Carbohydrate Polymers **23** (1994) 265–273
© 1994 Elsevier Science Limited.
Printed in Great Britain. All rights reserved
0144-8617/94/$07.00

Pumpkin pectin: gel formation at unusually low concentration

Nataliya M. Ptitchkina, Irina A. Danilova

The Institute of Chemistry of Saratov State University, Astrakhanskaya 83, Saratov 410601, Russia

Georgios Doxastakis,* Stefan Kasapis & Edwin R. Morris[†]

Department of Food Research and Technology, Cranfield University, Silsoe College, Silsoe, Bedford MK45 4DT, UK

(Received 19 August 1993; revised version received 9 December 1993; accepted 20 December 1993)

The gel properties of high-methoxy pectin from pumpkins have been investigated to assess the potential of this material as a hard-currency export from the former Soviet Union. Comparison was made with commercial slow-set, medium-set and rapid-set pectins from citrus peel. Gels were formed by cooling pectin solutions (pH 3·0; 60% (w/w) sucrose; 5% (w/w) corn syrup) from 95°C to 25°C, and the time–temperature course of network formation was monitored by small-deformation oscillatory measurements of storage modulus (G'). At concentrations above ~1% (w/w) the pumpkin pectin gave weaker gels than the other three samples, but its minimum critical gelling concentration (c_0) was found to be much lower (by at least a factor of five). Compression testing gave similar results, with pumpkin pectin giving useful breaking-stress ('hardness') at concentrations down to ~0·5% (w/w), about a factor of two lower than for the citrus samples. Its gelation was also less rapid, giving G' values below those of the other three samples at temperatures down to ~60°C, but then setting sharply; this behaviour could be useful in avoiding 'pregelation' in commercial processing. The commercial slow-set pectin showed typical 'weak gel' properties in the solution state at 95°C, with systematic reduction in gel-like character with increasing ester content in the other samples. The rigidity of the final gels also decreased systematically through the series: rapid-set < medium-set < slow-set. These observations are tentatively ascribed to stable association of unesterified galacturonate chain segments at low pH, where electrostatic repulsion is suppressed.

INTRODUCTION

Pectins have a very long history of practical use as gelling agents in food, with their molecular interactions being manipulated, largely unwittingly, in domestic production of jams and jellies (Whistler & BeMiller, 1993). The polymer backbone is based on (1 → 4)-linked α-D-galacturonate residues, a large, and varying, proportion of which occur as the methyl ester. The galacturonate chains, however, are interrupted by periodic insertion of (1 → 2)-linked rhamnosyl residues, which act as the point of attachment for neutral sugar side-chains linking the pectin molecules into the polysaccharide matrix of the plant cell wall (Selvendran, 1985). It has been shown that, at least for citrus pectins, the length of galacturonate sequences between rhamnosyl 'kinks' is virtually constant at ~25 residues (Powell *et al.*, 1982).

In commercial extraction of pectin (May, 1990) the sidechains are cleaved, and the proportion of esterified galacturonate residues (the 'degree of esterification', DE) altered to give the required gelling characteristics. Pectins of low ester content ('low methoxy'; DE < 50%) are normally gelled by calcium ions, and are often used in products where a low sugar content is required. Most applications, however, use pectins with a higher ester content, and gelation is induced by lowering the pH (to decrease intermolecular electrostatic repulsion between unesterified carboxyl groups) and by addition of high concentrations of co-solute (typically ~60% (w/w) sucrose) to reduce the solvent quality of the water. The

---

*Visiting professor from the Laboratory of Food Chemistry and Technology, Department of Chemistry, Aristotle University of Thessaloniki, Greece.
[†] To whom correspondence should be addressed.

rate of gelation on cooling hot solutions of 'high-methoxy' pectins under conditions of low pH and reduced water activity increases with ester content, and commercial materials are commonly classified as 'slow-set', 'medium-set' and 'rapid-set', with typical DE values (Christensen, 1986) of 62%, 68% and 72%, respectively.

The mechanism of calcium-induced gelation of low-methoxy pectin at neutral pH and in the presence of a large excess of monovalent cations is well understood. The junctions are dimeric assemblies of polygalacturonate in the 2_1 ('zig-zag') conformation, sandwiching an array of site-bound calcium ions (Morris *et al.*, 1982). The minimum sequence-length requirement for stable association is seven calcium ions bound to consecutive unesterified residues on each of the participating strands (Kohn, 1975; Powell *et al.*, 1982). Under more normal conditions of practical use, at acid pH (Gidley *et al.*, 1980) and with lower levels of other counterions (Powell *et al.*, 1982), however, the sequence-length requirements for ordered association are far less stringent, and the gelation mechanism is more complex and, as yet, incompletely understood.

Gelation of high-methoxy pectins is, arguably, even more complex. At the simplest level of interpretation, ester groups will facilitate intermolecular association by replacing ionisable carboxyl groups, and thus decreasing electostatic repulsion between chains. However, when comparisons are made between high-methoxy and low-methoxy samples brought to the same overall charge density by appropriate adjustment of pH (Morris *et al.*, 1980), it is clear that methyl substituents have an active role in promoting chain–chain association. Their likely mechanism of action is indicated by X-ray fibre diffraction analysis, which shows three-fold chains with interleaved arrays of methyl groups running between them (Walkinshaw & Arnott, 1981). Hydrophobic interactions (Tanford, 1980) may therefore make a significant contribution to junction stability. However, although the rate of gelation of high-methoxy pectins decreases as the ester content is lowered, the final gel strength increases (Doesburg & Grevers, 1960), suggesting that non-esterified residues may also have a role in intermolecular association.

