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Water-soluble yellow mustard (*Sinapis alba* L.) polysaccharides: partial characterization, molecular size distribution and rheological properties

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The water-soluble (WS) fraction of yellow mustard mucilage, exhibiting pronounced shear thinning behaviour in aqueous solutions, was separated into a CTAB-precipitated fraction (WSCP) and a CTAB-soluble fraction (WSCS) by precipitation with 5% CTAB (hexadecyltrimethylammonium bromide) under optimum conditions of complexation of CTAB with acidic polysaccharides. The chemical structure and molecular size distributions of WSCP and WSCS were determined by methylation, ^{13}C NMR spectra and gel filtration chromatography in order to examine their effect on the rheological properties of these materials in solution. The major fraction WSCP (52·0%) was a mixture of a pectic material consisting primarily of galacturonic acid, galactose and rhamnose and a 1,4-linked β-D-glucan. The minor fraction WSCS (34·0%) also was composed of two polysaccharide fractions differing in their molecular size and consisted mostly of neutral sugars and non-reducing end residues of glucuronic acid. Both WSCP and WSCS contribute to the rheological properties of WS solutions although they have different structures and molecular weight distributions. WSCP exhibited similar rheological behaviour to WS as assessed by both dynamic and steady shear flow measurements. In contrast, the WSCS fraction only showed a steady shear rheological pattern similar to WS.

INTRODUCTION

Aqueous dispersions of yellow mustard seed (*Sinapis alba* L.) mucilage and its fractions were previously reported to exhibit similar rheological responses to that of xanthan gum dispersions in terms of shear thinning properties and dynamic rheological patterns (Cui *et al.*, 1993). Of the yellow mustard mucilage fractions examined, a water-soluble (WS) fraction was the major component (55·6%) which in solution also showed the shear thinning behaviour of yellow mustard mucilage dispersions (Cui *et al.*, 1993). Substantial interfacial activity was observed for the WS fraction as assessed by its ability to reduce the surface tension of water and to stabilise water/oil emulsions and foams formed by 0·1% albumin solutions. WS appeared as a heterogeneous mixture of polysaccharides which consisted of both neutral sugars and uronic acids. The monosaccharides identified were glucose (22·3%), galactose (15·2%), mannose (6·3%), rhamnose (3·9%), xylose (1·8%) and arabinose (3·2%). Uronic acids (18·7%) consisted of both glucuronic and galacturonic acids (Cui *et al.*, unpublished) and this is contrary to earlier studies for mustard mucilage (Vose, 1974; Siddiqui *et al.*, 1986) in which only galacturonic acid was reported.

The structure and molecular weight distribution of natural hydrocolloids are important determinants of their physical properties (Dea & Clark, 1986). For

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example, in dilute polymer solutions or dispersions, the shear thinning behaviour is attributed to a decrease in the 'cross-link' density of the existing entangled network with increasing shear rate (Morris, 1990). The 'cross-link' density is in turn highly dependent upon the primary structure and conformation of the polymer molecules as well as the polymer–solvent and polymer–polymer interactions (Dea *et al.*, 1977). This paper reports on the primary structure, linkage pattern and molecular size distributions of two polysaccharide fractions (a CTAB-precipitated fraction, WSCP and a CTAB-soluble fraction, WSCS) of the water-soluble (WS) yellow mustard mucilage obtained by precipitation of the acidic polysaccharides with hexadecyltrimethylammonium bromide (CTAB). The rheological properties of these materials were also examined.

EXPERIMENTAL

Materials

The water-soluble fraction (WS) of yellow mustard mucilage was isolated according to the procedure described by Cui *et al.* (1993). All chemicals used were of reagent grade unless otherwise specified.

Fractionation of WS fraction

The WS was fractionated by co-precipitation with 5% CTAB (hexadecyltrimethylammonium bromide) (Scott, 1965). Optimization of the precipitation conditions was attained by stepwise addition of 5% CTAB (0·20 ml) to 0·20 g WS in 0·3% solution (Fig. 1). The precipitate was dissolved in 4 M NaCl, then precipitated in 3 volumes of 95% EtOH (×3), dialysed against running water (18°C) for 24 h, against distilled water at 35°C for 3 × 24 h, and finally freeze-dried. The supernatant was precipitated in 3 volumes of 95% EtOH (×2), dialysed against running water (18°C) for 24 h, against distilled water for 3 × 24 h at 35°C, and freeze-dried.

