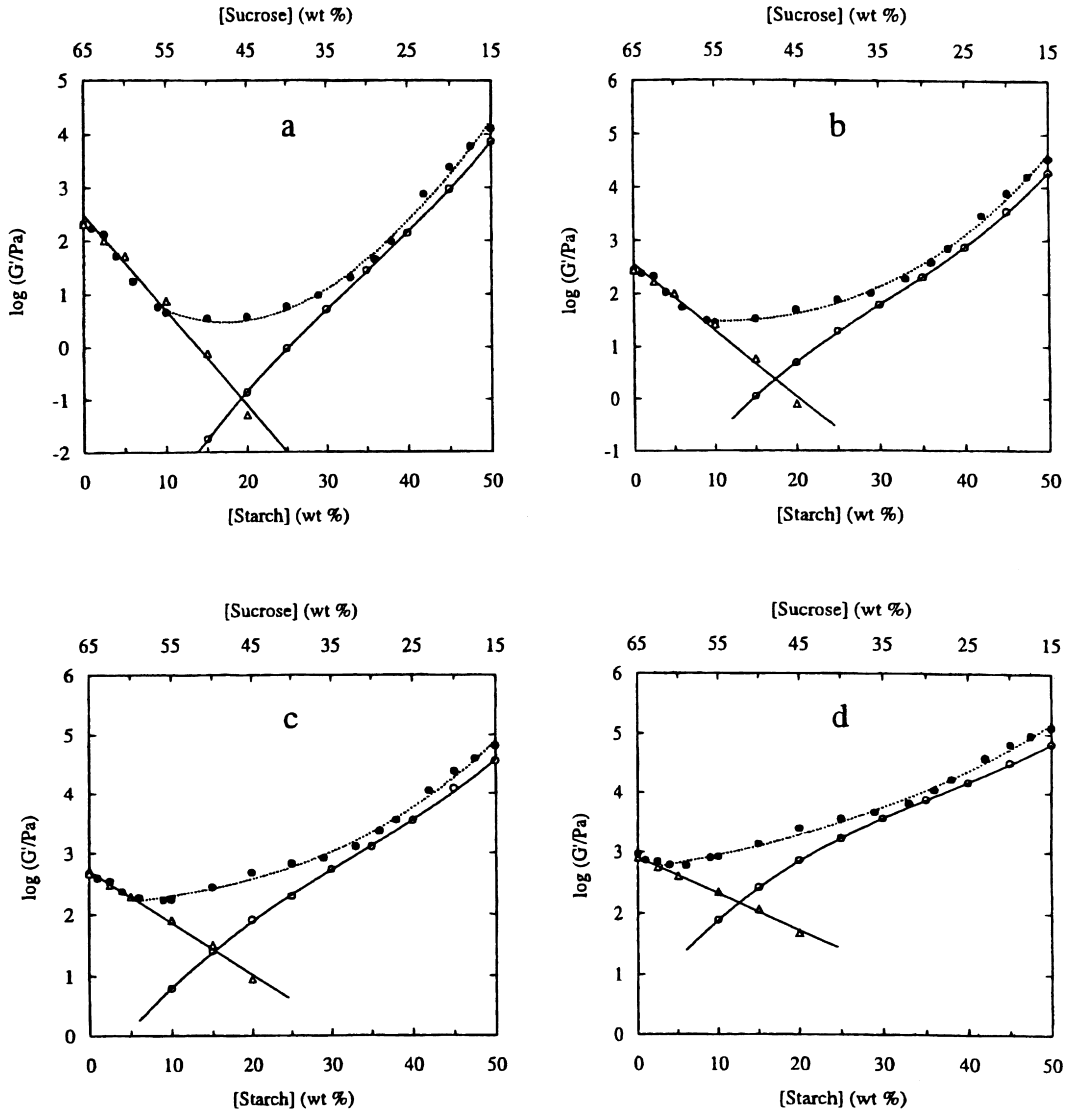

Fig. 12. Comparison of the composition-dependence of G' (1% strain; 5°C), measured at frequencies (rad s^{-1}) of: (a) 0.1; (b) 1.0; (c) 10; and (d) 100, for mixtures of 1.0 wt% high methoxy pectin (pH 3) with sucrose and oxidised starch at a combined concentration of 65 wt% (●); 1.0 wt% pectin (pH 3) with varying concentrations of sucrose in the absence of starch (△); and mixtures of oxidised starch and sucrose at a combined concentration of 65 wt% in the absence of pectin (○).