The function of the co-solute also seems more complex than simple competition for available water. In particular, substitution of corn syrup for sucrose causes a major increase in the temperature at which gelation occurs on cooling (Christensen, 1986), and studies of model systems using ethylene glycol as co-solute have shown that it may actually promote solubility of pectins with very high DE, rather than the normal converse effect of encouraging intermolecular association (Morris *et al.*, 1980; Jones, 1992). Finally, the possible role of residual neutral sugar side-chains in modulating network properties remains virtually unexplored (Hwang *et al.*, 1993).

The main sources of commercial pectins are citrus peel and apple pomace, produced as by-products of the fruit-juice and cider industries, respectively (Christensen, 1986). Recent work in Saratov has shown that good-quality pectin can be extracted from pumpkins by a commercially-viable route, with potential both for domestic use within Russia, and for development as a hard-currency export from the former Soviet Union.

Preliminary analysis of this material has shown that it has a galacturonate content of ~50% (in comparison with ~80% for normal citrus pectins), indicating an unusually high content of neutral sugar side-chains. Fuller chemical analysis is in progress, and will be published later. The purpose of the present work is to compare the gel properties of pumpkin pectin with those of existing high-quality commercial products.

MATERIALS AND METHODS

Pumpkin pectin was extracted and purified by the following procedure. Freshly-pressed pumpkin pulp (the residue from juice extraction) was washed three times with warm water (50°C) for 30 min, surplus water was squeezed out through cotton fabric, and the remaining insoluble material was pressed to dryness. The residue (50 g) was then suspended in 0·1 M HCl (500 ml), stirred for 2 h at 65°C, and filtered. The filtrate was cooled to ambient temperature, mixed with twice its own volume of 96% ethanol, and left to stand overnight (16 h) for precipitation of pectin. The precipitated pectin was collected by filtration, washed with acidified aqueous alcohol (10 ml HCl in 1 litre of 70% v/v ethanol), washed again with pure ethanol, pressed, dried in a current of warm air (30–40°C), ground and sieved.

Samples of slow-set, medium-set and rapid-set citrus pectin were kindly supplied by Hercules. Since these commercial materials are 'standardised' to specified gel properties by blending with sucrose, the polysaccharide content was determined by extensive dialysis against distilled water (four changes) and freeze-drying, with the ratio of final weight to initial weight being used to determine the nominal concentration of each material needed to give the required 'true' concentration of polymer. For consistency, the pumpkin pectin sample was treated in the same way.

The degree of esterification and proportion of galacturonate residues (esterified or non-esterified) in the citrus pectin samples were kindly provided by Hercules, and are listed in Table 1. The corresponding values for pumpkin pectin were obtained by gas-liquid chromatographic analysis (Lehrfeld, 1987; Maness *et al.*, 1990), using a Hewlett-Packard HP5890A chromatograph (Ultra-1 column; temperature programmed from 150 to 200°C at 5°C/min).

For preparation of pectin solutions, the polymer (as received) was first dry-blended with five times its own weight of sucrose (to aid dispersion), and dissolved in distilled water at ~90°C, using an Ultraturrax homogeniser at its lowest speed setting (6000 rev/min). The solution was then cooled to ambient temperature, adjusted to pH 3·0 with HCl, and re-heated to 95°C. Sucrose (normal food grade) and corn syrup (dextrose equivalent 42; Cerestar) were then added to final concentrations of 60 and 5% (w/w), respectively, with allowance being made for the sucrose used initially in dry-blending, and for the small amount (30–45%) present in the commercial samples, and dissolved by stirring for 3–4 min, again using the Ultraturrax at its lowest speed. The purpose of the corn syrup was to inhibit crystallisation of sucrose on cooling. The final solution was held at 95°C for ~30 min to de-gas, and was then used for the rheological studies described below.

Samples for compression-testing were filled into ring moulds (13-mm diameter; 13-mm height), sealed with lubricated cover-slips, and stored for 24 h at 5°C. Failure measurements were made at 5°C on a TA-XT2 texture analyser from Stable Microsystems. The samples were compressed at 0·8 mm/s between flat parallel plates, and the stress and strain at the yield point recorded. Each gel preparation was measured in triplicate, and the results averaged.

Low-amplitude oscillatory measurements (0·5% strain) were made using cone-and-plate geometry (50-mm diameter; 0·02 rad cone angle) on a sensitive prototype rheometer designed and constructed by Dr R.K. Richardson (Silsoe College). The hot solutions were loaded onto the rheometer at 95°C and network formation monitored on cooling to 25°C at 1°C/min. Measurements were then continued for a further 2 h at 25°C, and a full mechanical spectrum recorded to show the frequency-dependence of storage modulus (G'), loss modulus (G'') and complex dynamic viscosity ($\eta*$) for the final gel. The gels were then re-heated to 95°C (at 1°C/min), held there for ~3 min, and their mechanical response again measured as a function of frequency. The mechanical spectra of selected samples were also recorded immediately after loading at 95°C. A few comparative measurements of the strain-dependence of mechanical response were made using samples gelled in the same way on a Carri-Med CS500 controlled-stress rheometer (using cone-and-plate geometry of 50-mm diameter and 2° cone angle).

For determination of intrinsic viscosity of the pectin samples, stock solutions were dialysed extensively against 0·1 M NaCl (six changes), and the final dialysate was used for all subsequent dilutions. Measurements were made at 25°C on a Contraves Low Shear 30 viscometer, with cup-and-bob geometry of inner and outer radii 5·5 mm and 6·0 mm, respectively, using solutions with relative viscosities within the range $1{\cdot}2 < \eta_{rel} < 2{\cdot}0$. Intrinsic viscosity was determined by averaging 'single-point' values of $[2(\eta_{sp} - \ln \eta_{rel})]^{1/2}/c$ for four different concentrations of each sample (where $\eta_{rel} = \eta/\eta_s$ and $\eta_{sp} = \eta_{rel} - 1$, with η and η_s denoting the viscosity of the solution and the solvent, respectively). The applicability of the single-point procedure to pectin solutions has been demonstrated previously (Kravtchenko & Pilnik, 1990). The values obtained were also verified by Huggins and Kraemer extrapolations of η_{sp}/c and $\ln(\eta_{rel})/c$, respectively, to zero concentration (c).