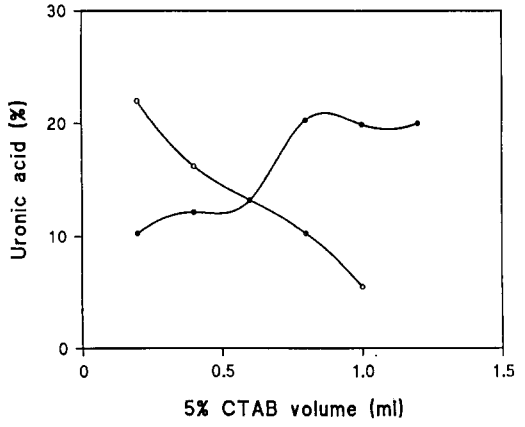

Fig. 1. Uronic acid content of the CTAB-precipitated (WSCP, ●—●) and the CTAB-soluble (WSCS, O—O) fractions as a function of CTAB (5%) added.

Chemical analysis and ^{13}C-NMR spectra

Neutral monosaccharides were determined following the procedure described by Englyst *et al.* (1982) on an SP-2330 glass capillary column, 30 m × 0·75 mm i.d. (Supelco Canada Ltd, Oakville, Canada). All samples were treated with 72% H_2SO_4 at 35°C for 30 min prior to the hydrolysis with 2 M H_2SO_4. Uronic acids and protein were estimated according to Blumenkrantz & Asboe-Hansen (1973) and Lowry's method (Lowry *et al.*, 1951), respectively. ^{13}C-NMR spectra (500 Hz) were recorded on a Bruker AMX500 FT spectrometer (Bruker, Rheinstetten, Germany) at 40°C: polymer concentration 2·0% (w/v) in 10% D_2O, 40 000 pulses, pulse repetition time 1·3 s and r.f. pulse angle 80·0°. Acetone was used as an internal standard.

Gel chromatography

Gel permeation chromatography was conducted on a Sephacryl S-500 (Pharmacia, Uppsala, Sweden) HR, 1·6 × 70 cm column and eluted with 0·1 M NaCl solution. Samples dissolved in the same buffer (1 mg/ml) were applied onto the column, and 3 ml fractions were collected. D-Glucose was used to determine the total volume, while linear dextrans T-500 and T-70 (Pharmacia Ltd, Montreal, Quebec) were used as relative molecular weight markers. Carbohydrates in the fractions were determined by the anthrone method (Loewus, 1952), and uronic acids were monitored by the method of Blumenkrantz and Asboe-Hansen (1973).

Methylation analysis

Methylation analysis was carried out as described by Ciucanu & Kerek (1984). The reduction of the carboxyl group after methylation was performed according to O'Neill *et al.* (1990). GC-MS was performed on an SP-2330 capillary column (60 m × 0·25 mm) while quantitative measurement of partially permethylated acetyl alditols was obtained on an SP-2330 capillary column (30 m × 0·75 mm), programmed from 160 to 210°C at 2°C per min; helium was used as carrier gas, at 15 psi.

Rheological properties

All rheological properties were determined on a Bohlin VOR Rheometer (Bohlin Reologi, Lund, Sweden). A concentric cylinder geometry, with a cylinder height of 63·0 mm and radii of the inner and outer containers of 12·5 and 13·75 mm, respectively, was used throughout the rheological study. In steady shear tests, samples were subjected to shear rate sweeps between 0·01 and

1164 s^{-1}. Viscosity measurements were conducted using aqueous solutions of 0·3, 0·5, 1·0 and 2·0% (w/w). The influence of temperature, pH, salt and sugar on the apparent viscosity of mucilage solutions was examined at 0·5% (w/w) polymer solutions. Dynamic rheological measurements were determined on 2·0% (w/w) mucilage solutions as a function of oscillatory frequency (f, 0·05–20·0 Hz) with a maximum input strain of 4% at 22°C. The dynamic rheological parameters used to evaluate the viscoelastic properties of these materials were the storage modulus (G'), loss modulus (G''), dynamic viscosity ($\eta' = G''/2\pi f$), complex viscosity ($\eta^* = [G'^2 + G''^2]^{0.5}/2\pi f$) and phase angle, δ ($\tan\delta = G''/G'$), as described previously (Cui *et al.*, 1993). All data presented are means of triplicate measurements.