recorded at the extremes of this range (Fig. 15) are both solution-like ($G'' > G'$ across the accessible frequency range). As shown in Fig. 16, the variation of log G' (Fig. 16a) and log G'' (Fig. 16b) with starch concentration across the range studied (7–20 wt%) is essentially linear at all frequencies accessed, allowing the concentration-dependence of modulus to be expressed by a relationship of the form:

$$A = p(1)c + p(0) \quad (2)$$

where $A = log(G'/\text{Pa})$ or $\log(G''/\text{Pa})$, c is starch concentration (wt%), and $p(1)$ and $p(0)$ denote, respectively, the slope and intercept of the line of best (root-mean-square) fit. The values of $p(1)$ and $p(0)$ for log G' and log G'' at 0.1, 1.0, 10 and 100 rad s^{-1} are listed in Table 1.

In the mixed systems prepared in 50 wt% sucrose, the pectin concentration was held constant at 1.0 wt% (pH 3), as in the main series of experiments (Figs. 3 and 4), and the concentration of oxidised starch was varied between 0 and 15 wt%. The upper end of this range corresponds to one of the samples in the main series (i.e. with a total cosolute concentration of 65 wt%). As shown in Fig. 2a, the rheological response for this composition is predominantly viscous ($G'' > G'$). At the opposite extreme (1.0 wt% pectin in 50 wt% sucrose, with no added starch), the mechanical spectrum (Fig. 17a) is also solution-like. In the centre of the composition range, however, the spectra approach (but do not reach) the form typical of a critically-crosslinked gel. This is illustrated in Fig. 17b for the sample prepared with 10 wt% oxidised starch (in combination with 1.0 wt% pectin and 50 wt% sucrose). The curvature of the individual plots of log G' and log G'' versus log ω is far less

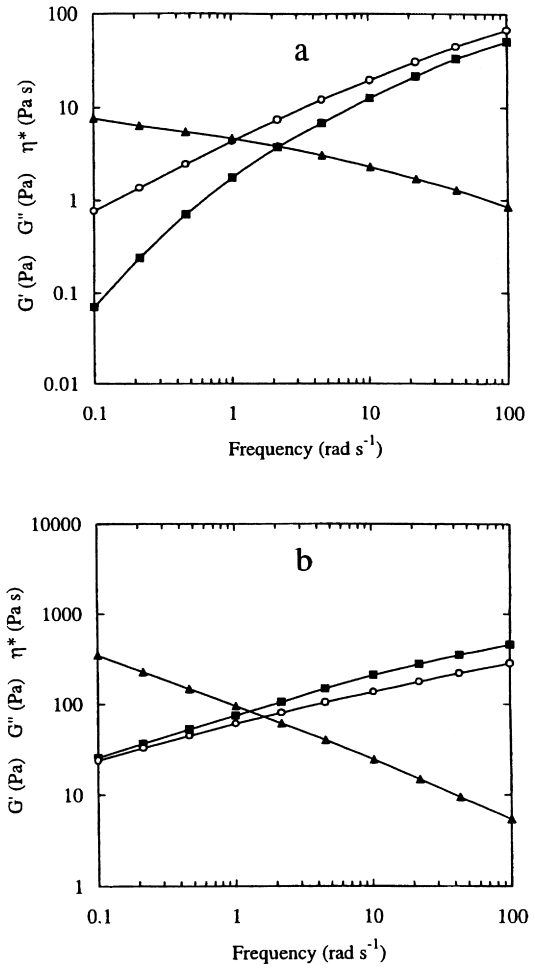

Fig. 13. Mechanical spectra (1% strain; 5°C) showing the frequency-dependence of G' (■); G'' (○); and η^* (▲) for (a) 0.7 wt% and (b) 2.0 wt% high methoxy pectin (pH 3) in 50 wt% sucrose.

