ELSEVIER

Carbohydrate Polymers 42 (2000) 245–259

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Effect of pH, sugar type and thermal annealing on high-methoxy pectin gels

V. Evageliou[a], R.K. Richardson[a], E.R. Morris[b,*]

[a]*Cranfield University, Silsoe College, Silsoe, Bedford MK45 4DT, UK*
[b]*Department of Food Science and Technology, University College Cork, Cork, Ireland*

Received 18 May 1999; accepted 29 October 1999

Abstract

The effect of cosolutes on the formation and properties of high-methoxy pectin gels (pectin concentration 0.5 wt%; DE 70.3) has been investigated by small-deformation oscillatory measurements of storage and loss moduli (G' and G'') and by compression testing at 5°C. Solutions were prepared at ~95°C and pH was varied by addition of citric acid. With 65 wt% sucrose as cosolute, a critically crosslinked network was formed on cooling to 5°C at pH 4.7. The changes in moduli observed for this composition during cooling were fully reversible on heating, with no thermal hysteresis. Progressive reduction in pH to below the pK_a of the galacturonic acid residues caused a sharp, sigmoidal increase in moduli at 5°C, attributed to association of pectin chains into a pectinic acid network. On heating to ~15°C, the gel moduli decreased, following the same temperature-course as the changes observed on cooling, but further increase in temperature caused large increases in moduli, consistent with hydrophobic association of methyl ester substituents. These increases became more pronounced as the strength of the gel structure at low temperature was decreased by reduction in sucrose concentration or increase in pH. Heating to 95°C and re-cooling caused a substantial increase in gel strength (G' from small-deformation measurements and breaking stress under compression), which is attributed to segregation of chain sequences of high and low ester content during thermal cycling, with the less highly esterified sequences giving stronger pectinic acid junctions at 5°C. Replacement of sucrose by glucose or fructose caused large changes in gelation temperature, in the order: fructose < sucrose < glucose. The departure from the normal order of effectiveness (fructose < glucose < sucrose) anticipated from compatibility with water structure and observed experimentally for the same sugars in combination with other biopolymers is attributed to inhibition of intermolecular association by strong hydrogen-bonding of primary alcohol groups on the sugars (2 per residue in fructofuranose, 1.5 per residue in sucrose, and 1 per residue in glucopyranose) to the carboxylic acid and methyl ester groups of pectin.

Keywords: High-methoxy pectin gels; Glucose; Fructose; Rheological properties

1. Introduction

High methoxy pectin is unique among gelling biopolymers with approval for food use in requiring high concentrations of a low molecular weight cosolute (normally sucrose) to induce gelation. The other requirement for gel formation is acidification (typically to ~pH 3). Gelation occurs spontaneously during cooling from the solution-state at high temperature. The resulting gels do not re-melt on heating (Christensen, 1986; Rolin, 1993).

In the two preceding papers (Evageliou, Richardson & Morris, 2000a,b), we showed that progressive replacement of sucrose by sub-gelling concentrations of oxidised starch (effectively partially depolymerised amylopectin) causes a large reduction in gel strength. The results from an analogous series of experiments using a different type of partially depolymerised starch (potato maltodextrin), however, indicated that maltodextrin, although less effective than sucrose, is capable of promoting self-association of high-methoxy pectin at acid pH. This was attributed, tentatively, to a substantial content of oligomeric fragments and short, linear chains in the maltodextrin preparation, but not in the oxidised starch.

In the present work, we have used small-deformation oscillatory measurements of storage modulus (G') and loss modulus (G'') to explore the effect of pH and sucrose concentration on the temperature-course of structure formation during cooling, and on subsequent thermal stability. We have also explored the changes that occur when sucrose is replaced by other cosolutes (glucose, glucose syrup, fructose, and glucose–fructose mixtures). Finally, we report an unusual "thermal annealing" effect observed

* Corresponding author. Tel.: +353-21-903625; fax: +353-21-270001.
E-mail address: ed.morris@ucc.ie (E.R. Morris).

0144-8617/00/$ - see front matter
PII: S0144-8617(99)00191-5

Table 1
Variation of sample pH with citric acid content

[Citric acid] (wt%)	pH (±0.05)
0.6	3.0
0.5	3.1
0.4	3.25
0.3	3.5
0.2	3.8
0.1	4.2
0.0	4.7

when gels were taken to high temperature and re-cooled.

2. Materials and methods

The pectin sample used was a laboratory preparation (X-4938) from the Copenhagen Pectin Division of Hercules. It has a pectin content of 97.7% of which 85.1% is galacturonate with a degree of methyl esterification (DE) of 70.3%. The unesterified carboxyl groups are predominantly in the un-ionised (acid) form, giving solutions of ~pH 3 or lower, depending on concentration. The same sample was used in the studies of mixtures of high-methoxy pectin with starch polysaccharides reported in the two preceding papers (Evageliou et al., 2000a,b). Glucose and fructose were Reagent grade from BDH; both are essentially anhydrous (<1% moisture). The sucrose used was normal food grade, purchased locally. Glucose syrup of dextrose equivalent 42 (i.e. with 42% of the glucose residues present as reducing end-groups) was a commercial preparation from Cerester (batch no NX 8472), with a water content of 19%. The concentration used is expressed on a dry-weight basis. Citric acid and trisodium citrate were AnalaR grade from BDH. Distilled deionised water was used throughout.

Sample preparation followed the procedure described in the preceding paper (Evageliou et al., 2000b). A pectin stock solution was prepared at ~5 wt%, and adjusted to pH 4 by addition of trisodium citrate. Mixtures were prepared at 95°C, by adding the required volume of the stock solution to a solution of the cosolute, and were brought to the required final weight by addition of water or continued evaporation, as appropriate. Citric acid (0–0.6 wt%) was then added, and the solution was immediately loaded into a rheometer pre-heated to 95°C, and/or filled into cylindrical moulds for compression testing. The effect of citric acid content on the pH of the final gels was determined by cooling samples to 5°C, disrupting the gel structure by vigorous manual stirring, and recording the pH values attained after prolonged equilibration at ambient temperature.

Small-deformation oscillatory measurements (0.5% strain) 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.). Temperature was varied at a fixed rate of 1°C/min, controlled by a Haake circulating water bath and measured using a thermocouple in contact with the stationary element. Measurements of G' and G'' during cooling and heating were made at a fixed frequency of 1 rad s^{-1}. After loading at 95°C, the samples were coated around their periphery with light silicone oil, to minimise evaporation, cooled to 5°C, heated to 90°C, and re-cooled to 5°C. At the end of each cooling or heating stage (i.e. at 5°C, 90°C and 5°C) a full mechanical spectrum was recorded to show the variation of G', G'' and η^* (complex dynamic viscosity) with frequency (ω).

Samples for compression testing were filled (at 95°C) into lubricated cylindrical moulds of diameter 13 mm and height 12 mm, and immediately sealed with lubricated foil. They were then allowed to cool naturally to ~30°C, transferred to a refrigerator at 5°C, and stored for 2 h. At this stage, three plugs were demoulded and compressed at a fixed rate of 0.8 mm s^{-1} on a TA-XT2 Texture Analyser (from Stable Microsystems), housed in a refrigerator at 5°C. The remaining plugs were then placed in an oven at 90°C for 1 h, cooled as before, and measured at 5°C. Duplicate preparations were made for each sample, and the results shown are mean values from the resulting six compression curves (two preparations, each run in triplicate). The extent of compression is expressed as "true strain", which is given (Ross-Murphy, 1984) by $\ln(L_0/L)$, where L_0 and L denote the height of the sample before and during compression, respectively. Stress (force per unit area) was similarly corrected for changes in the mean cross-sectional area of the sample during compression, by multiplying the initial area by L_0/L.

Optical rotation was measured at 365 nm on a Perkin–Elmer 241 polarimeter, using a jacketed cell of pathlength 10 mm. Temperature was controlled by a Haake circulating water bath and measured using a thermocouple in the neck of the cell, but out of the light path, and was held fixed at 25°C.

3. Results

3.1. Variation of pH at fixed sucrose concentration

In the first series of experiments, the sucrose content of the samples was held fixed at 65 wt% and the pH was varied, by varying the amount of citric acid added in the final stage of sample preparation. The concentrations of citric acid used were 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 wt%, and the corresponding values of pH for the resulting gels are listed in Table 1. As shown in Fig. 1, there is a smooth reduction in pH as the citric acid content is increased. The highest concentration (0.6 wt%), which was the value used in the investigation of mixtures of high-methoxy pectin with starch polysaccharides reported in the preceding paper (Evageliou et al., 2000b), gives a pH of 3.0. At the other extreme, the pH attained with no addition of citric acid is

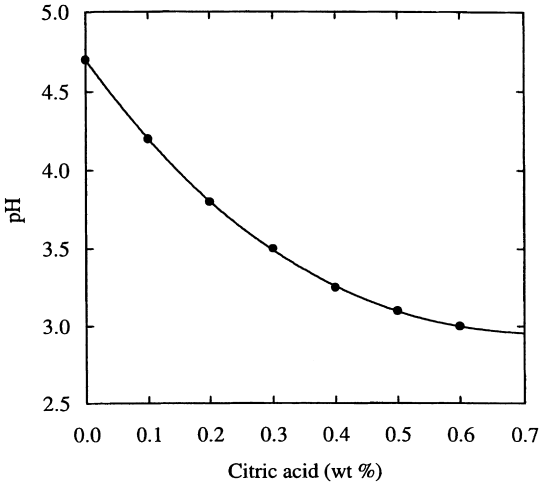

Fig. 1. Effect of citric acid content on the pH of high-methoxy pectin gels (0.5 wt% pectin; DE 70.3; 65 wt% sucrose) prepared using a pectin stock solution (5.0 wt%) at pH 4.

higher than that of the pectin stock solution (4.7 in comparison with 4.0), which can be explained by the reduction in pectin concentration from 5.0 wt% in the stock solution to 0.5 wt% in the mixtures with sucrose.