RESULTS AND DISCUSSION

Fractionation and chemical composition

The optimum precipitation conditions for the acidic polysaccharides of the WS fraction of yellow mustard mucilage was found when 0·8 ml of 5% CTAB was added to a 0·3% (w/v) solution (total weight of WS 0·20 g), as shown in Fig. 1. Exceeding this amount of CTAB resulted eventually in the complete precipitation of all polysaccharides. Under the optimum precipitation conditions, two fractions were obtained, a CTAB-precipitated fraction (WSCP) which contained 22·7% uronic acids (including both galacturonic and glucuronic acids) and a CTAB-soluble fraction (WSCS) which still contained 12·5% glucuronic acid (Table 1 and 2). There appeared to be a preference for CTAB to precipitate galacturonic-acid-containing polymers over those of glucuronic acid by methylation analysis and ^{13}C NMR spectra showed that galacturonic acid was only present in WSCP (Table 2 and Fig. 2). Although there was no clear-cut separation of the two uronic acids by CTAB precipitation, the two fractions obtained varied in composition, structure and rheological behaviour. The yield of WSCP was 52·0% and consisted mainly of pectic polysaccharides, while the yield of WSCS was 34·0% and was composed predominantly of 1,4-linked β-D-glucose polymer. There were no pectic-type polysaccharides associated with the WSCS fraction. A possible explanation could be that the pectic polysaccharides are selectively precipitated by CTAB under optimum conditions. The presence of glucuronic acid in the WSCP fraction may be due to its association to the pectic polysaccharides or coprecipitation of glucuronic-acid-containing polymers with the pectic polysaccharides. The separation of WS into CTAB-precipitated and CTAB-soluble subfractions allowed an investigation of the influence of the chemical composition and linkage patterns on the physical properties of these materials.

Table 1 shows that both WSCP and WSCS were mainly composed of glucose (22·9–24·2% in WSCP, 24·7–26·2% in WSCS), galactose (21·4–22·1% in WSCP and 20·3–21·0% in WSCS), mannose (6·3% in WSCP and 11% in WSCS), rhamnose (12·6% in WSCP and 3·5% in WSCS), arabinose (6·1% in WSCP and 8·9% in WSCS) and xylose (3·8% in WSCP and 3·3% in WSCS). Prolonged hydrolysis significantly increased the rhamnose content in the WSCP fraction, indicating that rhamnose was closely associated with the uronic acids. This was further supported by the methylation analysis shown in Table 2. All other sugar contents decreased slightly on prolonged hydrolysis, which can be attributed to the decomposition of the released sugars that were not closely associated to the acidic monosaccharide residues. The pre-treatment of the samples with 72% H_2SO_4 was found to be essential for the complete release of glucose, which would otherwise only yield 2–3%.

The ^{13}C NMR spectra (Fig. 2) confirmed that WSCP contained two uronic acids (δ = 174·95 ppm, galacturonic acid and δ = 175·75 ppm, glucuronic acid) and

Table 1. Composition of the water-soluble CTAB-precipitated (WSCP) and CTAB-soluble (WSCS) fractions of yellow mustard mucilage