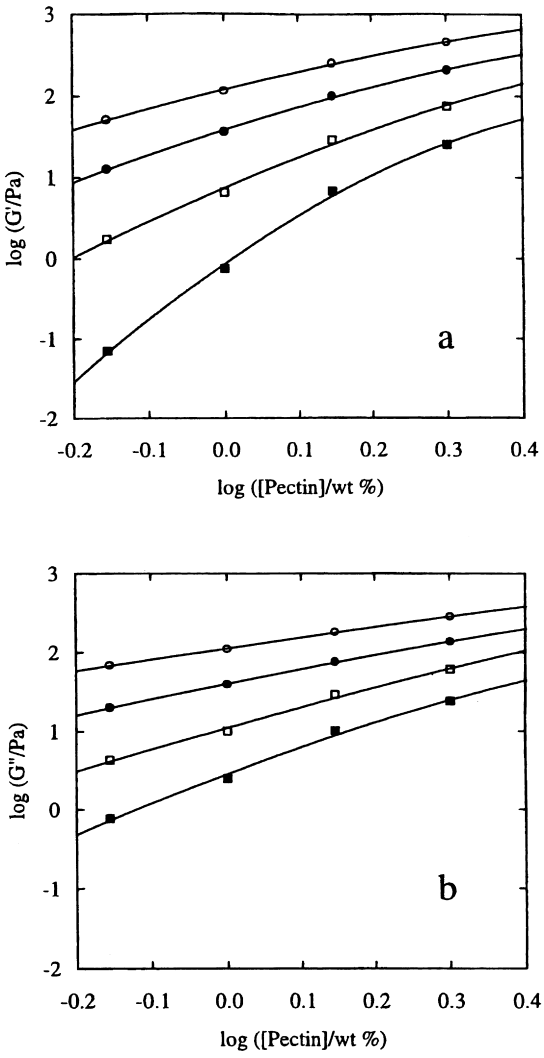

Fig. 14. Concentration-dependence of (a) G' and (b) G'' (1% strain; 5°C), measured at frequencies (rad s^{-1}) of 0.1 (■); 1.0 (□); 10 (●); and 100 (○), for high methoxy pectin (pH 3) in 50 wt% sucrose.

pronounced than for the samples with 0 or 15 wt% starch, and the two traces are roughly parallel.

The initial enhancement and subsequent reduction in gel-like character with increasing concentration of oxidised starch is shown more explicitly in Fig. 18, where tan δ at 0.1, 1.0, 10 and 100 rad s^{-1} is plotted against starch concentration. At high frequency (100 rad s^{-1}), where the overall response is likely to be dominated by topological entanglement, there is a monotonic increase in tan δ as the concentration of oxidised starch is raised. At low frequency (0.1 rad s^{-1}), by contrast, the increase in tan δ (i.e. in overall liquid-like response) at high starch concentration is preceded by a sharp decrease, consistent with some enhancement of the pectin network in response to segregative interactions with oxidised starch. Throughout the composition range studied, however, the rheological response at all frequencies (0.1–100 rad s^{-1}) is predominantly solution-like (tan $\delta > 1$).

Fig. 19 shows the concentration-dependence of solution rheology for oxidised starch in 50 wt% sucrose, in direct comparison with that of the mixtures with 1.0 wt% high methoxy pectin, using η^* as an index of overall response to oscillatory shear. At high frequency (100 rad s^{-1}; Fig. 19d) the increase in η^* with increasing starch concentration in the mixed systems runs roughly parallel to that of oxidised starch in the absence of pectin, again indicating a dominant contribution from topological entanglement of disordered starch. At low frequency (0.1 rad s^{-1}; Fig. 19a), the mixtures show a significant enhancement in η^* at starch concentrations where the corresponding values for oxidised starch alone are more than an order of magnitude lower, suggesting that the increase arises predominantly from the pectin component. These qualitative interpretations of overall response (as characterised by η^*) were explored further by quantitative analysis of the constituent moduli (G' and G'').

A common outcome of segregative interactions between two different biopolymers in the solution state is for the system to resolve into two separate phases, each enriched in one