Fig. 2a shows the effect of pH on the values of G' and G'' (1 rad s^{-1}; 0.5% strain) attained on completion of cooling to 5°C. There is a sharp reduction in both moduli as the pH is raised to above the pK_a of the galacturonate residues of the polymer (~pH 3.4; Plaschina, Braudo & Tolstoguzov, 1978). As illustrated in Fig. 3a, the mechanical spectra recorded at 5°C for samples prepared at lower pH (<3.4) are typically gel-like ($G' \gg G''$; little frequency-dependence of either modulus; linear decrease in log η^* with increasing log ω, with a slope close to -1). At the highest pH studied (4.7, obtained with no addition of citric acid), the corresponding spectrum (Fig. 3b) has the form characteristic (Durrand, Delsanti, Adam & Luck, 1987; te Nijenhuis & Winter, 1989) of a critically crosslinked network: log G' and log G'' vary linearly with log ω across the whole of the accessible frequency-range, with the same slope (~0.63) for both moduli. Thus pH 4.7 is the point at which there is just sufficient self-association of pectin chains to give a continuous network.

As shown in Fig. 4, the changes in G' and G'' observed during cooling at this pH are fully reversible on heating, with no detectable thermal hysteresis. At the opposite extreme of the pH range studied (3.0, obtained using 0.6 wt% citric acid), there is an initial slight reduction in G' and G'' on heating (Fig. 5a), again following the same temperature-course as the changes observed during cooling, but at temperatures above ~30°C both moduli remain virtually constant, consistent with the well-established thermal stability of high-methoxy pectin gels under these conditions (0.5 wt% pectin; 65 wt% sucrose; pH 3).

At intermediate pH values (i.e. between 4.7 and 3.0), however, the initial reduction in moduli is followed by a substantial increase at higher temperatures, suggesting that the overall thermal stability may arise from formation of new intermolecular associations during heating, rather than from simple preservation of the structures formed initially during cooling. This behaviour is illustrated in Fig. 5b for the sample prepared at pH 3.5 (citric acid content 0.3 wt%). The pH-dependence of the final moduli at 90°C is shown in Fig. 2b.

The extent of thermal hysteresis between cooling and heating is indicated in Fig. 6, which shows the vaues of G' and G'' obtained on completion of heating to 90°C, divided by the corresponding values recorded at the same temperature during initial cooling from the solution state. The results are somewhat scattered, probably due to the low values of the initial moduli at high temperature, but there is a clear trend to greater hysteresis as the pH is reduced (with, as shown in Fig. 4, no detectable hysteresis for the critically crosslinked network formed at pH 4.7).

As a further indication of the rheological changes that occur during heating, Fig. 7 shows the moduli at 5°C (Fig.

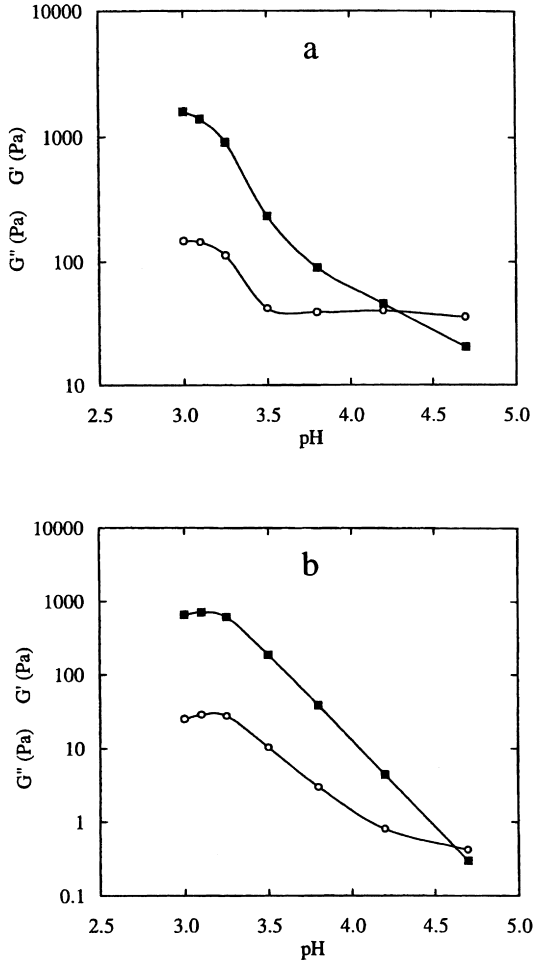

Fig. 2. pH-Dependence of G' (■) and G'' (○), measured at 1 rad s^{-1} and 0.5% strain, for 0.5 wt% high-methoxy pectin (DE 70.3) with 65 wt% sucrose, (a) after cooling to 5°C from the solution state at 95°C, and (b) after subsequent heating to 90°C.

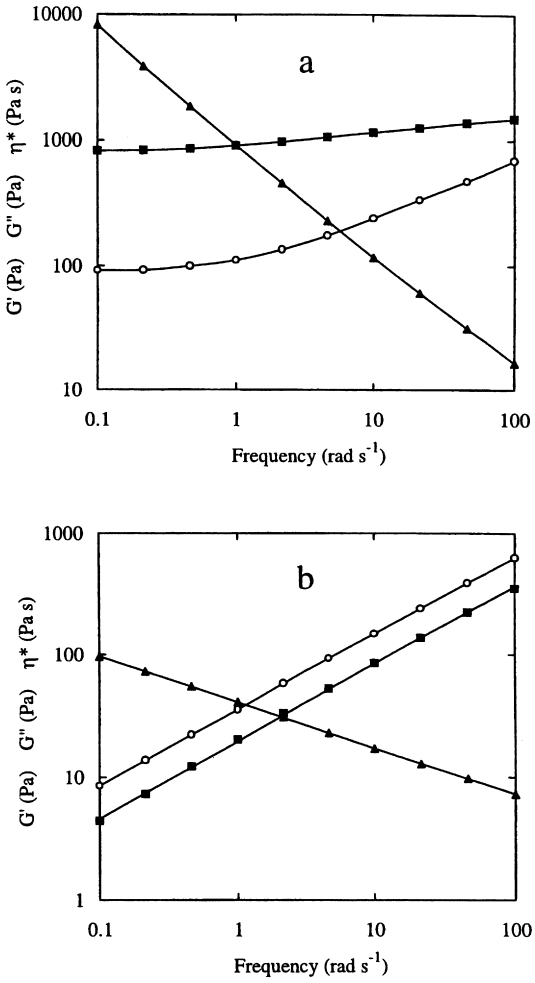

Fig. 3. Mechanical spectra (0.5% strain) showing the frequency-dependence of G' (■), G'' (○) and η^* (▲) for 0.5 wt% high-methoxy pectin at (a) pH 3.1 and (b) pH 4.7 after cooling to 5°C in the presence of 65 wt% sucrose.

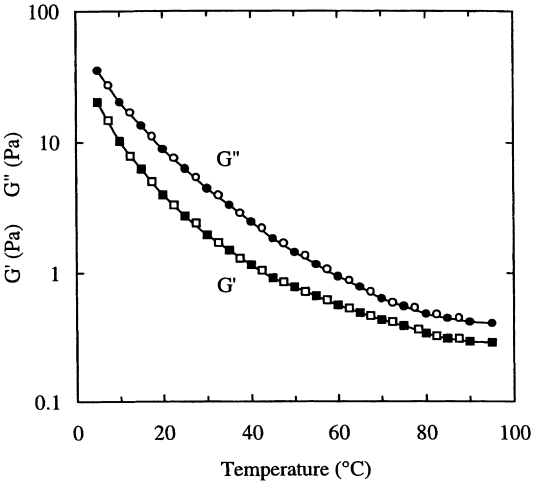

Fig. 4. Variation of G' (squares) and G'' (circles) for 0.5 wt% high-methoxy pectin in the presence of 65 wt% sucrose at pH 4.7, during initial cooling from 95 to 5°C (filled symbols) and subsequent heating to 90°C (open symbols).

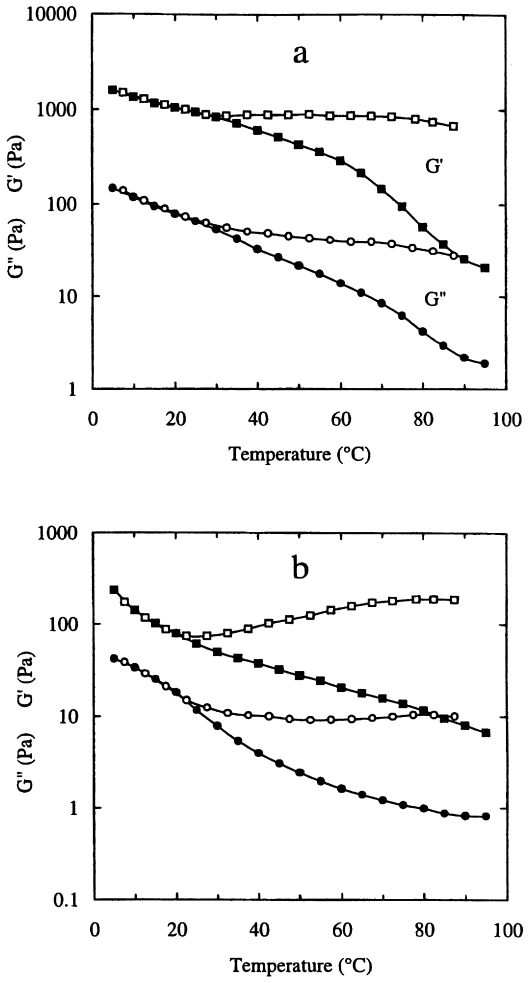

Fig. 5. Variation of G' (squares) and G'' (circles) during initial cooling from 95 to 5°C (filled symbols) and subsequent heating to 90°C (open symbols) for 0.5 wt% high-methoxy pectin with 65 wt% sucrose at (a) pH 3.0 and (b) pH 3.5.

2a) divided by the corresponding values at 90°C (Fig. 2b). The divergence is smallest (i.e. the ratio is lowest) at around the pK_a of the galacturonate residues, increasing steeply at higher pH and also increasing slightly under more acidic conditions. At all pH values studied, the ratios are greater than 1 (i.e. the moduli at 90°C are lower than at 5°C). The decrease however, is much greater for G'' than for G' (i.e. the ratio of the low-temperature/high-temperature values is higher).