	WSCP		WSCS	
Yield (%)	52·0		34·0	
Uronic Acid (%)[a]	22·72 ± 2·04		12·50 ± 0·56	
Protein (%)[a]	1·84 ± 0·06		3·28 ± 0·10	
Monosaccharide[a]	2 h	(6 h)	2 h	(6 h)
Rhamnose (%)	4·75 ± 0·40	(12·67 ± 0·34)	2·58 ± 0·16	(3·55 ± 0·04)
Arabinose (%)	6·12 ± 0·42	(5·23 ± 0·01)	8·88 ± 0·16	(7·34 ± 0·30)
Xylose (%)	3·82 ± 0·11	(2·62 ± 0·13)	4·34 ± 0·01	(3·17 ± 0·28)
Mannose (%)	6·15 ± 0·40	(6·35 ± 0·08)	10·98 ± 0·06	(10·50 ± 0·05)
Galactose (%)	22·14 ± 0·34	(21·44 ± 0·18)	21·00 ± 0·11	(20·29 ± 0·19)
Glucose (%)	24·23 ± 0·07	(22·86 ± 0·28)	26·24 ± 0·14	(24·74 ± 0·27)

[a] n = 2, mean ± SD.

Table 2. Relative retention times and molar ratios of partially permethylated acetyl alditols of the water-soluble CTAB-precipitated (WSCP) and CTAB-soluble (WSCS) fractions of yellow mustard mucilage

	Rf[a]	Molar ratio (%)[b]				Diagnostic fragment ions
		WSCP	WSCPR[c]	WSCS	WSCSR[c]	
2,3,5-Me_3-Ara	0·66	3·1	1·1	3·7	2·6	117 118 161 162
2,3-Me_2-Ara	1·19	7·0	3·2	7·1	6·7	118 129 189
Total methyl ethers of arabinose		10·1	4·3	10·8	9·3	
2,3-Me_2-Xyl	1·32	5·7	3·1	5·3	3·1	118 129 189
Xyl $(acet)_5$	2·55	trace	8·0	trace	trace	115 145 188 218 290
Total methyl ethers of xylose		6·5	11·1	6·4	3·6	
2,3,4,6-Me_4-Glc	1·00	trace	trace	2·0	2·2	118 129 145 162
2,3,6-Me_3-Glc	1·82	17·1	10·5	32·4	22·7	118 162 233
2,3-Me_2-Glc	2·35	5·7	3·9	8·3	7·8	118 201 261
Total methyl ethers of glucose		23·9	14·4	42·7	32·7	
2,3,4-Me_3-Glc ($6D_2$)	1·74	n.d.	8·9	n.d.	9·3	118 131 162 191 235
2,3,4,6-Me_4-Gal	1·17	5·9	1·9	3·0	3·0	118 129 145 162
3,4,6-Me_3-Gal	1·71	2·6	trace	6·5	8·4	129 130 161 190
2,3,4-Me_3-Gal	2·03	26·5	22·1	14·6	16·8	118 129 189 233
2,4-Me_2-Gal	2·67	4·0	2·8	3·6	3·1	118 129 189 234
Total methyl ethers of galactose		39·0	26·8	29·3	31·3	
2,3-Me_2-Gal ($6D_2$)	2·62	n.d.	6·5	n.d.	trace	118 129 203 263
2,3,6-Me_3-Man	1·63	5·5	4·0	8·9	8·4	118 162 233
3,4-Me_2-Rham	0·94	4·9	3·5	trace	n.d.	131 190
3-Me-Rham	1·48	10·2	20·3	1·9	3·5	130 143 190 203
Total methyl ethers of rhamnose		15·1	23·8	1·9	3·5	

[a]Typical relative retention time of partially permethylated acetyl alditols on a SP-2330 glass capillary column (30 m × 0·75 mm), programmed from 160 to 210°C at 2°C per min; carrier gas: helium, 15 psi.
[b]Relative molar ratio, calculated from the ratio of peak areas.
[c]WSCPR and WSCSR are carboxyl-reduced WSCP and WSCS, respectively.
n.d. = Not determined.