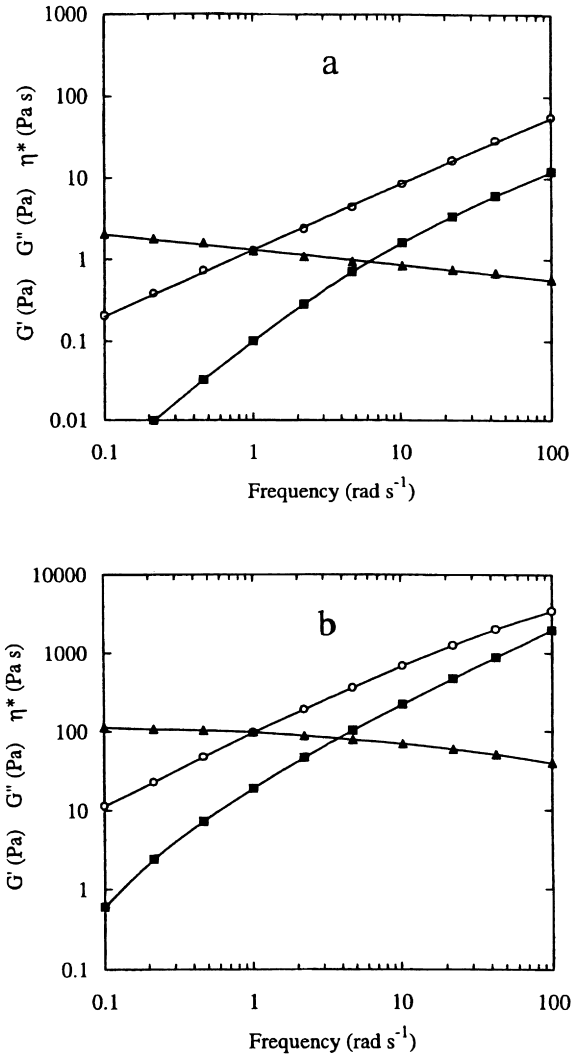

Fig. 15. Mechanical spectra (1% strain; 5°C) showing the frequency-dependence of G' (■); G'' (○); and η^* (▲) for (a) 7 wt% and (b) 20 wt% oxidised starch in 50 wt% sucrose.

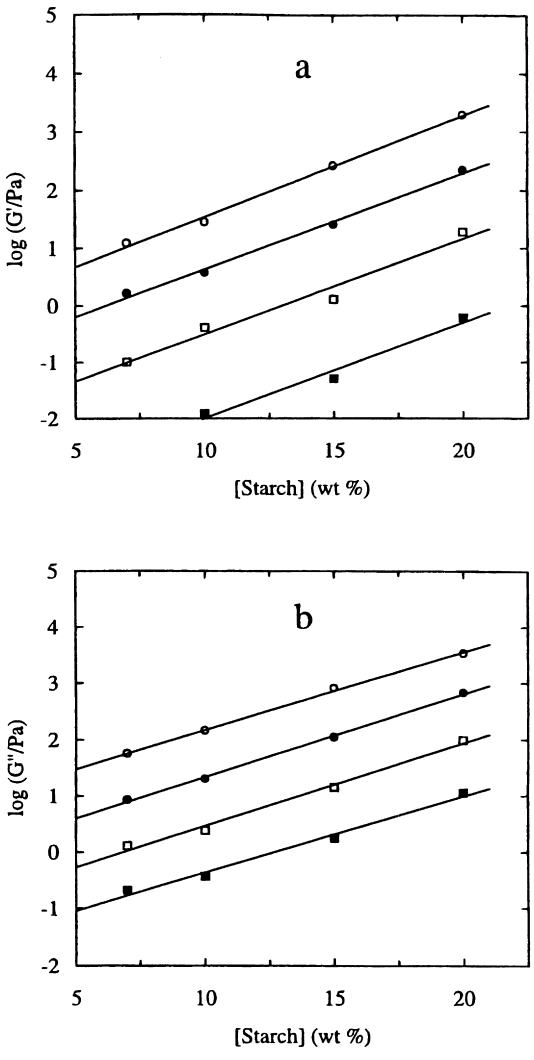

Fig. 16. Concentration-dependence of (a) G' and (b) G'' (1% strain; 5°C), measured at frequencies (rad s^{-1}) of 0.1 (■); 1.0 (□); 10 (●); and 100 (○), for oxidised starch in 50 wt% sucrose.

polymer and depleted in the other (e.g. Piculell et al., 1994; Tolstoguzov, 1986). Normally, one phase forms a continuous matrix with the other dispersed through it as a "water-in-water emulsion". The droplet size for the dispersed phase is typically in the range 10–100 μm, allowing the phase structure to be visualised by light microscopy (e.g. Abeysekera & Robards, 1995; Clark, Richardson, Ross-Murphy & Stubbs, 1983).