RESULTS

Figure 1 shows the time–temperature course of gel formation for all four samples at the same concentration of polymer (1%, w/w). The rapid-set pectin gave an immediate increase in G' as soon as cooling commenced from 95°C, but the medium-set and, particularly, the slow-set samples showed an initial 'lag period' with little change in modulus before the onset of the steep increase. The initial lag period is particularly evident for the pumpkin pectin, with development of G' showing distinct sigmoidal character. Network formation for all four samples was largely confined to the cooling step, with only limited further increase in modulus on holding at 25°C.

As observed by 'sag' measurements (Doesburg & Grevers, 1960), the final moduli of the commercial samples decreased systematically through the series: slow-set > medium-set > rapid-set. The same progression is evident in the moduli recorded at 95°C, immedi-

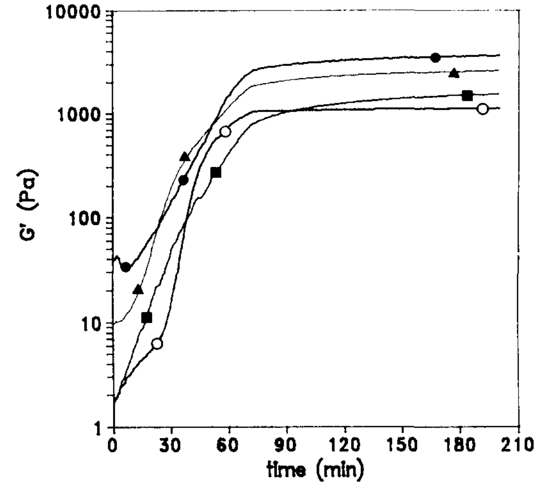

Fig. 1. Time–temperature course of gel formation for 1% (w/w) preparations of pumpkin pectin (○) and slow-set (●), medium-set (▲) and rapid-set (■) citrus pectins. The first 70 min corresponds to cooling from 95°C to 25°C at 1°C/min; the samples were then held at 25°C. Measurements were made at 1-min intervals; the symbols are included solely to identify individual curves.

ately after the samples were loaded onto the rheometer.

Figure 2 shows the mechanical spectrum (variation of G', G'' and $\eta*$ with frequency, ω) for the slow-set sample (1%, w/w) in the high-temperature solution state. The spectrum has obvious gel-like character ($G' > G''$; little frequency-dependence in either modulus; linear decrease in log $\eta*$ with increasing log ω). Indeed, it is closely similar to the spectra observed for the 'weak gels' formed by xanthan in its ordered conformation (Ross-Murphy *et al.,* 1983). Gel-like response was progressively less evident for solutions of the medium-set and rapid-set materials. As indicated in Fig. 1, the behaviour of pumpkin pectin at 1% (w/w) is closely similar to that of the rapid-set citrus pectin sample at both high and low temperature, although the time–temperature course of gel formation is substantially different.

Figure 3 shows the gelation curves from Fig. 1 rescaled by dividing throughout by the initial modulus at 95°C. The expected increase in gelation rate through the series — slow-set < medium-set < rapid-set — is now evident, and the difference in the gelation behaviour of the pumpkin pectin is clear.

As illustrated in Fig. 4, the gels retain their structural integrity on re-heating to 95°C, although with a significant reduction in modulus. Figures 5, 6 and 7 show a direct comparison of mechanical spectra at 25°C and 95°C for pumpkin pectin gels at polymer concentrations of 4·0, 2·0 and 0·3% (w/w), respectively. Throughout this concentration range, the high-temperature spectra remain typically gel-like (Ross-Murphy, 1984).

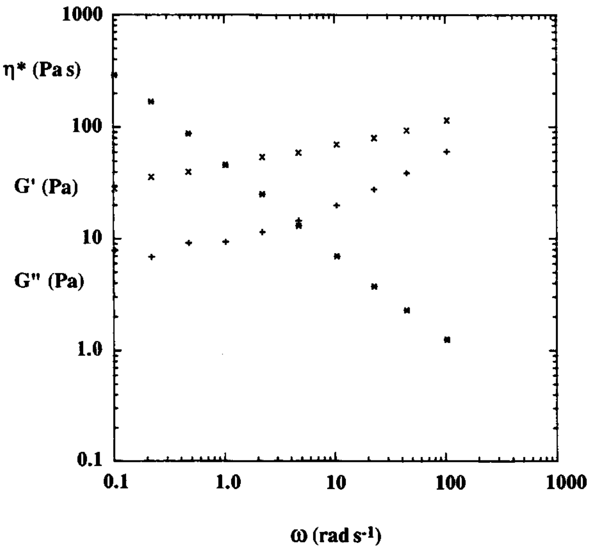

Fig. 2. Frequency (ω) dependence of G' (×), G'' (+) and $\eta*$ (*) for a 1% (w/w) preparation of slow-set citrus pectin (pH 3·0; 65% (w/w) co-solute) in the solution state at 95°C.