rhamnose (δ = 17·26 ppm), while WSCS only contained glucuronic acid (δ = 175·74 ppm) with the absence of rhamnose signals. The anomeric regions (δ = 90–109 ppm) of the ^{13}C NMR spectra of WSCP and WSCS are significantly different from each other, although WSCP and WSCS both had 102·7 and 103·5 ppm peaks as the most abundant components. Chemical shifts between δ = 108 to 109·5 ppm can be attributed to the α 1-C of arabinoses which are weak in WSCP but significantly stronger in WSCS. Moreover, the resonances at δ = 103·5 ppm and 102·7 ppm could be attributed to 1-C of β-galactose and 1-C of 1,4-linked β-D-glucose, respectively (Bock *et al.*, 1984; Doco *et al.*, 1990; Goldberg *et al.*, 1991). Two peaks were absent for WSCP (δ = 104·66 and 100·18 ppm) compared to WSCS, although an additional peak was evident at δ = 97·85 ppm for WSCP. The relative intensities of glucuronic acid in both WSCP and WSCS are in agreement with the methylation results, where glucuronic acid was higher in both WSCP (8·9%) and WSCS (9·3%) compared to galacturonic acid, which was 6·5% in WSCP only (Table 2). There appeared to be some differences in resonances within the region δ = 50–90 ppm of WSCP and WSCS, although further fractionation and purification of the polymeric constituents of these fractions are necessary for a complete assignment of the ^{13}C NMR spectra.

The gel chromatographic profiles (Fig. 3) showed high molecular weight regions (at the void volume) for both WSCP and WSCS fractions, which were composed mainly of neutral species. In addition to the high molecular weight neutral fraction, WSCP contained an acidic fraction with an elution volume smaller than that of Dextran T-500 as well as a well-distributed acidic fraction with much larger peak elution volume (between T-70 and T-500). The WSCS exhibited a low molecular weight fraction (less than T-70) as the major component, which contains high amounts of glucuronic acid (Fig. 3).

Methylation analysis

Table 2 shows the relative retention times and molar proportions of partially permethylated acetyl alditols (PPAA) of WSCP and WSCS. Mass spectra obtained were compared with the literature (Carpita & Shea, 1990) and the corresponding diagnostic fragment ions for each derivative are also presented. Two uronic acids were determined following reduction of the carboxyl group with lithium triethylborodeuteride (1·0 M solution