Table 1
Polynomial parameters for the concentration-dependence of modulus for high methoxy pectin Eq. (1) and oxidised starch Eq. (2) in 50 wt% sucrose

Modulus	Frequency (rad s^{-1})	Pectin: log G vs. log c			Starch: log G vs. c	
		$p(2)$	$p(1)$	$p(0)$	$p(1)$	$p(0)$
G'	0.1	−3.144	6.194	−0.105	0.170	−3.70
	1.0	−1.708	3.909	0.869	0.167	−2.17
	10	−1.284	2.897	1.577	0.166	−1.03
	100	−1.028	2.261	2.077	0.174	−0.20
G''	0.1	−1.524	3.576	0.455	0.136	−1.73
	1.0	−0.477	2.648	1.041	0.148	−1.01
	10	−0.416	1.910	1.600	0.147	−0.14
	100	−0.126	1.387	2.048	0.139	0.79

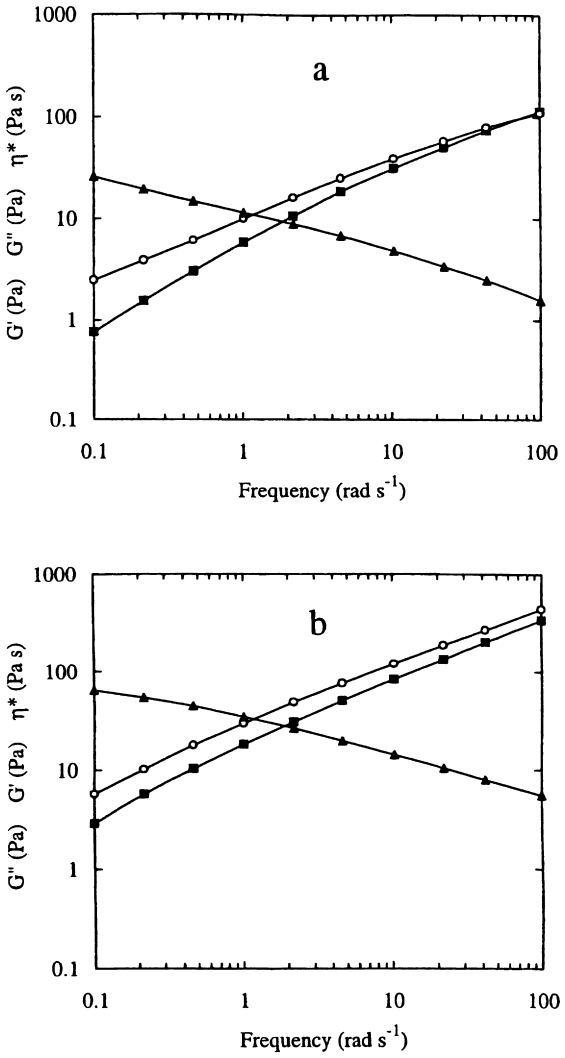

Fig. 17. Mechanical spectra (1% strain; 5°C) showing the frequency-dependence of G' (■); G'' (○); and η^* (▲) for 1.0 wt% high methoxy pectin (pH 3) in 50 wt% sucrose: (a) with no added starch; and (b) with 10 wt% oxidised starch. The corresponding spectrum for a starch concentration of 15 wt% is shown in Fig. 2a.

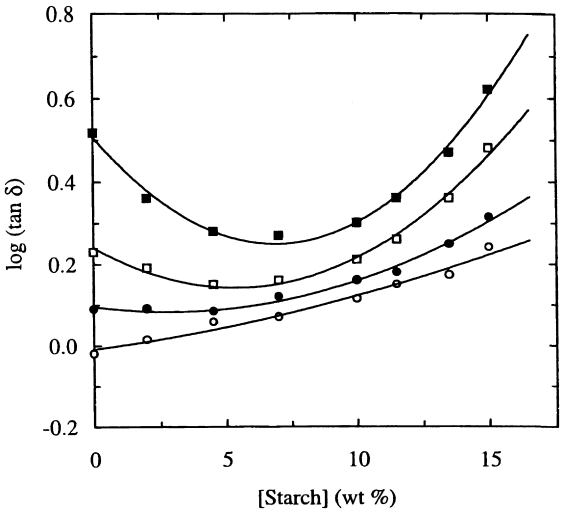

Fig. 18. Variation of tan δ (1% strain; 5°C), measured at frequencies (rad s^{-1}) of 0.1 (■); 1.0 (□); 10 (●); and 100 (○), with increasing concentration of oxidised starch in mixtures with 1.0 wt% high methoxy pectin (pH 3) in 50 wt% sucrose.