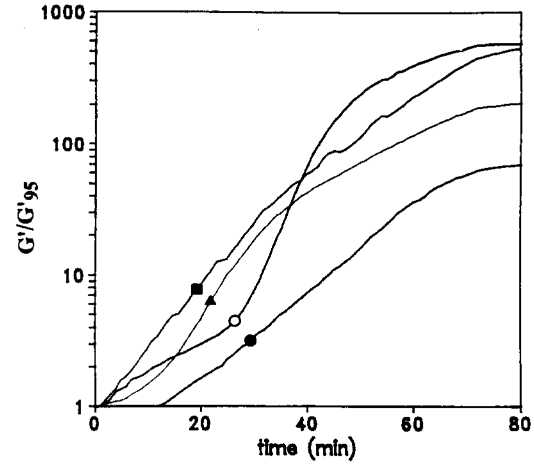

Fig. 3. Time–temperature curves from Fig. 1, re-scaled by dividing throughout by the initial modulus at 95°C (G'_{95}): slow-set (●); medium-set (▲); rapid-set (■); pumpkin (○).

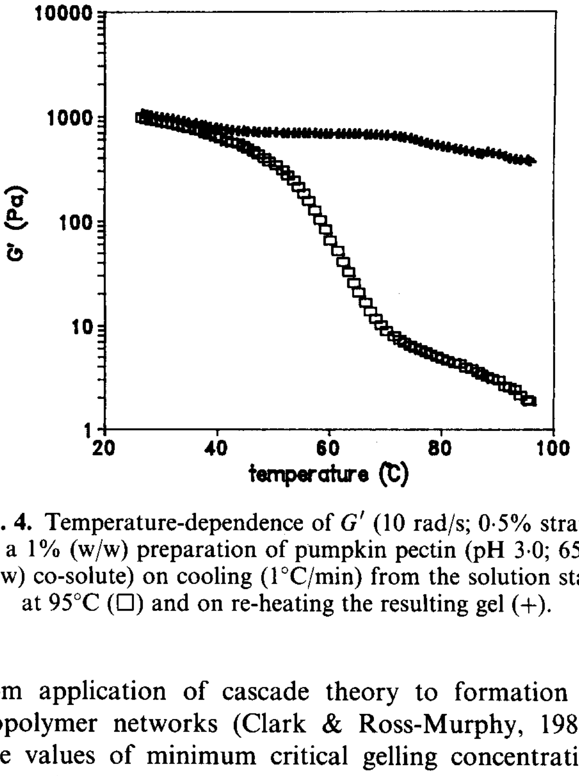

Fig. 4. Temperature-dependence of G' (10 rad/s; 0·5% strain) for a 1% (w/w) preparation of pumpkin pectin (pH 3·0; 65% (w/w) co-solute) on cooling (1°C/min) from the solution state at 95°C (□) and on re-heating the resulting gel (+).

The concentration-dependence of G' for the fully-formed gels at 25°C is shown in Fig. 8 for all four samples. In each case, the results can be fitted, with acceptable precision, to the general form anticipated from application of cascade theory to formation of biopolymer networks (Clark & Ross-Murphy, 1985). The values of minimum critical gelling concentration (c_0) and number of binding sites per chain ('functionality', f) derived from the computer fit to the experimental data are listed in Table 1.

The failure properties of the gels under progressively increasing amplitude of oscillatory shear are illustrated in Fig. 9 for 2% (w/w) preparations of pumpkin pectin and rapid-set citrus pectin. It is clear that the strain of 0·5% used as standard in the experiments described above is well within the region of linear response (i.e. where moduli are independent of frequency). For both samples shown in Fig. 9, breakdown begins at ~20% strain, and is marked by an initial rise in G'' as large cross-linked fragments are separated from the network and released into the sol fraction. At higher strains, as

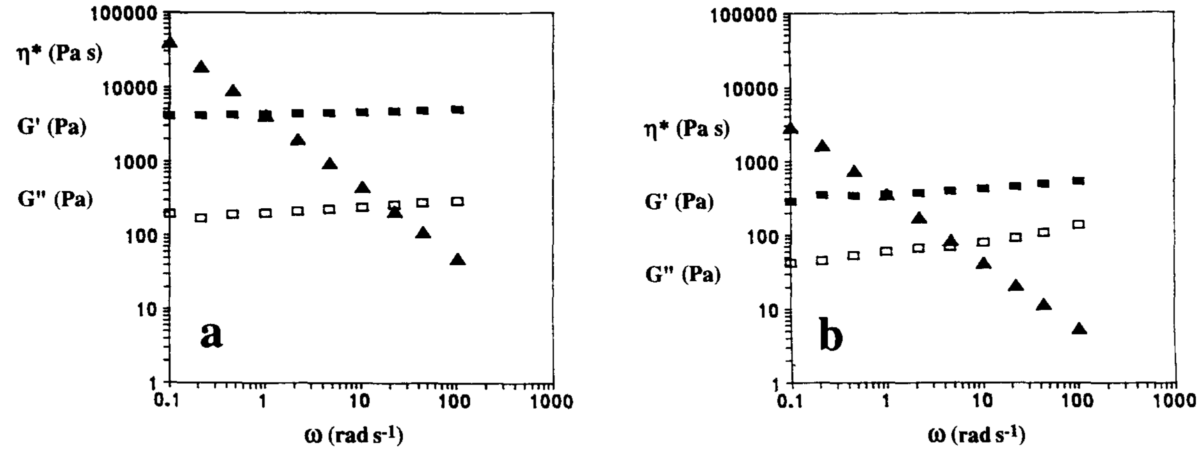

Fig. 5. Mechanical spectra (0·5% strain) for 4·0% (w/w) pumpkin pectin in the gel state (pH 3·0; 65% co-solute) at (a) 25°C and (b) 95°C, showing the variation of G' (■), G'' (□) and $\eta *$ (▲) with frequency (ω).