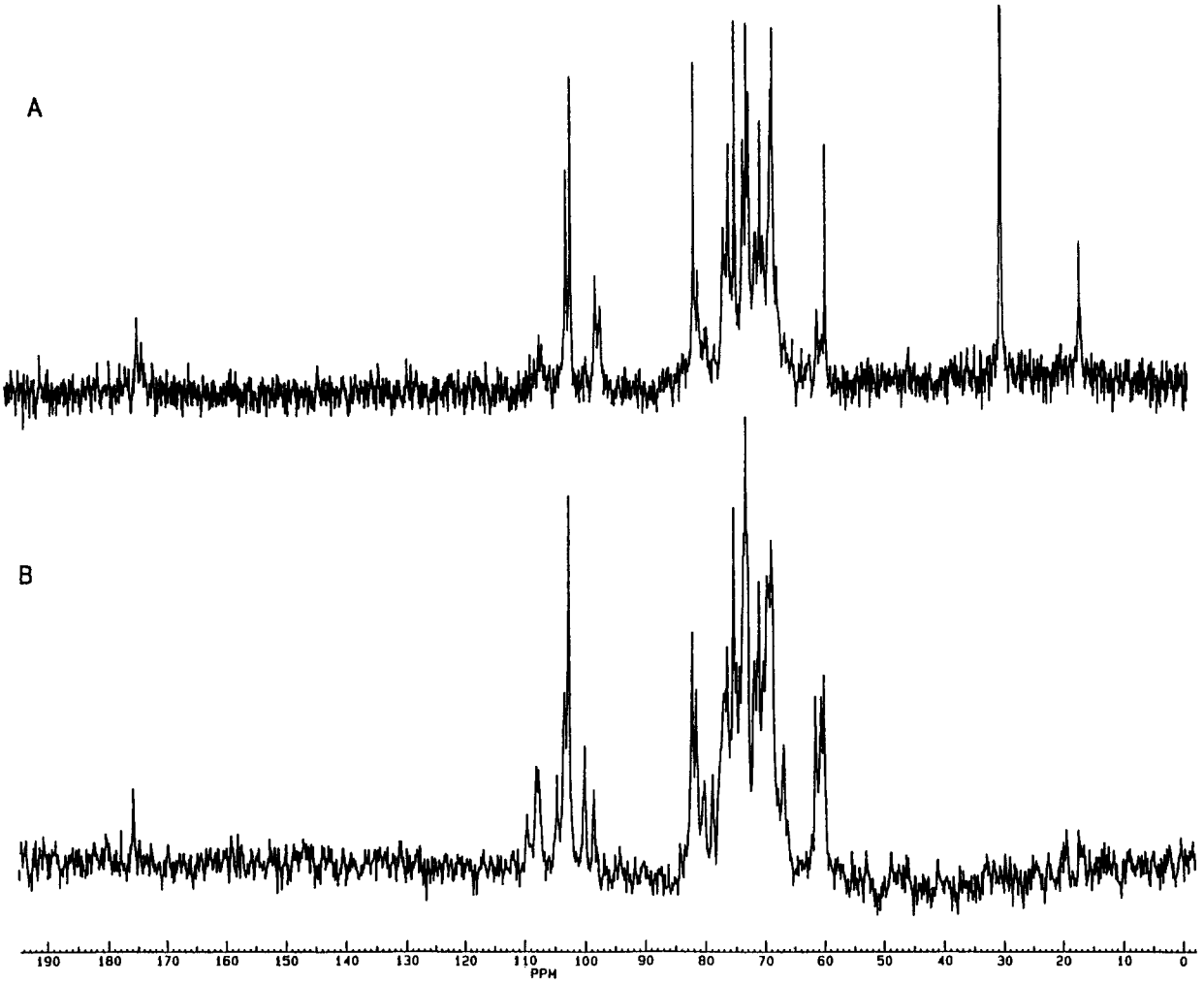

Fig. 2. Comparison of ^{13}C-NMR spectra of the CTAB-precipitated (WSCP, A) and the CTAB-soluble (WSCS, B) fractions of water-soluble yellow mustard mucilage. Acetone was used as an internal standard (δ = 30·511 ppm).

in tetrahydrofuran) (O'Neill *et al.*, 1990). A non-reducing end glucuronic acid was found having a relative retention time of Rf = 2·35 on the SP-2330 capillary column (30 m × 0·75 mm). The diagnostic fragment ions for carboxyl-reduced non-reducing end glucuronic acid were 118, 131, 162, 191 and 235 (Table 2). The determination of 1,4-linked galacturonic acid was established by its Rf = 2·62 and diagnostic fragments (118, 129, 203 and 263).

Two major polysaccharides were found in the WSCP fraction: a pectic-like polysaccharide and a neutral polysaccharide. In the pectic polysaccharide of WSCP, 1,2,4-linked rhamnose and fully substituted xylose were closely associated with 1,4-linked galacturonic acid and possibly glucuronic acid. This was based on the significant increases of 3-Me-rham (10·2 to 20·3%) and xyl (acetate)$_5$ (trace to 8·0%) upon reduction of the carboxyl group of the uronic acids. Among the residues identified, a substantial amount of 1,4-linked β-glucose was found (14·4%, Table 2) which originates primarily from the high molecular size eluting species in Fig. 3(A), as evidenced by monosaccharide analysis (Table 3).

In contrast to WSCP, WSCS appeared to consist of polysaccharides containing non-reducing end glucuronic acid with trace amount of pectic polysaccharides. In addition, 1,4-linked β-D-glucose (22·7%) was found as the major component of WSCS, particularly in the high molecular size material eluting in the void volume of Sephacryl S-500 column (Fig. 3(B)) as indicated by monosaccharide analysis (Table 3). Other sugars present in the WSCS fraction were 1,6-linked galactose (16·8%), 1,4-linked mannose (8·4%) and non-reducing end glucuronic acid (9·3%). It appeared that some of the 1,2- and 1,6-linked galactoses were connected with the non-reducing end glucuronic acid, as shown by their slight increase upon reduction of the carboxyl group of glucuronic acid after methylation (Table 2).