As part of the LINK project, selected samples from the series shown in Figs. 3 and 4 (i.e. 1.0 wt% pectin in the presence of sucrose and oxidised starch at a combined concentration of 65 wt%) were prepared by essentially the same procedure as used in the present work, and examined by bright-field microscopy, with iodine staining for starch (Abeysekera, Institute for Applied Biology, University of York, unpublished). At low concentrations of starch (i.e. high concentrations of sucrose) the micrographs were featureless, indicating homogeneous distribution of both polymers. As the sucrose concentration was decreased to ~60 wt% (5 wt% starch), the high methoxy pectin network became visible as a honeycomb structure, similar to that observed (Picout et al., 2000a) for "collapsed" networks of calcium pectinate in the presence of oxidised starch, with pores of diameter ~10 μm occupied by starch. At starch concentrations above ~10 wt% (i.e. sucrose concentrations below ~55 wt%), however, the micrographs became featureless again, indicating that the mixed solutions (and starch gels) obtained after loss of high methoxy pectin gel structure by reduction of sucrose concentration were monophasic.

The analysis of the observed moduli for the samples in 50 wt% sucrose was therefore based on the following working assumptions: (i) that both polymers are present in a single phase, (ii) that the overall moduli can therefore be equated to the sum of the moduli for the individual constituents (i.e. with each polymer making a simple, additive, contribution to the overall resistance to deformation), and (iii) that the effective concentration of each polymer is raised by mutual exclusion with the other, giving rise to the obvious non-additivity in Figs. 12 and 19.

This model was tested by attempting to fit the observed values of G' and G'' for the mixtures in 50 wt% sucrose by using two variable parameters, V_p and V_s, to characterise the volume occupied (i.e. made unavailable to the other polymer) by, respectively, 1 g of pectin and 1 g of oxidised starch. For simplicity, the calculations were carried out on a weight basis, so that, strictly, V_p and V_s denote the mass of solvent (50 wt% sucrose) in the excluded volume for each 1 g of the individual polymers. Thus the effective concentrations (wt%) of pectin and starch, [P] and [S], are related to the nominal concentrations, $[P]_0$ and $[S]_0$, by:

$$[P] = \{[P]_0 \times 100\}/\{100 - ([S]_0 \times V_s)\} \quad (3)$$

$$[S] = \{[S]_0 \times 100\}/\{100 - ([P]_0 \times V_p)\} \quad (4)$$

The values of G' and G'' at these concentrations can then be determined from the calibration curves in Figs. 14 and 16