The change in character of the gel networks during heating is illustrated more directly in Fig. 8, which shows the values of tan δ (G''/G') at 5°C and at 90°C. Throughout the pH range studied, tan δ at 90°C is lower than at 5°C. The divergence is greatest for the sample prepared at pH 3.8 (using 0.2 wt% citric acid). Fig. 9 shows the mechanical spectra obtained for this preparation after initial cooling to 5°C and after subsequent heating to 90°C. The high-temperature spectrum (Fig. 9b) has the form typical of a normal biopolymer gel. Although the moduli at 5°C are substantially higher, the mechanical spectrum (Fig. 9a) is far less gel-like: the separation between G' and G'' is much

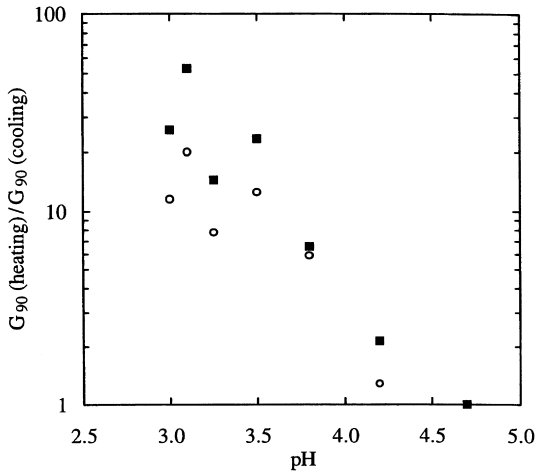

Fig. 6. Effect of pH on the values of G' (■) and G'' (○) for 0.5 wt% high-methoxy pectin with 65 wt% sucrose after cooling to 5°C and heating to 90°C, divided by the corresponding moduli at 90°C during initial cooling.

smaller, and the frequency-dependence of both moduli is greater. This striking difference in network character between low and high temperature is again indicative of changes in intermolecular association during heating.

3.2. Variation in sucrose concentration at fixed pH

Fig. 10 shows the results of a brief investigation in which pH was held constant at 3.0 (using 0.6 wt% citric acid) and sucrose content was varied. The concentrations of sucrose used were 50, 55 and 60 wt%, in addition to the concentration of 65 wt% used in the study of pH-dependence described above. As reported previously (Evageliou et al., 2000b), reduction in sucrose content causes a massive reduction in initial moduli at the loading temperature of 95°C, and displaces the sharp increase in G' (Fig. 10a) and G'' (Fig. 10b) during cooling to progressively lower temperatures, with accompanying reduction in the moduli reached at 5°C.

As found for samples prepared at 65 wt% sucrose but with a lower content of citric acid (Fig. 5b), the preparations with lower sucrose content show a substantial increase in moduli during heating. Indeed, the final values of G' (Fig. 10a) at 90°C for the pectin–sucrose mixtures at sucrose concentrations of 50 and 55 wt% are appreciably higher than the corresponding values at 5°C, giving direct evidence of the formation of additional intermolecular associations as the temperature is raised.

3.3. Thermal annealing

Further changes in network properties were observed when samples that had been heated to 90°C were re-cooled to 5°C. Fig. 11 shows the variations in G' and G'' during heating and cooling for 0.5 wt% pectin with 65 wt% sucrose and 0.6 wt% citric acid (pH 3.0). Similar changes were seen for mixtures prepared at higher pH (0–0.5 wt% citric acid). In the initial stages of cooling, the moduli follow the same temperature-course as the changes observed during heating, but then diverge to higher values at lower temperature.

One obvious, trivial, explanation might be that the higher values of G' and G'' at 5°C after heating to 90°C arise from evaporation of water at elevated temperature, despite the coating of silicon oil around the periphery of the samples. This possibility was explored by compression-testing of samples prepared in tightly sealed moulds. Fig. 12 shows the stress–strain curves obtained for the same formulation as in Fig. 11 (0.5 wt% pectin; 65 wt% sucrose, pH 3.0), measured at 5°C before and after heating to 90°C. The difference in the large-deformation measurements is less pronounced than the changes in small-deformation response (Fig. 11), but there is a significant increase in breaking stress after "thermal annealing", with an accompanying reduction

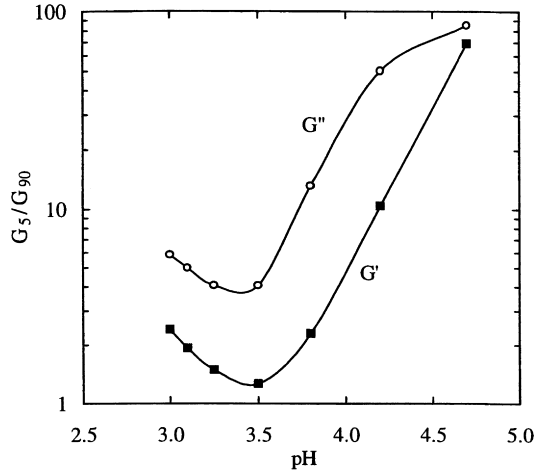

Fig. 7. Effect of pH on the values of G' (■) and G'' (○) for 0.5 wt% high-methoxy pectin with 65 wt% sucrose after cooling to 5°C, divided by the corresponding moduli after subsequent heating to 90°C.

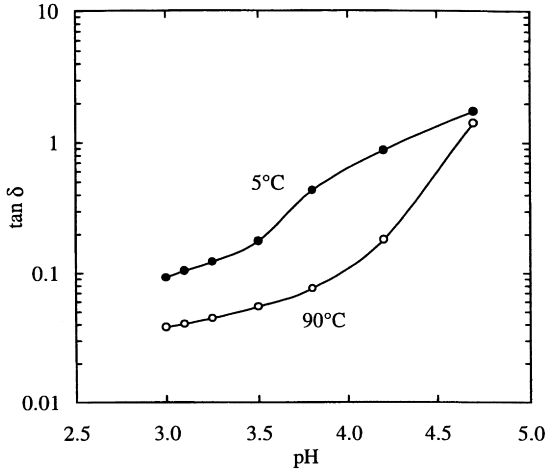

Fig. 8. Effect of pH on the values of tan δ (1 rad s^{-1}; 0.5% strain) observed for 0.5 wt% high-methoxy pectin with 65 wt% sucrose, after initial cooling to 5°C (●) and after subsequent heating to 90°C (○).

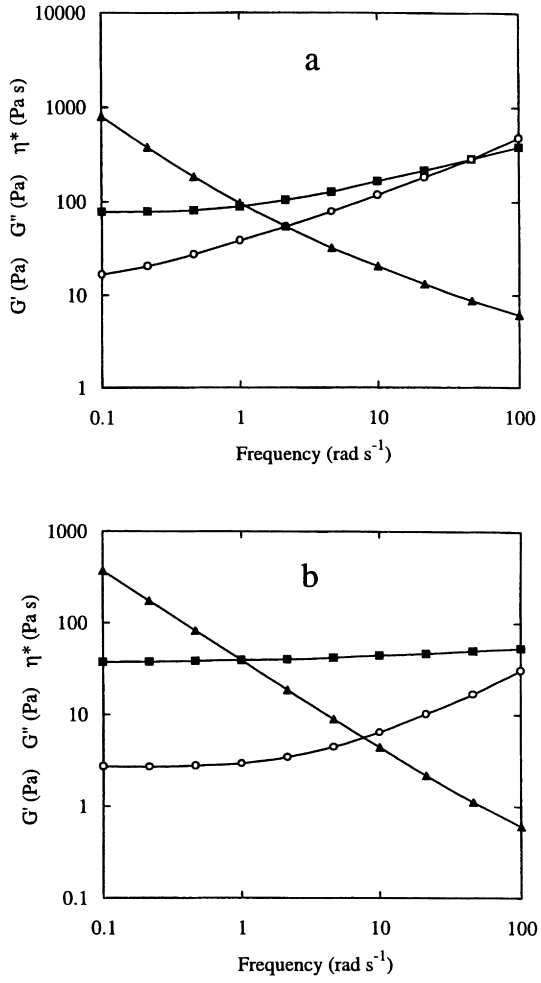

Fig. 9. Mechanical spectra (0.5% strain) showing the frequency-dependence of G' (■), G'' (○) and η^* (▲) for 0.5 wt% high-methoxy pectin with 65 wt% sucrose at pH 3.8 (a) after initial cooling to 5°C and (b) after subsequent heating to 90°C.

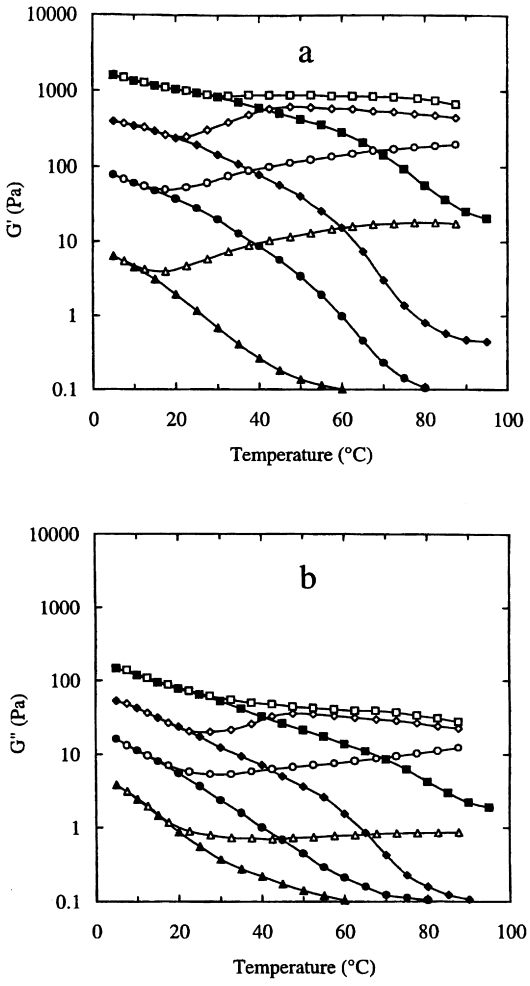

Fig. 10. Temperature-dependence of (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain) during initial cooling from 95 to 5°C (filled symbols) and subsequent heating to 90°C (open symbols) for 0.5 wt% high-methoxy pectin (pH 3.0) in the presence of sucrose at concentrations (wt%) of 50 (triangles), 55 (circles), 60 (diamonds) and 65 (squares).

in strain at the point of failure (i.e. the sample has become firmer and more brittle).

Perhaps more convincingly, samples prepared at the same pH (3.0), but with a lower content of sucrose (60 wt%), were too weak to be demoulded without breaking after initial cooling to 5°C. When the same preparations were heated to 90°C and re-cooled to 5°C, however, the resulting gels were obviously more cohesive, and could be readily extracted from the moulds, yielding the compression values shown in Fig. 13. As would be anticipated from the lower content of cosolute, failure occurred at substantially lower stress, and lower strain, than for the preparations at 65 wt% sucrose (Fig. 12), but the stress–strain curve has the form expected for fracture of a normal gel network.