Although WSCP and WSCS are still heterogeneous mixtures of polysaccharides and their structural information is limited, there seem to be significant

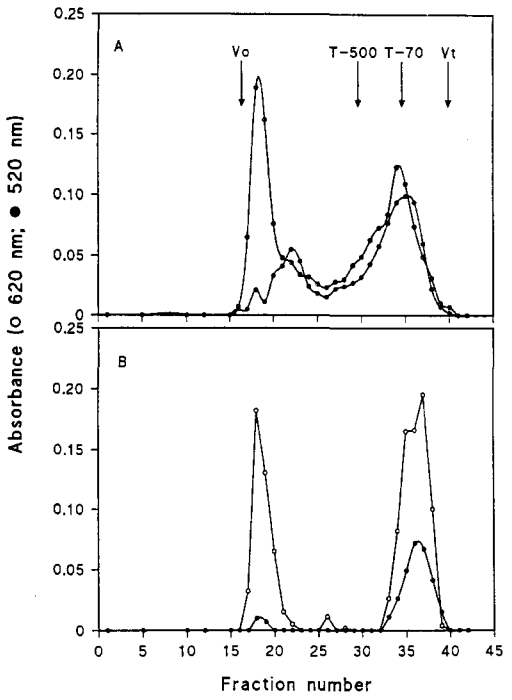

Fig. 3. Gel filtration chromatography of the CTAB-precipitated (WSCP, A) and the CTAB-soluble (WSCS, B) fractions of water-soluble yellow mustard mucilage on a Sephacryl HR S-500 column (1·6 × 70 cm) eluted with 0·1 N NaCl solution, flow rate 1 ml/min, temperature 23°C; arrows indicate peak elution volumes of dextran standards (Blue dextran T-2000, *Vo*; T-500; T-70; Glucose, *Vt*) used as molecular weight markers (O—O, total carbohydrate; ●—●, uronic acids).

Table 3. Monosaccharide (neutral) molar ratios of yellow mustard mucilage water-soluble CTAB-precipitated (WSCP) and CTAB-soluble (WSCS) fractions collected from gel filtration chromatography

Sugars[a]	WSCP-H[b]	WSCP-L[b]	WSCS-H[b]	WSCS-L[b]
Rhamnose	1·05	0·81	0·10	0·28
Arabinose	0·50	0·18	1·33	0·30
Xylose	n.d.	0·07	0·22	0·10
Mannose	0·40	0·15	0·54	0·44
Galactose	1·00	1·00	1·00	1·00
Glucose	3·42	0·27	1·51	0·42

[a]Sugar molar ratios were calculated from peak heights.
[b]Collected fractions from gel filtration chromatography (Fig. 3): H, higher molecular size peak; L, lower molecular size peak.
n.d. = Not determined.

differences in chemical composition, molecular size distribution as well as linkage pattern among these fractions.

Rheological properties

Steady shear rheological test

The shear-rate dependent flow behaviour of WS, WSCP and WSCS is shown in Fig. 4. No Newtonian