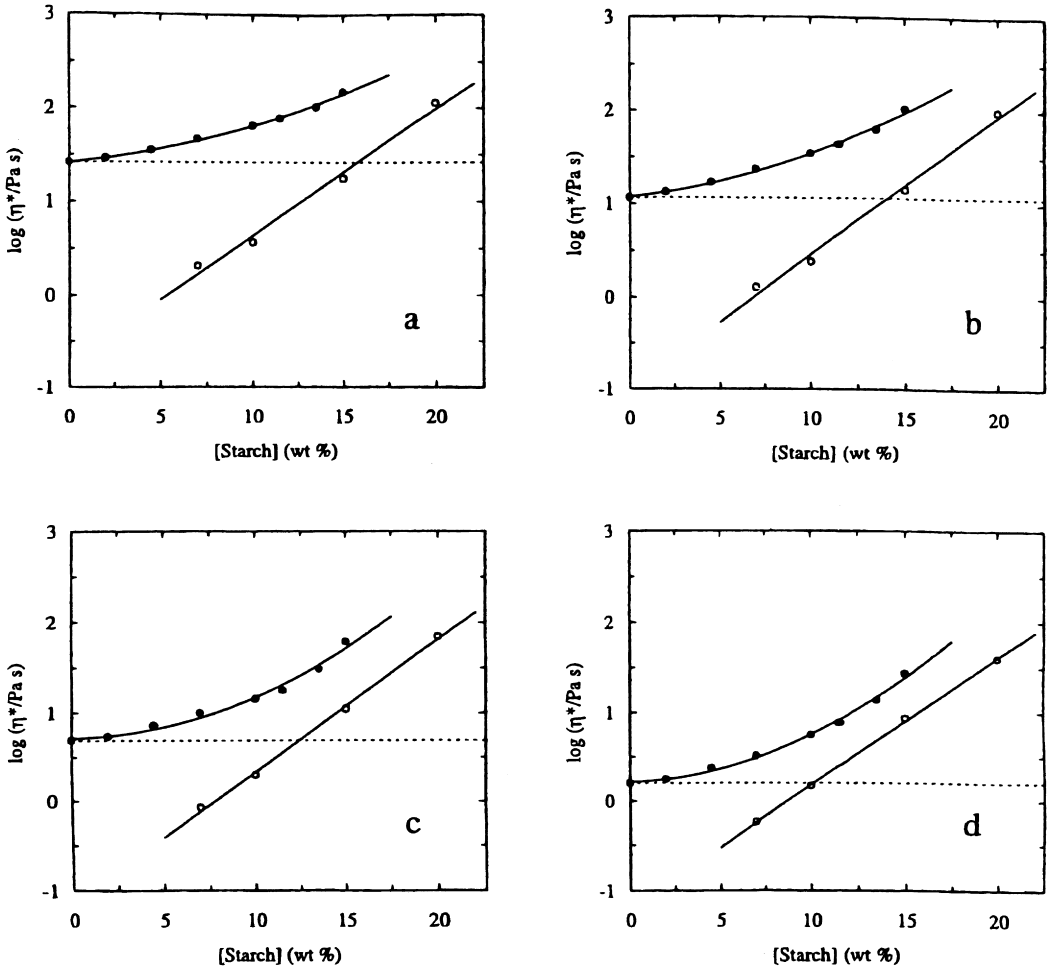

Fig. 19. Variation of η^* (1% strain; 5°C), measured at frequencies (rad s^{-1}) of: (a) 0.1; (b) 1.0; (c) 10; and (d) 100, with increasing concentration of oxidised starch in 50 wt% sucrose, in the presence (●) and absence (○) of 1.0 wt% high methoxy pectin (pH 3). The horizontal dotted lines shown η^* for pectin in the absence of starch.

by using Eq. (1) for pectin and Eq. (2) for oxidised starch (in conjunction with the polynomial parameters from Table 1), with the calculated values of overall moduli then being obtained by simple addition.

The fit was implemented by using the Microsoft Excel "Solver" routine to minimise the overall root-mean-square difference between observed and calculated values of log G' and log G'' at 0.1, 1.0, 10 and 100 rad s^{-1} for the mixed systems (1.0 wt% pectin in 50 wt% sucrose at pH 3, with 0–15 wt% oxidised starch), by varying V_p and V_s. The standard of agreement obtained is shown in Fig. 20. The discrepancies between the observed and fitted values of log G' (Fig. 20a) and log G'' (Fig. 20b) are all virtually within the experimental scatter of the data. The maximum effective concentrations of pectin and starch generated in the analysis were, respectively, [P] ≈ 1.4 wt% and [S] ≈ 19 wt%. These are well within the range of experimental concentrations used for calibration (up to 2.0 wt% for pectin; Fig. 14, and up to 20 wt% for oxidised starch; Fig. 16). Thus the empirical relationships derived for the concentration-dependence of moduli for the individual polymers Eqs. (1) and (2) were used solely for interpolation.

The values of the adjustable parameters yielding the fit shown in Fig. 20 were $V_p = 20.4$ and $V_s = 1.75$. It seems physically realistic that the excluded volume for oxidised starch (which is, effectively, partially depolymerised amylopectin, and therefore densely branched) should be lower than that for (linear) pectin. The value for pectin (~20 g solvent per 1 g of polymer), however, is perhaps higher than would have been anticipated. A possible interpretation is that, even after loss of network continuity by reduction in sucrose concentration, there is still some association of pectin chains into local regions of microgel structure, and that the microgel particles are impermeable to starch. Indeed, the initial reduction in tan δ at low frequency with increasing concentration of oxidised starch in mixtures with 1.0 wt% high methoxy pectin in 50 wt% sucrose (Fig. 18) is entirely consistent with enhancement of the gel-like character of the pectin component as its effective concentration is raised by the excluded volume of the starch.