The indications of a harder but more brittle structure after heating and cooling (Fig. 12) are reflected in the mechanical spectra obtained for the same preparation (0.5 wt% pectin; 65 wt% sucrose; pH 3.0). As shown in Fig. 14a, the spectrum recorded after initial cooling at 5°C has the form typical of a reasonably strong polysaccharide gel. Heating

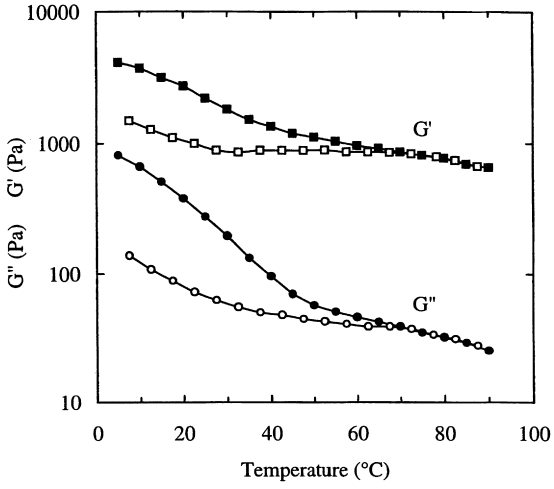

Fig. 11. Temperature-dependence of G' (squares) and G'' (circles) for high-methoxy pectin gels (0.5 wt% pectin; 65 wt% sucrose; pH 3.0) formed by cooling to 5°C, during heating to 90°C (open symbols) and on subsequent re-cooling to 5°C (filled symbols).

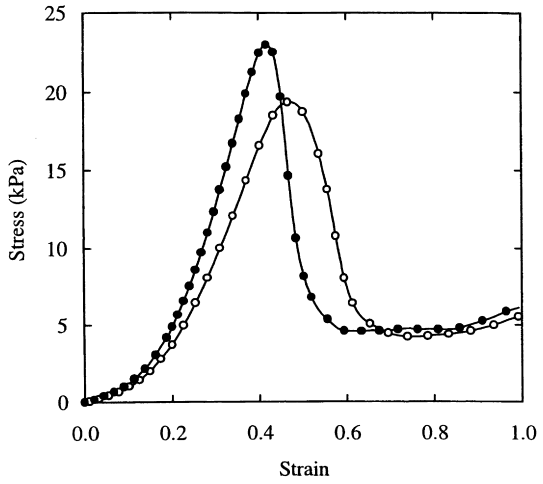

Fig. 12. Compression curves for 0.5 wt% pectin in combination with 65 wt% sucrose at pH 3.0, measured at 5°C after initial cooling from the solution state (○) and after subsequent heating to 90°C (●).

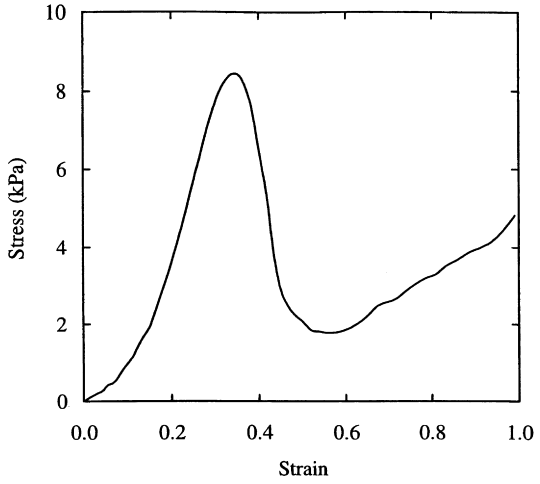

Fig. 13. Compression curve for 0.5 wt% pectin (pH 3.0) in combination with 60 wt% sucrose, after cooling from 95 to 5°C, heating to 90°C, and re-cooling to 5°C. The gels formed after initial cooling to 5°C were too weak to be removed intact from the moulds.

to 90°C and re-cooling raises the overall moduli, but the resulting mechanical spectrum (Fig. 14b) shows clear indications of a higher sol fraction (i.e. a greater proportion of polymeric material that does not contribute to the elastic character of the network, but increases the viscous response): the separation of G' and G'' is smaller than after initial cooling and there is a substantial increase in both moduli at high frequency, as found for polymer solutions (Ross-Murphy 1984).

A possible interpretation of these differences might be that they arise from progressive crystallisation of sucrose during thermal cycling, with the sugar crystals raising the effective concentration of pectin (by reducing the volume of the solution phase) but causing physical damage to the gel network. This possibility was tested using a preparation with the same pH (3.0) and cosolute concentration (65 wt%), but with sucrose replaced by glucose syrup, which is resistant to crystallisation. The mechanical spectra recorded at 5°C before and after heating to 90°C are shown in Fig. 15. The changes observed for the sample incorporating 65 wt% sucrose (Fig. 14) are again evident, but are more pronounced with glucose syrup as cosolute. It seems clear, therefore, that the higher overall moduli and increase in proportion of solution-like response after heating and cooling are not associated with crystallisation, but reflect a genuine change in structure of the pectin network.

3.4. Effect of changes in cosolute

The final series of experiments originated from an ill-considered attempt to characterise the molecular processes underlying the observed changes in rheology during cooling and heating by using optical rotation to monitor chain conformation. The optical rotation values, however, instead of giving the sigmoidal changes expected for co-operative transitions, showed a progressive decrease, which continued uninterrupted when the direction of temperature change was reversed. The obvious explanation is, of course, that this monotonic decrease was caused by hydrolysis of sucrose at acidic pH, which prompted us to consider the extent of hydrolysis likely to have occurred during the rheological studies, and its possible effect on the observed moduli.

To characterise the maximum rate of hydrolysis at elevated temperature, a solution of 65 wt% sucrose was prepared at ~95°C, acidified to pH 3.0 with citric acid, sealed in an autoclave bottle, and placed in a boiling water bath. Samples were withdrawn at 15 min intervals, up to 1 h, and loaded into a polarimeter cell (1 cm pathlength) thermostatted at 25°C. Optical rotation was measured at 365 nm, and recorded when temperature had equilibrated and stable readings were attained. The hydrolysis reaction can be expressed as:

$$\begin{array}{ccccccccc} C_{12}H_{22}O_{11} & + & H_2O & \rightarrow & C_6H_{12}O_6 & + & C_6H_{12}O_6 & & \\ \text{sucrose} & + & \text{water} & \rightarrow & \text{glucose} & + & \text{fructose} & & (1) \\ (MW = 342) & & (MW = 18) & & (MW = 180) & & (MW = 180) & & \end{array}$$

Thus complete hydrolysis of 65 wt% sucrose would yield a solution containing $65 \times 180/342 = 34.2$ wt% glucose and 34.2 wt% fructose. A solution of this composition was prepared, and gave an optical rotation (365 nm, 1 cm pathlength; 25°C) of −5.44°. The corresponding value for 65 wt% sucrose was 16.05°. Thus the toal change in optical rotation for complete hydrolysis would be −21.49°, with the fractional change at each sampling time giving the

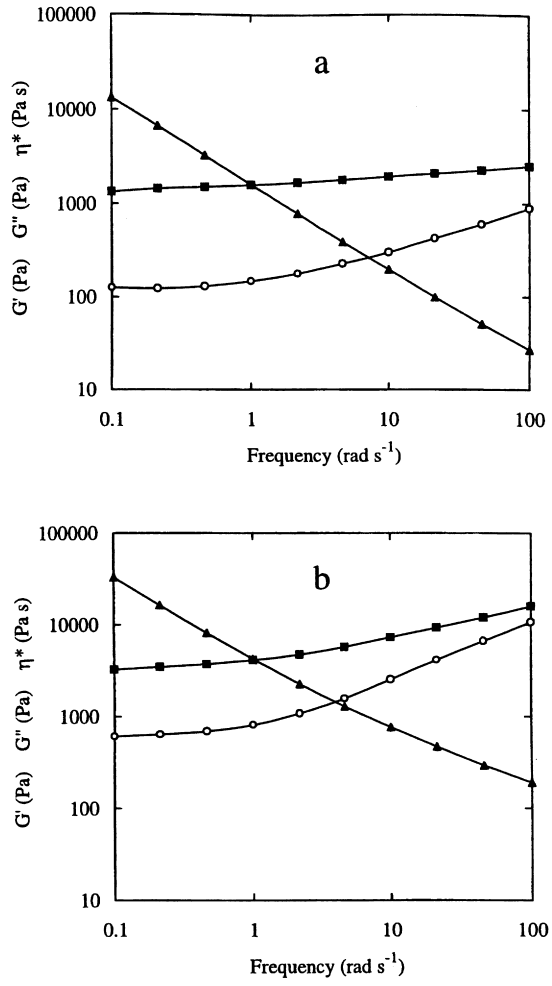

Fig. 14. Mechanical spectra (0.5% strain) showing the frequency-dependence of G' (■), G'' (○) and η^* (▲) for 0.5 wt% high-methoxy pectin with 65 wt% sucrose at pH 3.0 (a) after initial cooling to 5°C, and (b) after subsequent heating to 90°C and re-cooling to 5°C.

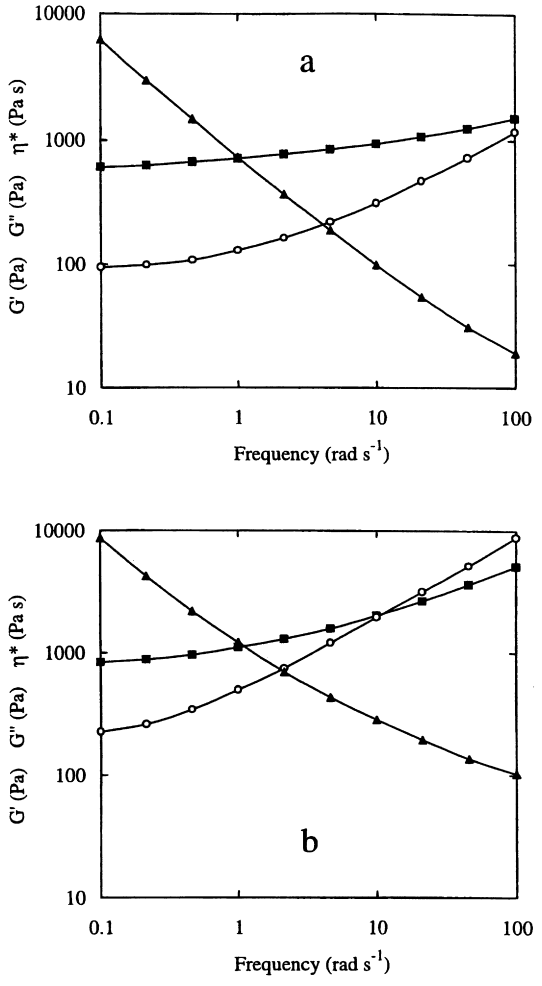

Fig. 15. Mechanical spectra (0.5% strain) showing the frequency-dependence of G' (■), G'' (○) and η^* (▲) for 0.5 wt% high-methoxy pectin with 65 wt% glucose syrup at pH 3.0 (a) after initial cooling to 5°C, and (b) after subsequent heating to 90°C and re-cooling to 5°C.

extent of hydrolysis that had occurred. As shown in Fig. 16, ~10% of the sucrose molecules in a 65 wt% solution at pH 3.0 are split into glucose and fructose after 30 min at ~100°C, and the extent of hydrolysis after 1 h is ~18%.