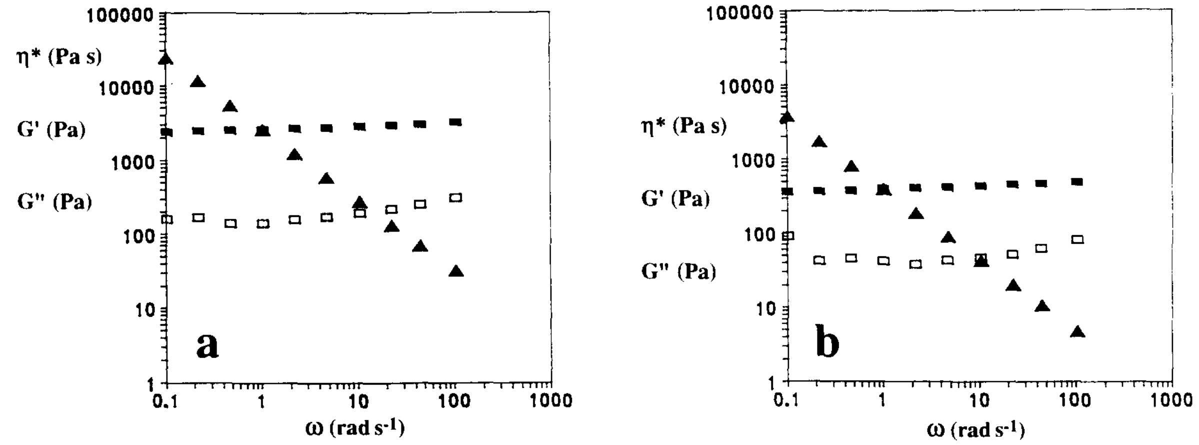

Fig. 6. Mechanical spectra (0·5% strain) for 2·0% (w/w) pumpkin pectin in the gel state (pH 3·0, 65% co-solute) at (a) 25°C and (b) 95°C, showing the variation of G' (■), G'' (□) and $\eta *$ (▲) with frequency (ω).

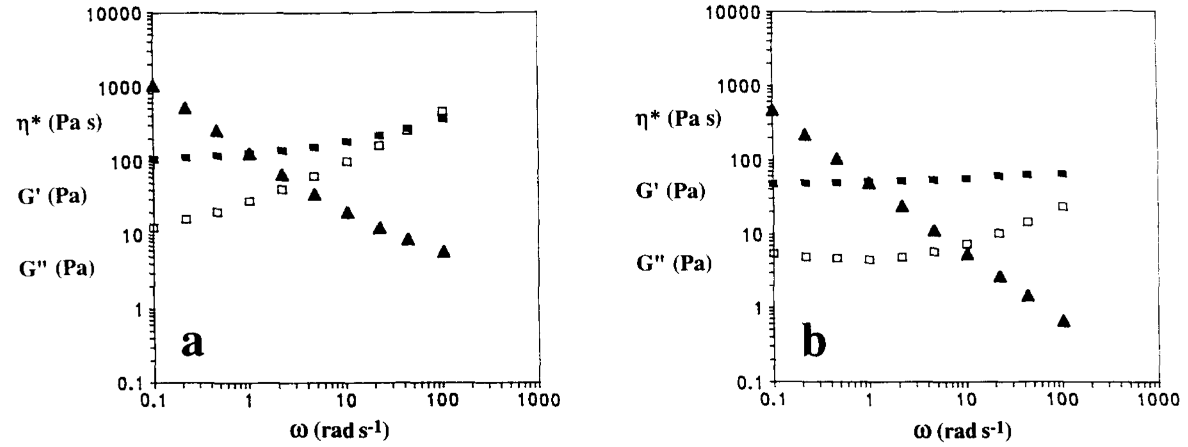

Fig. 7. Mechanical spectra (0·5% strain) for 0·3% (w/w) pumpkin pectin in the gel state (pH 3·0, 65% co-solute) at (a) 25°C and (b) 95°C, showing the variation of G' (■), G'' (□) and $\eta *$ (▲) with frequency (ω).

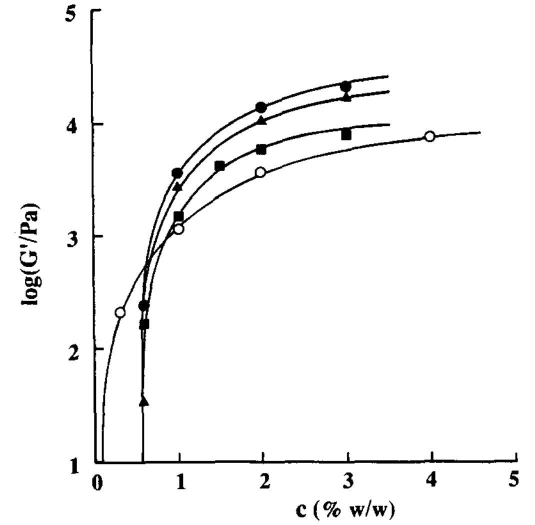

Fig. 8. Concentration-dependence of G' (10 rad/s; 0·5% strain; 25°C) for gels (pH 3·0; 65% co-solute) of pumpkin pectin (○) and slow-set (●), medium-set (▲) and rapid-set (■) citrus pectin prepared by cooling from 95°C to 25°C at 1°C/min and holding at 25°C for a further 2 h. The solid lines show cascade fits, yielding the values of functionality (f) and minimum critical gelling concentration (c_0) listed in Table 1.