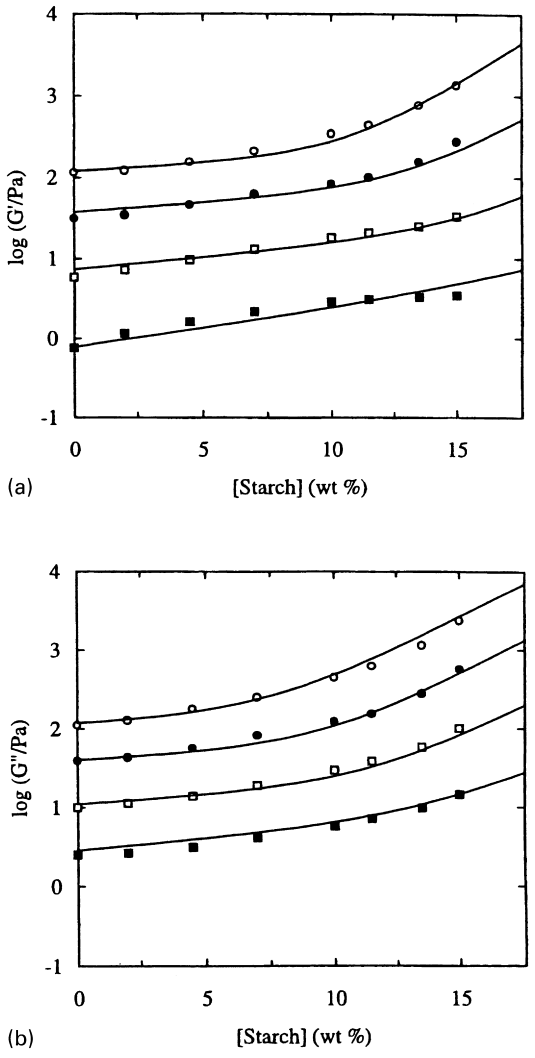

Fig. 20. Observed values of: (a) G'; and (b) G'' (1% strain; 5°C), measured at frequencies (rad s^{-1}) of: 0.1 (■); 1.0 (□); 10 (●); and 100 (○), for 1.0 wt% high methoxy pectin (pH 3) in the presence of 50 wt% sucrose and varying concentrations of oxidised starch, in comparison with fitted values (—) derived by the excluded volume analysis described in the text.

4. Discussion

The initial reductions in modulus seen on progressive replacement of sucrose by oxidised starch (Fig. 3) are at first sight similar to the reductions seen on progressive incorporation of oxidised starch in gelling mixtures with low methoxy pectin in the presence of Ca^{2+} (Picout et al., 2000a,b). In the low methoxy pectin systems, however, loss of gel strength was traced to "over-association" of calcium pectinate into large aggregates, in response to segregative interactions with starch. Similar "coarsening" of network structure was observed (Abeysekera, unpublished) for the high methoxy pectin systems studied in the present work, but the dominant factor in loss of gel strength (Fig. 3) appears to be the reduction in sucrose concentration, rather than the accompanying increase in starch content. Comparison of the tan δ values in Figs. 4 and 7 shows that the transition from predominantly elastic to predominantly viscous response occurs at roughly the same sucrose concentration for high methoxy pectin in the presence or absence of oxidised starch.

Re-establishment of gel structure (i.e. predominantly elastic response) at high concentrations of oxidised starch also occurs at roughly the same starch concentration in the presence (Fig. 4) and absence (Fig. 10) of pectin. In the intermediate range of composition, between ~10 and ~40 wt% starch (~55 to ~25 wt% sucrose), the rheological response of the mixed systems is predominantly viscous, but the mechanical spectra (e.g. Fig. 2) show less frequency-dependence of G' than for normal polysaccharide solutions (Ross-Murphy, 1984), indicating some residual gel-like character.

The overall moduli in this intermediate region are substantially higher than the sum of the moduli for the individual constituents (Fig. 12). The analysis of mixtures prepared at a fixed sucrose concentration of 50 wt% (Fig. 20), however, is consistent with simple additivity of the constituent moduli if allowance is made for mutual exclusion of two thermodynamically-incompatible polymers present within a single phase. The effect of excluded volume would also explain why the moduli of the mixed systems at high concentrations of oxidised starch (Fig. 12) are somewhat higher than those of the starch component in the absence of pectin.

In summary, therefore, the rheology of mixtures of 1.0 wt% high methoxy pectin (pH 3) with sucrose and oxidised starch at a combined concentration of 65 wt% is dominated by the behaviour of pectin at low concentrations of starch, and by the behaviour of starch at high concentrations. In the intermediate region, where the mixtures are fluid, the effective concentration of each polymer is raised by mutual exclusion with the other.

Acknowledgements

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