The effect of replacing sucrose by glucose or fructose, or by mixtures of the two monosaccharides, was explored at a fixed pectin concentration of 0.5 wt% (as in all the studies reported in previous sections) and at a fixed pH of 3.0. Mixtures were prepared at glucose: fructose ratios of 0:4, 1:3, 2:2, 3:1 and 4:0. For ease of comparison, the total monosaccharide concentrations (Table 2) were based on concentrations of sucrose solutions with the same content of monosaccharide residues. Thus, for example, comparisons with 65 wt% sucrose were made using glucose/fructose solutions prepared at 68.4 wt%. The "equivalent sucrose concentration" is smaller than the absolute concentration of monosaccharides by a factor of 0.95 (342/360; Eq. (1)). Fig. 17 shows the cooling curves obtained at a monosaccharide concentration equivalent to 65 wt% sucrose. As

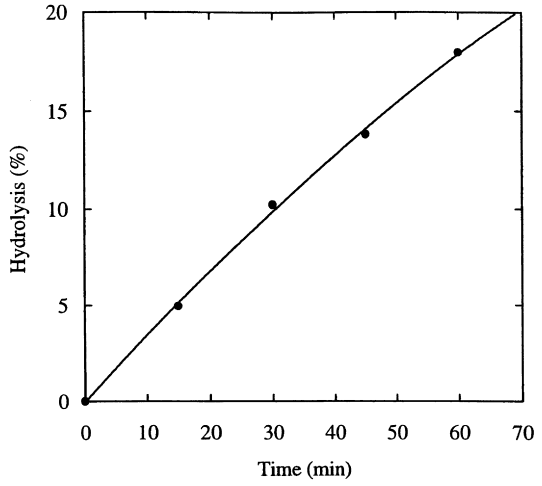

Fig. 16. Time-course of hydrolysis for sucrose (65 wt%) acidified to pH 3.0 with citric acid and held in a water bath at ~100°C.

Table 2
Concentrations of monosaccharide solutions that would be obtained from complete hydrolysis of sucrose

Sucrose concentration (wt%)	Monosaccharide concentration (wt%)
50.0	52.6
52.5	55.3
55.0	57.9
60.0	63.2
65.0	68.4

the glucose content is increased from 0 to 100% of the total cosolute (with corresponding reduction in fructose content from 100 to 0%), the sigmoidal increase in G' (Fig. 17a) and G'' (Fig. 17b) is displaced to progressively higher temperature, with an accompanying progressive increase in the initial values of both moduli at the loading temperature of 95°C. The temperature-course of structure formation with 65 wt% sucrose as cosolute lies between the curves obtained at glucose:fructose ratios of 3:1 and 4:0.

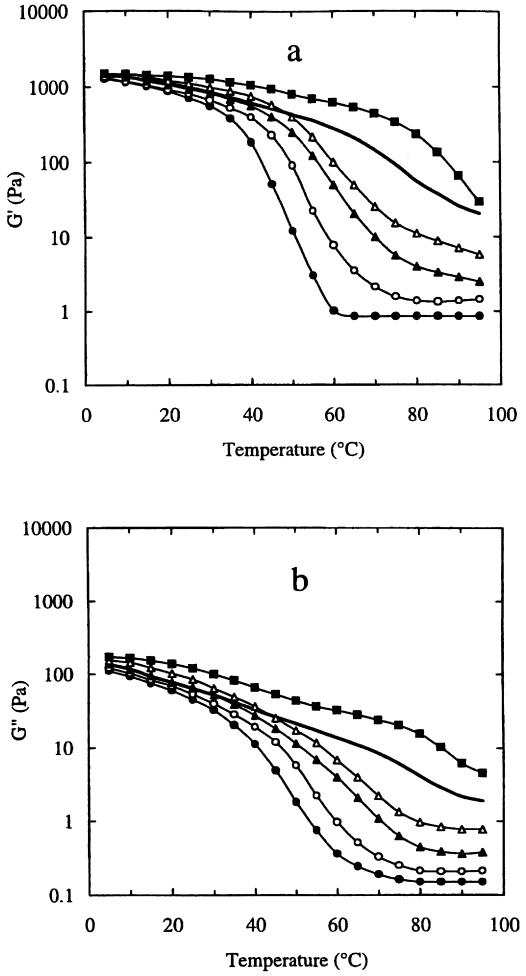

Fig. 17. Variation of (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain) during initial cooling from 95 to 5°C for 0.5 wt% high-methoxy pectin (pH 3.0) in combination with glucose/fructose cosolutes (concentration 68.4 wt%, equivalent to 65 wt% sucrose) with glucose content (% total monosaccharide) of 0 (●), 25 (○), 50 (▲), 75 (△) and 100 (■). The bold line with no symbols shows the changes observed (Fig. 5) with 65 wt% sucrose as cosolute.

At these high concentrations of cosolute, however, the nature of the sugar(s) has little effect on the mechanical properties attained on completion of cooling to 5°C. Fig. 18a shows the mechanical spectrum for 0.5 wt% pectin (pH 3.0) at 5°C with 68.4 wt% glucose (equivalent to 65 wt% sucrose) as cosolute. The corresponding spectrum for an equivalent preparation incorporating the same concentration of fructose is shown in Fig. 18b. There is little difference between the two spectra, and both have the form typical of a normal polysaccharide gel (Ross-Murphy, 1984). Fig. 19 shows the compression curves recorded for the same compositions, in comparison with the corresponding trace for 0.5 wt% pectin with 65 wt% sucrose as cosolute. As found for the small-deformation moduli at low temperature (Fig. 17), the three compression curves are virtually identical.

At lower concentrations of cosolute, however, changes in sugar type have a large effect on gel moduli at low temperature. Fig. 20a shows the value of G' observed for 0.5 wt% pectin (pH 3.0) after cooling to 5°C in the presence of glucose/fructose cosolutes at concentrations of 52.6, 55.3, 57.9, 63.2 and 68.4 wt%, which are equivalent (Table 2) to 50, 52.5, 55, 60 and 65 wt% sucrose. The corresponding values of G'' are shown in Fig. 20b. There is a general trend to higher moduli as the glucose content of the cosolute is increased, particularly at intermediate concentrations (~55–63 wt% monosaccharide).

Fig. 21 shows the changes in G' (Fig. 21a) and G'' (Fig. 21b) during cooling for the samples prepared using glucose alone as cosolute. The corresponding cooling curves for 0.5 wt% pectin (pH 3.0) with fructose as cosolute are shown in Fig. 22. In both cases, the increase in moduli is displaced to progressively lower temperatures as the concentration of monosaccharide is decreased, but gelation occurs at systematically lower temperatures with fructose than with equivalent concentrations of glucose, and, as shown in Fig. 20, the resulting moduli at 5°C are correspondingly lower. The greatest overall differences between the two cosolutes occur at a monosaccharide concentration of ~58 wt%.

Fig. 23 shows the mechanical spectra obtained for 0.5 wt% pectin (pH 3.0) after completion of cooling to 5°C in the presence of glucose or fructose at a concentration of 57.9 wt% (equivalent to 55 wt% sucrose). As found (Fig. 18) using a higher concentration of either monosaccharide (68.4 wt%, equivalent to 65 wt% sucrose), the rheological response with 57.9 wt% glucose as cosolute (Fig. 23a) is typically gel-like. The spectrum obtained with 57.9 wt% fructose as cosolute (Fig. 23b), however, indicates a substantially higher sol-fraction (smaller separation of G' and G''; greater frequency-dependence of both moduli, particularly at high frequency), and the individual moduli are much smaller.

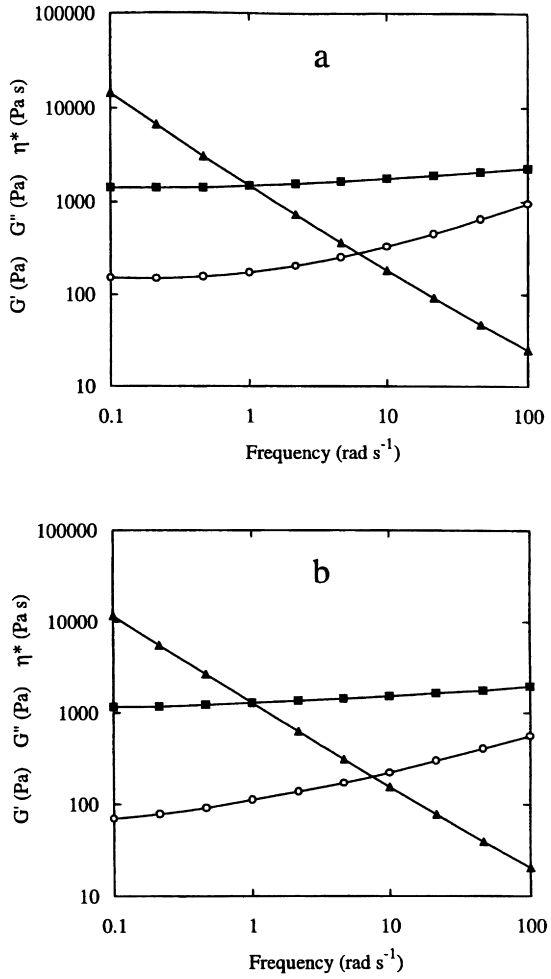

Fig. 18. Mechanical spectra (0.5% strain) showing the frequency-dependence of G' (■), G'' (○) and η^* (▲) for 0.5 wt% high-methoxy pectin (pH 3.0) after cooling from 95 to 5°C in the presence of (a) glucose and (b) fructose at a concentration of 68.4 wt% (equivalent to 65 wt% sucrose).