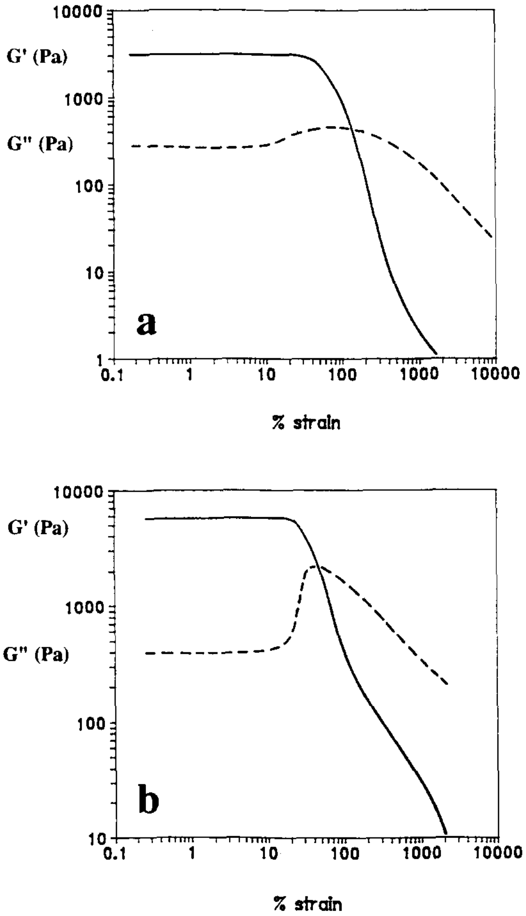

Fig. 9. Strain-dependence of G' (——) and G'' (-----), at 10 rad/s and 25°C, for 2% (w/w) gels of (a) pumpkin pectin and (b) rapid-set citrus pectin.

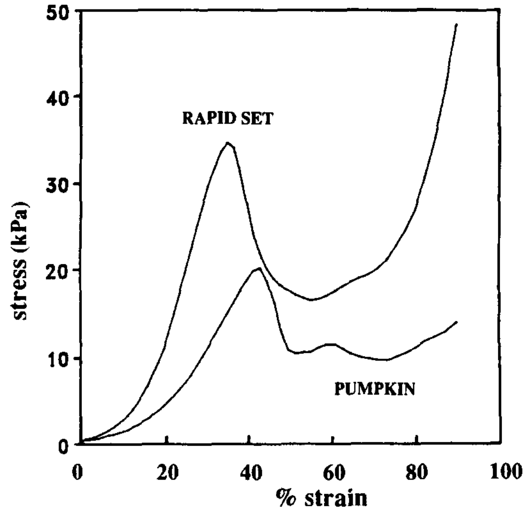

Fig. 10. Illustrative stress–strain curves from compression testing (5°C; 0·8 mm/s; initial height of sample 13 mm) for 1% (w/w) gels of pumpkin pectin (lower curve) and rapid-set citrus pectin (upper curve).

the fragments are broken down further, G'' drops again to below its initial value for the intact gel. The initial rise and subsequent decrease in G'' are both accompanied by a sharp reduction in G', as the overall cohesion of the network is destroyed.

A more detailed study of the failure properties of gels produced by all four pectin samples over a wide range of concentration was made using compression testing. Figure 10 shows illustrative stress–strain curves for pumpkin pectin and rapid-set citrus pectin (at 1%, w/w). Both show a well-defined 'yield point' at a strain comparable to the onset of the steep decrease in G' under oscillatory shear (Fig. 9). Failure curves closely similar in form to those shown in Fig. 10 were observed for all other samples.

The strain required to break the network ('yield strain') showed no systematic variation with concentration or pectin type, the mean value for all samples being 0·40 ± 0·05. The corresponding values of stress at the point of failure ('yield stress') are shown in Fig. 11. Yield stress values for the three citrus pectin samples drop sharply towards zero at concentrations below ~1% (w/w). This is higher than the corresponding range of steep decrease in G' (Fig. 8), as would be expected in comparison of large-deformation and small-deformation properties, but the overall form of concentration-dependence is similar, with again a systematic decrease in gel strength through the series: slow-set > medium-set > rapid-set.

The concentration-dependence of yield stress for pumpkin pectin is quite different in form, with the force required to break the network increasing linearly with polymer concentration. In particular, it proved possible to produce demouldable gels of pumpkin pectin at

Table 1. Composition and properties of the pectin samples studied

Pectin sample	Degree of esterification[a] (%)	Galacturonate content[a] (%)	Intrinsic viscosity[b] (dl g^{-1})	c_0 (%, w/w)[c]	f
Slow-set	64	81	3·28 ± 0·07	0·38	7·3
Medium-set	67	83	3·31 ± 0·22	0·49	4·3
Rapid-set	70	83	3·42 ± 0·22	0·32	2·6
Pumpkin	65	50	3·37 ± 0·04	0·052	12·3

[a] Analytical values for the citrus pectin samples were kindly supplied by Dr Claus Rolin, Copenhagen Pectin Division of Hercules Inc.

[b] Intrinsic viscosity was measured at a salt (NaCl) concentration of 0·1 M.

[c] Values of minimum critical gelling concentration (c_0) and functionality (f) were obtained from a cascade-fit to the concentration-dependence of G' for samples cooled from 95°C to 25°C at 1°C/min and held at 25°C for a further 2 h.

concentrations where the citrus pectin preparations remained fluid (Fig. 11).

To determine whether or not this difference in gelation behaviour could be attributed to a difference in chain length, the intrinsic viscosity of all four samples was measured (at a constant ionic strength of 0·1). Figure 12 illustrates the satisfactory convergence of Huggins, Kraemer and single-point values (Morris, 1984). The mean and standard deviation of single-point values at four concentrations of each material are listed in Table 1. There is a slight decrease in $[\eta]$ for the citrus pectin samples through the series: rapid-set > medium-set > slow-set, as might be expected from the progressively harsher hydrolysis conditions required to remove a higher proportion of the ester substituents, but the differences are within the experimental scatter of results for the individual samples. More significantly, the intrinsic viscosity obtained for the pumpkin pectin is virtually identical to the mean of the values for the other three samples, showing that there are no significant differences in molecular size.