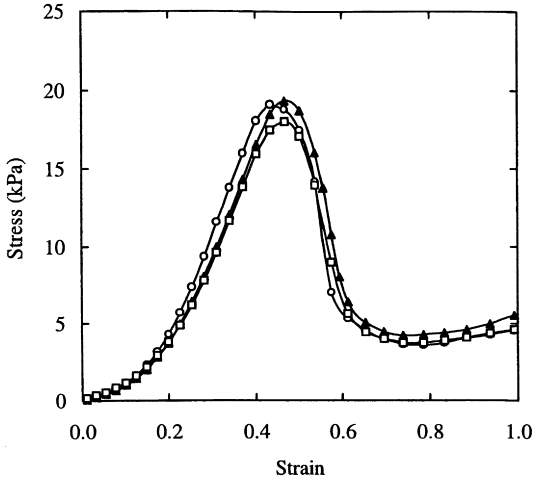

Fig. 19. Compression curves for 0.5 wt% pectin (pH 3.0) after initial cooling to 5°C in the presence of glucose (□) or fructose (○) at a concentration of 68.4 wt% (equivalent to 65 wt% sucrose), in comparison with the curve obtained (Fig. 12) using 65 wt% sucrose as cosolute (▲).

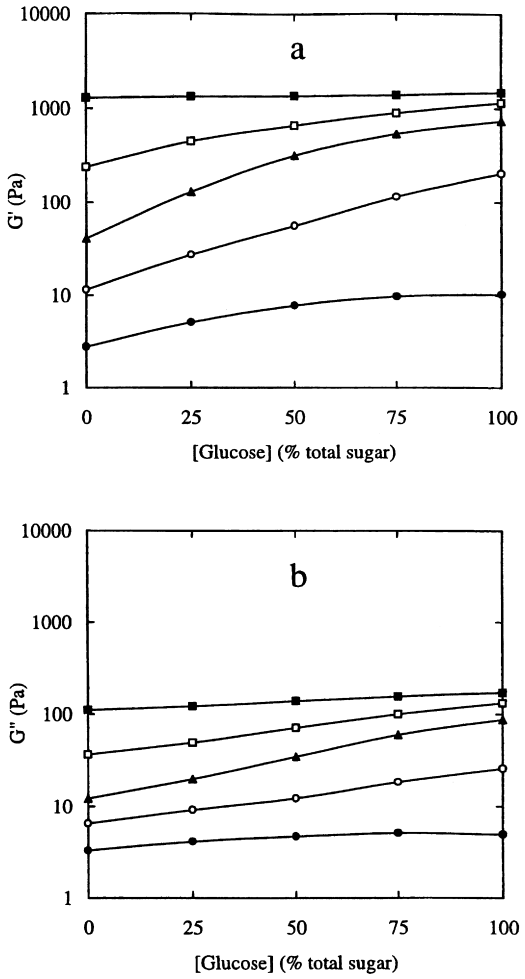

Fig. 20. Effect of glucose content (% total monosaccharide) on the values of (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain) observed for 0.5 wt% high-methoxy pectin (pH 3.0) after initial cooling to 5°C in the presence of glucose/fructose cosolutes at concentrations (wt%) of 52.6 (●), 55.3 (○), 57.9 (▲), 63.2 (□) and 68.4 (■), which are equivalent to 50, 52.5, 55, 60 and 65 wt% sucrose, respectively.

Fig. 24 shows a direct comparision of values of G' (Fig. 24a) and G'' (Fig. 24b) observed for 0.5 wt% high-methoxy pectin (pH 3.0) after cooling to 5°C in the presence of various concentrations of sucrose (50–65 wt%), and the corresponding values obtained using equivalent concentrations (Table 2) of glucose, fructose, or equimolar mixtures of both (as would be obtained by complete hydrolysis of sucrose). As shown previously (Figs. 17–19), changes in sugar-type at high concentration of cosolute (65 wt% sucrose, or equivalent monosaccharide concentration) have little effect on gel properties at 5°C. As the cosolute concentration is decreased, the moduli obtained using equimolar mixtures of glucose and fructose rise above those observed at equivalent concentrations of sucrose, but converge again at lower concentrations (50 wt%, and equivalent). Throughout this range of cosolute concentrations, however, the differences in moduli that would occur from complete hydrolysis of sucrose into an

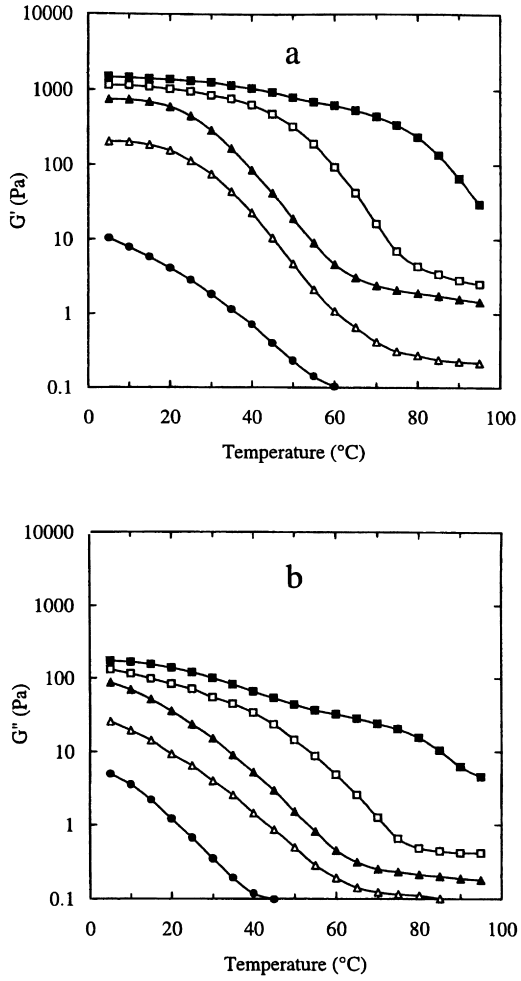

Fig. 21. Variation of (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain) during initial cooling from 95 to 5°C for 0.5 wt% high-methoxy pectin (pH 3.0) in the presence of glucose at concentrations (wt%) of 52.6 (●), 55.3 (○), 57.9 (▲), 63.2 (□) and 68.4 (■), which are equivalent to 50, 52.5, 55, 60 and 65 wt% sucrose, respectively.

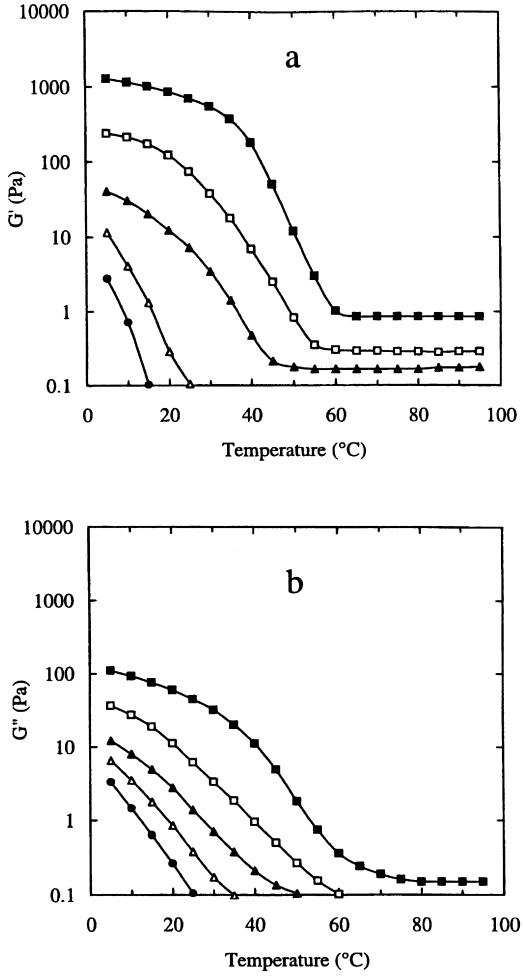

Fig. 22. Variation of (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain) during initial cooling from 95 to 5°C for 0.5 wt% high-methoxy pectin (pH 3.0) in the presence of fructose at concentrations (wt%) of 52.6 (●), 55.3 (○), 57.9 (▲), 63.2 (□) and 68.4 (■), which are equivalent to 50, 52.5, 55, 60 and 65 wt% sucrose, respectively.

equimolar mixture of glucose and fructose are comparatively small, reaching a maximum of ~3-fold enhancement in G' and ~2-fold increase in G'' at ~55 wt% sucrose. Thus the extent of hydrolysis found (Fig. 16) for mixtures held for an hour at around the boiling point would be unlikely to cause any significant change in final gel properties at 5°C. As shown in Fig. 17, however, a comparatively low content of fructose in the cosolute may cause a significant reduction in initial moduli at high temperature, and in the onset temperature for steep increase in moduli during cooling.

4. Discussion

There are two obvious barriers to self-association of pectin chains into gel junctions: (i) intermolecular electrostatic repulsion between charged carboxyl groups, and (ii) polymer–water interactions acting in competition with polymer–polymer interactions. The first of these obstacles can be overcome by reduction in charge density and the second by partial replacement of water by a material with less capacity for forming hydrogen bonds and other enthalpically favourable associations with the polymer chains. To a first level of approximation, these simple considerations explain the large increases in moduli observed on: (i) reduction in pH to below the pK_a of galacturonic acid (Fig. 2) and (ii) progressive increase in sucrose concentration (Fig. 10). The detail of the structures and processes involved, however, is more complex.

For 0.5 wt% pectin (DE 70.3) in the presence of 65 wt% sucrose at pH 4.7, where the extent of interchain association is just sufficient to give a continuous network (Fig. 3b) at 5°C, there is no detectable difference between the changes in moduli observed on cooling and on heating (Fig. 4). As indicated in Fig. 6, however, substantial hysteresis develops on progressive reduction in pH. Thermal hysteresis between formation and melting of polysaccharide gels is normally

associated with development of large aggregates, which stabilise the constituent ordered structures to temperatures above those at which they will form in isolation (Morris & Norton, 1983). Previous studies (Morris, Gidley, Murray, Powell & Rees, 1980) of the ability of short chain segments to reduce gel strength by "competitive inhibition" of associations between intact chains suggest that the junctions in high-methoxy pectin gels do indeed consist of large, aggregated assemblies. The changes in moduli observed during heating of samples studied in the present work (Figs. 5b and 10), however, indicate formation of new intermolecular junctions, rather than simple stabilisation of the structures formed initially during cooling.

An increase in gel strength with increasing temperature has been reported previously (Oakenfull & Scott, 1984) from penetration testing of high-methoxy pectin gels (0.36 wt%; DE 69.7; 55 wt% sucrose; 0.21 wt% citric acid) prepared and measured in constant-temperature rooms. Our present results show that the enhancement in gel strength occurs rapidly during heating (rather than developing during storage) and that it extends to a wide range of pH values and sucrose concentrations. Under the experimental conditions used by Oakenfull and Scott (1984), the increase in rupture strength occurred over the approximate temperature range 15–30°C. It was preceded by a reduction in strength at lower temperatures, as found here (Figs. 5 and 10), and followed by a further decrease at higher temperatures (~35 to ~50°C), which was not observed in the small-deformation studies carried out in the present work.

The central conclusion from both investigations, however, is that two types of interaction are involved in intermolecular association of high-methoxy pectin, one of which is destabilised by increasing temperature, while the other becomes more stable. The interpretation proposed by Oakenfull and Scott (1984) was that these interactions correspond to hydrogen bonding and hydrophobic association, respectively. We suggest that the first assignment should be broadened to include other types of thermally labile non-covalent interactions (dipolar and van der Waals), but agree fully with the second.

There is normally a slight enthalpic advantage in apolar groups (such as the methyl substituents of the ester groups in pectin) being surrounded by water, rather than by other groups of the same type. The drive to self-association (e.g. Tanford, 1980) comes from reduction in the number of possible hydrogen bonds that can be formed by water molecules in the vicinity of the apolar species (which, of course, cannot participate in hydrogen bonding), with consequent loss of entropy. The entropic disadvantage of exposure of apolar groups to the aqueous environment becomes progressively more significant as the temperature is raised ($\Delta G = \Delta H - T\Delta S$), and can ultimately outweigh the enthalpic advantage of hydration, with consequent clustering of the apolar ("hydrophobic") species.

Pectin chains exist in the solid state in a 3-fold ordered conformation. Analysis of X-ray fibre diffraction data by computer modelling (Walkinshaw & Arnott, 1981) suggests that the methyl groups of the esterified residues are grouped together in trigonal channels formed by three adjacent (parallel) chains in a hexagonal lattice. Development of this type of structure under hydrated conditions would explain the increases in moduli observed (Figs. 5 and 10) when high-methoxy pectin gels are heated.

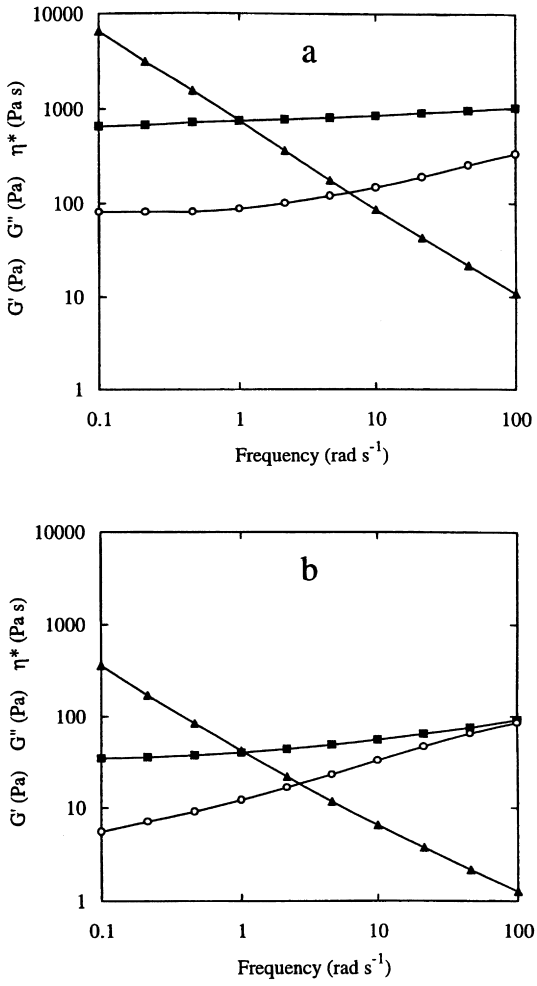

Fig. 23. Mechanical spectra (0.5% strain) showing the frequency-dependence of G' (■), G'' (○) and η^* (▲) for 0.5 wt% high-methoxy pectin (pH 3.0) after cooling from 95 to 5°C in the presence of (a) glucose and (b) fructose at a concentration of 57.9 wt% (equivalent to 55 wt% sucrose).

The concept of two different mechanisms of interchain association may also explain the "thermal annealing" effects illustrated in Figs. 11–13. The increases in moduli attributable to hydrophobic association during heating begin (Figs. 5 and 10) at ~15–20°C. It seems likely, therefore, that hydrophobic junctions present initially in the solid state will remain intact during sample preparation at high temperature (95°C), thus preventing the polymer from forming a true solution of individual chains. During initial cooling from 95 to 5°C, these pre-existing junctions will dissociate. The development of a gel network, however, shows that they are replaced by another type of intermolecular association.

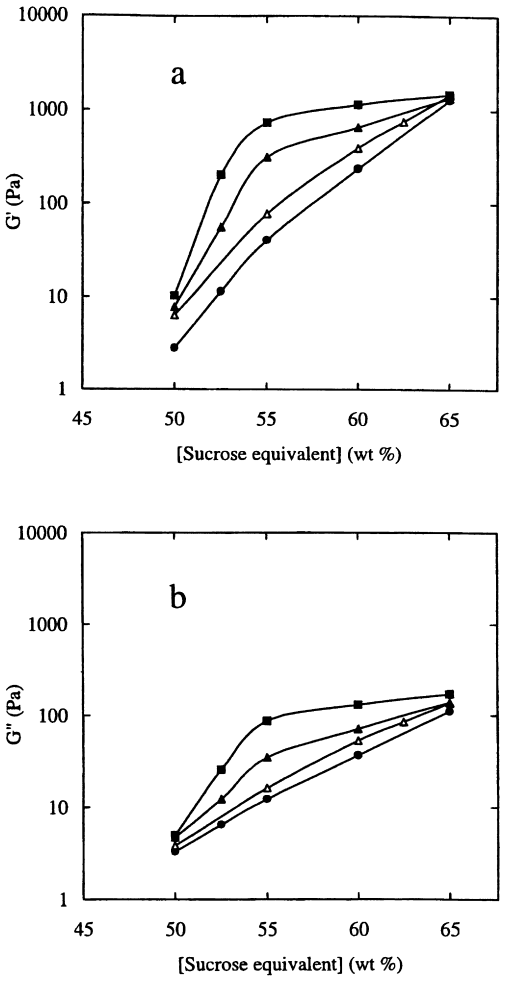

Fig. 24. Observed values of (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain) for 0.5 wt% high-methoxy pectin (pH 3.0) after initial cooling from 95 to 5°C in the presence of 50–65 wt% sucrose (△), in comparison with the moduli obtained using equivalent concentrations of glucose (■), fructose (●) or equimolar mixtures of both (▲).

Recent studies (Gilsenan, Richardson & Morris, 2000) have shown that low methoxy pectin at acidic pH gives thermally reversible gels that form and melt over approximately the same range of temperatures as the gelling transitions shown in Figs. 5 and 10 for high-methoxy pectin. It seems reasonable to conclude that the same process is involved in both the cases (i.e. formation of a pectinic acid gel). The thermoreversibility of the pectinic acid network would then explain the initial reductions in moduli during heating, which follow the same temperature-course as the changes observed on cooling (Figs. 5 and 10).

Incorporation of esterified residues into pectinic acid junctions will involve a greater reduction in entropy than for unesterified residues, because of loss of rotational mobility about the C–O–CH_3 bonds, and the physical presence of the methyl groups may also hinder close-packing of the polymer chains. It seems likely, therefore, that the stability of the pectinic acid junctions will decrease with increasing ester content, whereas the stability of hydrophobic junctions will, of course, increase with ester content. Thus, the hydrophobic structures that dissociate most readily during cooling will be the ones best suited to formation of a pectinic acid gel, and conversely the acidic junctions that dissociate most readily during heating will have the greatest capacity for hydrophobic association. The increases in gel strength produced by thermal cycling may therefore arise from progressive "polarisation" into regions with lower than average ester content, involved preferentially in acid-induced gelation, and regions of higher than average ester content, involved preferentially in hydrophobic association, with consequent increase in the stability of the pectinic acid network at low temperature and of the hydrophobic network at high temperature.

Conversely, however, the extent of dissociation of each type of structure at the opposite extremes of the temperature range would be expected to increase with progressive segregation into junctions of high and low ester content. This may explain the increased sol fraction (Figs. 14 and 15) after thermal annealing (i.e. arising from greater release of disordered chains on dissociation of hydrophobic junctions preferentially enriched in esterified residues). The concept of interchange between acidic and hydrophobic junctions might also explain why the reduction in gel strength at low temperature with decreasing sucrose content (Fig. 10) or increasing pH (Fig. 5) is accompanied by an increase in the extent of hydrophobic association on heating.

Finally, the large changes observed (Figs. 17 and 20–22) on replacement of sucrose by glucose or fructose (or by mixtures of the two monosaccharides) are particularly intriguing. As outlined at the beginning of the this section, the primary effect of introducing large concentrations of a cosolute into aqueous biopolymer systems is to reduce the concentration of water, with consequent decrease in the effectiveness of polymer–solvent interactions in competing with polymer–polymer interactions, thus promoting self-association of the polymer chains. Different cosolutes, however, differ substantially in their effectiveness in promoting association of biopolymers into intermolecular junctions. These differences are normally attributed to modification of water structure by the cosolute, with consequent modification of the competition between polymer–water and polymer–polymer interactions.

For sugars, the order of effectiveness can often be correlated with the content of equatorial hydroxyl groups, whose spacing is particularly compatible with the "lattice" structure of water (Tait, Suggett, Franks, Ablett & Quickenden, 1972). In terms of this interpretation, the order expected for the three sugars studied in the present work would be: fructose $<$ glucose $<$ sucrose (e.g. Watase, Kohyama & Nishinari, 1992). This sequence has indeed been observed experimentally for a number of gelling biopolymers, including gelatin (Oakenfull & Scott, 1986), κ-carrageenan (Nishinari & Watase, 1992) and agarose (Watase et al., 1992), and for initial conformational ordering

of the partially depolymerised amylopectin (oxidised starch) material studied in the investigation reported in the following paper (Evageliou, Richardson & Morris, 2000c).