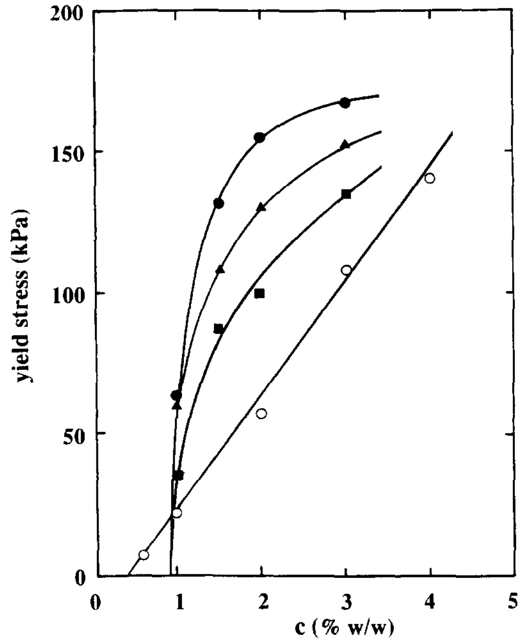

Fig. 11. Concentration-dependence of yield stress ('hardness') for gels (pH 3·0; 65% co-solute) of pumpkin pectin (○) and slow-set (●), medium-set (▲) and rapid-set (■) citrus pectin set for 24 h at 5°C and measured at the same temperature.

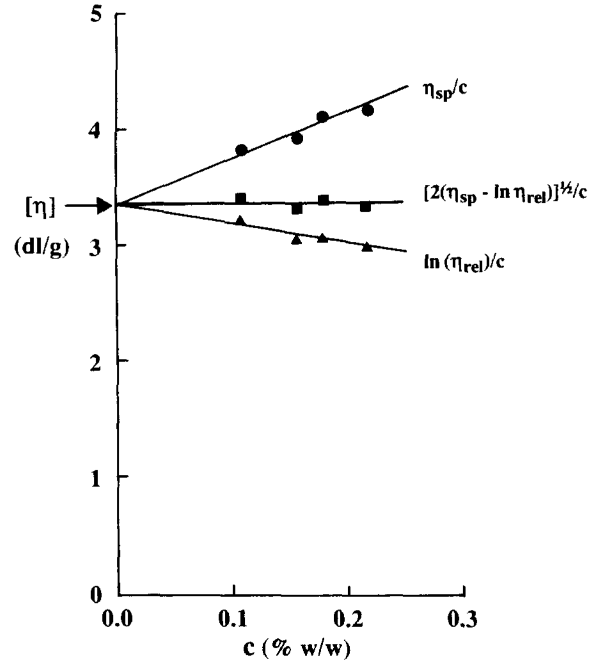

Fig. 12. Determination of intrinsic viscosity ($[\eta]$; 0·1 M NaCl), illustrated for pumpkin pectin, showing the agreement between Huggins (●), Kraemer (▲) and single-point (■) values. Similar plots were obtained for the citrus pectin samples. The mean and standard deviation of the single-point values for all four samples are listed in Table 1.

DISCUSSION

One immediate conclusion from this work is that the solutions of slow-set citrus pectin had already developed substantial 'weak gel' character at 95°C (Fig. 2) prior to formation of a cohesive 'true' gel network on cooling.

The magnitude of this effect decreases with increasing ester content (Fig. 1), and is reflected in the ultimate strength of the resulting gels at low temperature (Figs 8 and 11).

A possible interpretation is that, under conditions of reduced water activity, and at pH values well below the pK_a of galacturonic acid (~3·4), non-esterified chain sequences are capable of forming stable associations at high temperature, and that these associations persist on cooling, to augment the additional junctions formed between esterified residues at lower temperature. The ability of polygalacturonate sequences to form intermolecular associations at low pH, even in simple aqueous solution, has been demonstrated directly by light-scattering studies of de-esterified pectins (Sawayama *et al.*, 1988). Also, calorimetric studies of pectins in 50% glycerol (Sheyenson *et al.*, 1992), where thermal dissociation occurs within an accessible temperature range, suggest a qualitative change in the nature of intermolecular association as the ester content is reduced, with endotherms moving to lower temperature, as expected, but also becoming much sharper, indicating the progressive involvement of a different, more co-operative, mechanism of chain–chain association.

Although highly speculative, this proposal is consistent with the functionality values listed in Table 1. Since the cascade formalism was developed for networks formed by polymerisation of monomer units each capable of linking to only a specific (integer) number of other units, the absolute values obtained when it is applied to biopolymers must be treated with caution. However, there seems clear evidence of a systematic increase in the average number of junctions formed by each (citrus) pectin chain as the ester content is decreased.

The degree of esterification of the pumpkin pectin is very close to that of the slow-set citrus sample, but the value obtained for its functionality is substantially higher. More strikingly, the concentration-dependence of yield stress (Fig. 11) and time–temperature course of gelation (Fig. 3) for pumpkin pectin are different in form from those observed for the citrus pectins, and its minimum critical gelling concentration is far lower, although intrinsic viscosity measurements show that it is closely similar in molecular size (Table 1).

A further peculiarity of the pumpkin pectin system is that, while the gels formed at high concentration show the expected reduction in gel-like character on heating to elevated temperature (Fig. 5), those formed at low concentration, below c_0 for the citrus samples, become appreciably more gel-like (Fig. 7): although there is a slight reduction in overall moduli, the frequency-dependence becomes much less pronounced and the amount by which G' exceeds G'' increases. These observations indicate the involvement of some different mechanism of interaction, perhaps hydrophobic in character.

One possibility is that neutral sugar side-chains, which, from the unusually low content of galacturonate (Table 1), appear to be present at high concentration in pumpkin pectin, are directly involved in the gelation mechanism, particularly at the very low concentrations where citrus pectins of comparable molecular size do not gel.

Further interpretation of the possible role of neutral chains in intermolecular association must await determination of their primary structure. For the moment, however, the main conclusions from the present work are that pumpkin pectin, under conditions typical of normal industrial use, shows little development of structure at temperatures above ~60°C (Fig. 4), but then sets sharply on slight further cooling, and gives useful gel strength at concentrations substantially lower (by about a factor of two; Fig. 11) than high-quality industrial pectins from citrus peel. This combination of economy of use and likely ease of processing suggests that pumpkin pectin could be an attractive material for development as a commercial gelling agent.

ACKNOWLEDGEMENTS

We thank Professor S.B. Ross-Murphy for kindly providing a copy of his computer program for fitting modulus–concentration data to the cascade model. We also thank Dr C. Rolin for analytical data on the citrus pectins, Dr R.K. Richardson for generous access to his prototype rheometer, and Mr I. Chronakis for expert advice and practical assistance in carrying out the rheological measurements.

REFERENCES

Christensen, S.H. (1986). Pectins. In *Food Hydrocolloids, Vol. 3*, ed. M. Glicksman. CRC Press, Boca Raton, FL, USA, pp. 205–30.

Clark, A.H. & Ross-Murphy, S.B. (1985). The concentration dependence of biopolymer gel modulus. *Br. Polym. J.*, **17**, 164–8.

Doesburg, J.J. & Grevers, G. (1960). Setting time and setting temperature of pectin jellies. *Food Res.*, **25**, 634–45.

Gidley, M.J., Morris, E.R., Murray, E.J., Powell, D.A. & Rees, D.A. (1980). Evidence for two mechanisms of interchain association in calcium pectate gels. *Int. J. Biol. Macromol.*, **2**, 332–4.

Hwang, J., Pyun, Y.R. & Kokini, J.L. (1993). Sidechains of pectins: some thoughts on their role in plant cell walls and foods. *Food Hydrocolloids*, **7**, 39–53.

Jones, A.K. (1992). Hydrophobicity in polysaccharide gelation. PhD Thesis, Cranfield Institute of Technology, Silsoe College, Silsoe, Bedford, UK.

Kohn, R. (1975). Ion binding on polyuronates — alginate and pectin. *Pure Appl. Chem.*, **42**, 371–97.

Kravtchenko, T.P. & Pilnik, W. (1990). A simplified method for determination of the intrinsic viscosity of pectin solutions by classical viscosimetry. In *Gums and Stabilisers for the Food Industry 5*, eds G.O. Phillips, D.J. Wedlock & P.A. Williams. IRL Press, Oxford, UK, pp. 281–5.

Lehrfeld, J. (1987). Simultaneous gas-liquid chromatographic determination of aldoses and alduronic acids. *J. Chromatography*, **408**, 245–53.

Maness, N.O., Ryan, J.D. & Mort, A.J. (1990). Determination of the degree of methyl esterification of pectins in small samples by selectrive reduction of esterified galacturonic acid to galactose. *Analyt. Biochem.*, **185**, 346–52.

May, C.D. (1990). Industrial pectins: sources, production and applications. *Carbohydr. Polym.*, **12**, 79–99.

Morris, E.R. (1984). Rheology of hydrocolloids. In *Gums and Stabilisers for the Food Industry 2*, eds G.O. Phillips, D.J. Wedlock & P.A. Williams. Pergamon Press, Oxford, UK, pp. 57–78.

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. *Int. J. Biol. Macromol.*, **2**, 327–30.

Morris, E.R., Powell, D.A., Gidley, M.J. & Rees, D.A. (1982). Conformations and interactions of pectins I. Polymorphism between gel and solid states of calcium polygalacturonate. *J. Mol. Biol.*, **155**, 507–16.

Powell, D.A., Morris, E.R., Gidley, M.J. & Rees, D.A. (1982). Conformations and interactions of pectins II. Influence of residue sequence on chain association in calcium pectate gels. *J. Mol. Biol.*, **155**, 517–31.

Ross-Murphy, S.B. (1984). Rheological methods. In *Biophysical Methods in Food Research*, ed. H.W.-S. Chan. Critical Reports on Applied Chemistry, SCI, London, pp. 138–99.

Ross-Murphy, S.B., Morris, V.J. & Morris, E.R. (1983). Molecular viscoelasticity of xanthan polysaccharide. *Faraday Symp. Chem. Soc.*, **18**, 115–29.

Sawayama, S., Kawabata, A., Nakahara, H. & Kamata, T. (1988). A light scattering study of the effects of pH on pectin aggregation in aqueous solution. *Food Hydrocolloids*, **2**, 31–7.

Selvendran, R.R. (1985). Developments in the chemistry and biochemistry of pectin and hemicellulosic polymers. *J. Cell Sci. Suppl.*, **2**, 51–88.

Sheyenson, V.A., Khomutov, L.I., Lashek, N.A. & Ptitchkina, N.M. (1992). The role of crystallisation in the process of pectin/glycerine solution gelation. In *Gums and Stabilisers for the Food Industry 7*, eds G.O. Phillips, P.A. Williams & D.J. Wedlock. IRL Press, Oxford, UK, pp. 311–5.

Tanford, C. (1980). *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*. Wiley, New York.

Walkinshaw, M.D. & Arnott, S. (1981). Conformations and interactions of pectins II. Models for junction zones in pectinic acid and calcium pectate gels. *J. Mol. Biol.*, **153**, 1075–85.

Whistler, R.L. & BeMiller, J.N. (1993). *Induustrial Gums: Polysaccharides and their Derivatives*, 3rd edn. Academic Press, San Diego, CA, USA.