As shown in Fig. 17, however, the order of effectiveness in promoting gelation of high-methoxy pectin at acidic pH follows the sequence: fructose < sucrose < glucose. The same order of effectiveness has been observed previously (May & Stainsby, 1986) for glucose syrups and high-fructose syrups in comparison with sucrose. The possible origin of this departure from the normal sequence (fructose < glucose < sucrose) is suggested by an elegant theoretical and experimental study by Nilsson, Piculell and Malmsten (1990). The polymer studied was agarose, and it was found that the order of effectiveness of a wide range of cosolutes in inducing helix formation (as characterised by the onset-temperature for conformational ordering on cooling) was strongly correlated to their order of elution from an agarose column, thus clearly indicating that direct interactions between polymer and cosolute have an important role in modifying conformational stability.

The effect of one cosolute (urea) was explored in detail, using a lattice model in which all possible pairwise interactions between the three constituents were considered explicity. The central conclusions was that water–cosolute interactions may dictate the overall properties of the system (thus explaining why the same order of effectiveness is often found for the same cosolutes in combination with different biopolymers), but that they act indirectly, by competing with polymer–cosolute interactions, which are the direct determinant of changes in conformational stability. Departures from the general order of effectiveness, which would be hard to explain in terms of modification of water structure, then become an expected consequence of particularly favourable, or particularly unfavourable, interactions between the polymer and the cosolute. The concept of displacement of conformational equilibria by enhancement (or depletion) of the concentration of cosolute molecules surrounding the polymer chains would also explain why changes in sugar type have a much greater effect on the sol–gel transition temperatures (Fig. 17) than on the final moduli of the fully formed gel networks (Fig. 18).

The order of effectiveness observed in the present work implies that association with the pectin chain (inhibiting formation of intermolecular junctions) is strongest for fructose and weakest for glucose, with sucrose showing intermediate adsorption. An explanation of this sequence is suggested by a study of high-methoxy pectin in methanol–water mixtures (Plashchina et al., 1986), in which it was concluded that the hydroxyl groups of methanol form strong hydrogen bonds with the lone-pair electrons of the carbonyl moiety at C(6), giving a stoichiometric complex. The particular effectiveness of methanol was attributed to its high acidity in comparison with other aliphatic alcohols. The same concept of increasing strength of hydrogen bonding with increasing polarity (acidity) of the hydroxyl group would imply that primary alcohols should bind more strongly than secondary alcohols. Fructose, in its normal furanose ring form, has two primary alcohol groups (at C(1) and C(6)); glucose (in the preferred pyranose ring form) has only one (at C(6)); the average number per sugar ring in sucrose is therefore 1.5, giving the sequence observed experimentally (Fig. 17) for relative effectiveness in promoting gelation of high-methoxy pectin.

The other factor in promoting stable association of methanol with pectin (Plaschina et al., 1986) is the polarity of the carbonyl function, making it more effective as a receptor for hydrogen bonding than the hydroxyl groups of the sugar rings. Other polysaccharides with a high content of substituents in which this moiety is also present (carboxylic acid, ester and acyl groups) might therefore be expected to show the same sequence of response to the presence of sucrose, glucose or fructose as cosolute. The reduced viscosity of hyaluronic acid (which has carboxyl and *N*-acetyl substituents on alternate residues) in the presence of increasing concentrations of these three cosolutes has indeed been observed (Nakamura, 1999) to change in the sequence expected from increasing condensation of cosolute along the polymer chain, in the order: glucose < sucrose < fructose.

As an additional test of the proposed model, a brief investigation has recently been carried out (Tsoga, Richardson & Morris, unpublished) to explore the relative effectiveness of the same three sugars in inducing conformational ordering of high acyl gellan, which contains both *O*-acetyl and *O*-glyceryl substituents, in addition to glucuronic acid (Jansson, Lindberg & Sandford, 1983; Kuo, Mort & Dell, 1986; O'Neill, Selvendran & Morris, 1983). The onset-temperature for gel formation was found to follow the same order as in the high-methoxy pectin samples studied in the present work (fructose < sucrose < glucose). Further tests using different carboxylated and acylated polysaccharides are planned. For the moment, however, we conclude that the differences in gelation temperature shown in Fig. 17, and the associated changes in moduli at 5°C (Fig. 20), probably arise from progressive increase in hydrogen bonding of cosolute molecules to the polymer chain as their content of primary hydroxyl groups increases.

Acknowledgements

This work was carried out as part of an industrial–academic LINK project, "Behaviour of Biopolymer Mixtures in Structuring Food Products". We thank the participating companies (Unilever, Nestlé, Cerestar, Hercules and SKW Biosystems), and the UK Ministry of Agriculture, Fisheries and Food, for financial support. We also thank Dr. K. Nakamura, Rakuno Gakuen University, Japan for helpful discussions and communication of research results prior to publication.

References

Christensen, S. H. (1986). Pectins. In M. Glicksman, *Food Hydrocolloids*, vol. 3 (pp. 205–230). Boca Raton, FL: CRC Press.

Durrand, D., Delsanti, M., Adam, M., & Luck, J. M. (1987). Frequency dependence of viscoelastic properties of branched polymers near gelation threshold. *Europhysics Letters*, *3*, 297–301.

Evageliou, V., Richardson, R. K., & Morris, E. R. (2000a). Effect of oxidised starch on high methoxy pectin–sucrose gels formed by rapid quenching. *Carbohydrate Polymers*, *42*, 219–232.

Evageliou, V., Richardson, R. K., & Morris, E. R. (2000b). Co-gelation of high methoxy pectin with oxidised starch or potato maltodextrin. *Carbohydrate Polymers*, *42*, 233–243.

Evageliou, V., Richardson, R. K., & Morris, E. R. (2000c). Effect of sucrose, glucose and fructose on gelation of oxidised starch. *Carbohydrate Polymers*, *42*, 261–272.

Gilsenan, P. M., Richardson, R. K., & Morris, E. R. (2000). Thermally-reversible acid-induced gelation of low-methoxy pectin, *Carbohydrate Polymers*, *41*, 339–349.

Jansson, P.-E., Lindberg, B., & Sandford, P. A. (1983). Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea*. *Carbohydrate Research*, *124*, 135–139.

Kuo, M.-S., Mort, A. J., & Dell, A. (1986). Identification and location of L-glycerate, an unusual acyl substituent in gellan gum. *Carbohydrate Research*, *156*, 173–187.

May, C. D., & Stainsby, G. (1986). Factors affecting pectin gelation. In G. O. Phillips, D. J. Wedlock & P. A. Williams, *Gums and Stabilisers for the Food Industry 3* (pp. 515–523). London: Elsevier.

Morris, E. R., & Norton, I. T. (1983). Polysaccharide aggregation in solutions and gels. In E. Wyn-Jones & J. Gormally, *Aggregation Processes in Solution* (pp. 549–593). Amsterdam: Elsevier.

Morris, E. R., Gidley, M. J., Murray, E. J., Powell, D. A., & Rees, D. A. (1980). Characterisation of pectin gelation under conditions of low water activity, by circular dichroism, competitive inhibition and mechanical properties. *International Journal of Biological Macromolecules*, *2*, 327–330.

Nakamura, K. (2000). Physico-chemical properties of aqueous sodium hyaluronate solutions. In K. Nisinari, *Proceedings of 4th International Conference on Hydrocolloids*, Osaka, Japan.

Nilsson, S., Piculell, L., & Malmsten, M. (1990). Nature of macromolecular denaturation by urea and other cosolutes: experiments on agarose interpreted within a lattice model for adsorption from a mixed solvent. *Journal of Physical Chemistry*, *94*, 5149–5154.

Nishinari, K., & Watase, M. (1992). Effects of sugars and polyols on the gel–sol transition of kappa-carrageenan gels. *Thermochimica Acta*, *206*, 149–162.

Oakenfull, D., & Scott, A. (1984). Hydrophobic interactions in the gelation of high methoxy pectins. *Journal of Food Science*, *49*, 1093–1098.

Oakenfull, D., & Scott, A. (1986). Stabilisation of gelatin gels by sugars and polyols. *Food Hydrocolloids*, *1*, 163–175.

O'Neill, M. A., Selvendran, R. R., & Morris, V. J. (1983). Structure of the acidic extracellular gelling polysaccharide produced by *Pseudomonas elodea*. *Carbohydrate Research*, *124*, 123–133.

Plashchina, I. G., Braudo, E. E., & Tolstoguzov, V. B. (1978). Circular dichroism studies of pectin solutions. *Carbohydrate Research*, *60*, 1–8.

Plashchina, I. G., Semenova, M. G., Slovokhotov, Yu. L., Struchkov, Yu. T., Braudo, E. E., Tolstoguzov, V. B., & Ignatov, G. I. (1986). Studies of the interaction of methanol with pectin in aqueous solution. *Carbohydrate Polymers*, *6*, 1–13.

Rolin, C. (1993). Pectin. In R. L. Whistler & J. N. BeMiller, *Industrial Gums: Polysaccharides and their Derivatives* (3rd Ed.) (pp. 257–293). San Diego, CA: Academic Press.

Ross-Murphy, S. B. (1984). Rheological methods. In H. W.-S. Chan, *Biophysical Methods in Food Research* (pp. 195–290). *Critical reports on applied chemistry*. London: SCI.

Tait, M. J., Suggett, A., Franks, F., Ablett, S., & Quickenden, P. A. (1972). Hydration of monosaccharides: a study by dielectric and nuclear magnetic relaxation. *Journal of Solution Chemistry*, *1*, 131–151.

Tanford, C. (1980). *The hydrophobic effect: formation of micelles and biological membranes*. New York: Wiley.

Te Nijenhuis, K., & Winter, H. H. (1989). Mechanical properties at the gel point of a crystallizing poly (vinyl chloride) solution. *Macromolecules*, *22*, 411–414.

Walkinshaw, M. D., & Arnott, S. (1981). Conformations and interactions of pectins II. Pectinic acid and calcium pectate gels. *Journal of Molecular Biology*, *153*, 1075–1085.

Watase, M., Kohyama, K., & Nishinari, K. (1992). Effects of sugars and polyols on the gel–sol transition of agarose by differential scanning calorimetry. *Thermochimica Acta*, *206*, 163–173.