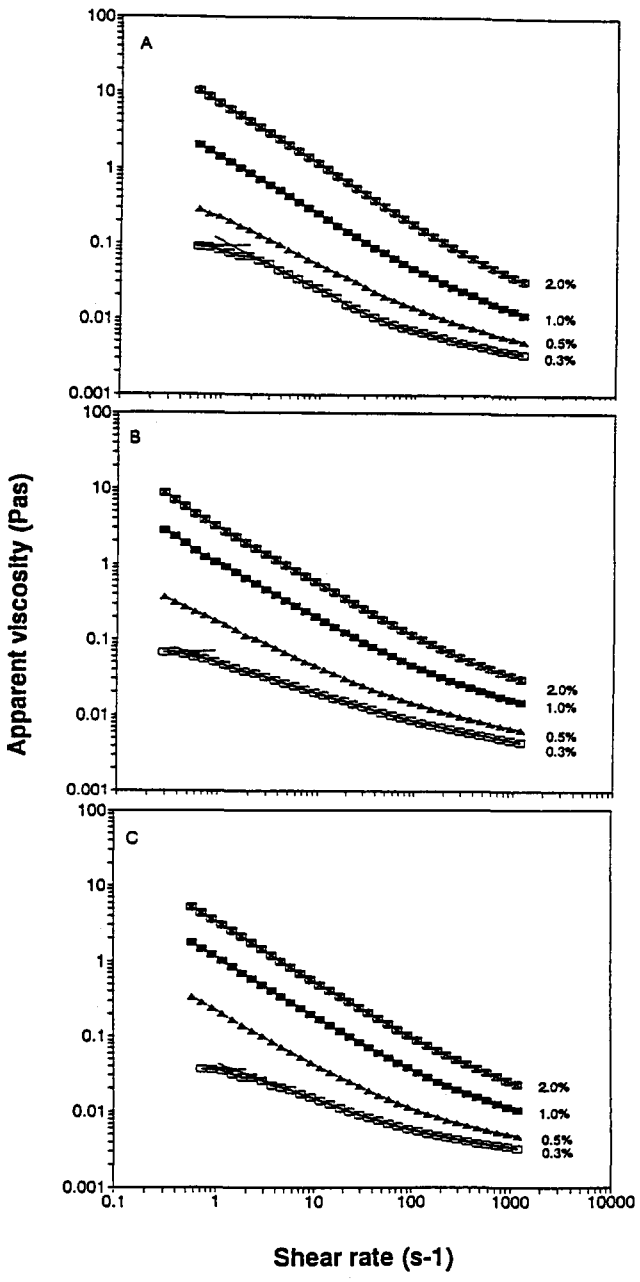

Fig. 4. Steady shear flow curves of the yellow mustard mucilage water-soluble fraction (WS, A) and its subfractions: the CTAB-precipitated fraction (WSCP, B) and the CTAB-soluble fraction (WSCS, C) at concentrations between 0·3 and 2·0%, 22·0 ± 0·1°C.

plateau is evident at concentrations between 0·5 and 2·0% for all samples over the shear rate range investigated (0·1–1162 s^{-1}). However, (upper) Newtonian plateaus were observed at a concentration of 0·3%, and both the zero-shear-rate viscosity and the shear-rate value ($\dot{\gamma}$) at which the onset shear-thinning behaviour occurs could be evaluated, as shown in Table 4. The zero-shear-rate viscosity was found highest for WS, followed by WSCP and WSCS, while the $\dot{\gamma}$ value was found highest for WSCS, lowest for WSCP and in between for WS. The lowest $\dot{\gamma}$ value of WSCP suggested

Table 4. A comparison of zero-shear-rate viscosity (η_0) and shear-rate ($\dot{\gamma}$) value at which onset shear thinning occurred for the water-soluble fraction (WS) and its subfractions: CTAB-precipitated fraction (WSCP) and CTAB-soluble fraction (WSCS) at 0·3% (22°C)

	η_0 (mPa s)	$\dot{\gamma}$ value (s^{-1})
WS	94·4	1·16
WSCP	68·2	0·46
WSCS	36·4	1·47

that WSCP solutions are the most elastic, while WSCS the least. Both WSCP and WSCS seem to contribute to the viscoelastic character of WS solutions.

By applying the power-law model (Witcomb *et al.*, 1980), the consistency index (K) and flow index (n), defined from the equation $\eta = K\dot{\gamma}^{n-1}$, were obtained (Table 5). As concentration increases, K increases and n decreases. The increase in K with increasing concentration suggests that a more viscous system is obtained at higher concentrations. On the other hand, the decrease in n with increasing concentration implies a more pronounced shear thinning of the system. The WS exhibited the highest K value and the lowest n value, as compared to those of WSCP and WSCS, suggesting the presence of polysaccharide–polysaccharide interactions between some of the components present in WSCP and WSCS. This phenomenon is particularly significant at 2·0% polymer solutions.

Dynamic oscillatory shear experiments

The viscoelastic spectra of WS, WSCP and WSCS at 2·0% (w/w) are presented in Fig. 5. The viscoelastic behaviour of yellow mustard mucilage and its fractions are typical of a 'weak gel' with $G' > G''$ over the frequency range investigated (Cui *et al.*, 1993). A typical liquid system has $G' \propto \omega^2$ and $G'' \propto \omega^1$, while for a 'weak gel' system, both G' and G'' are only slightly dependent upon frequency (Navarini *et al.*, 1992). Both WSCP and WSCS exhibited a similar dynamic rheological pattern to that of WS while the maximum frequency dependence is approximately $G' \propto \omega^{0.15}$ and

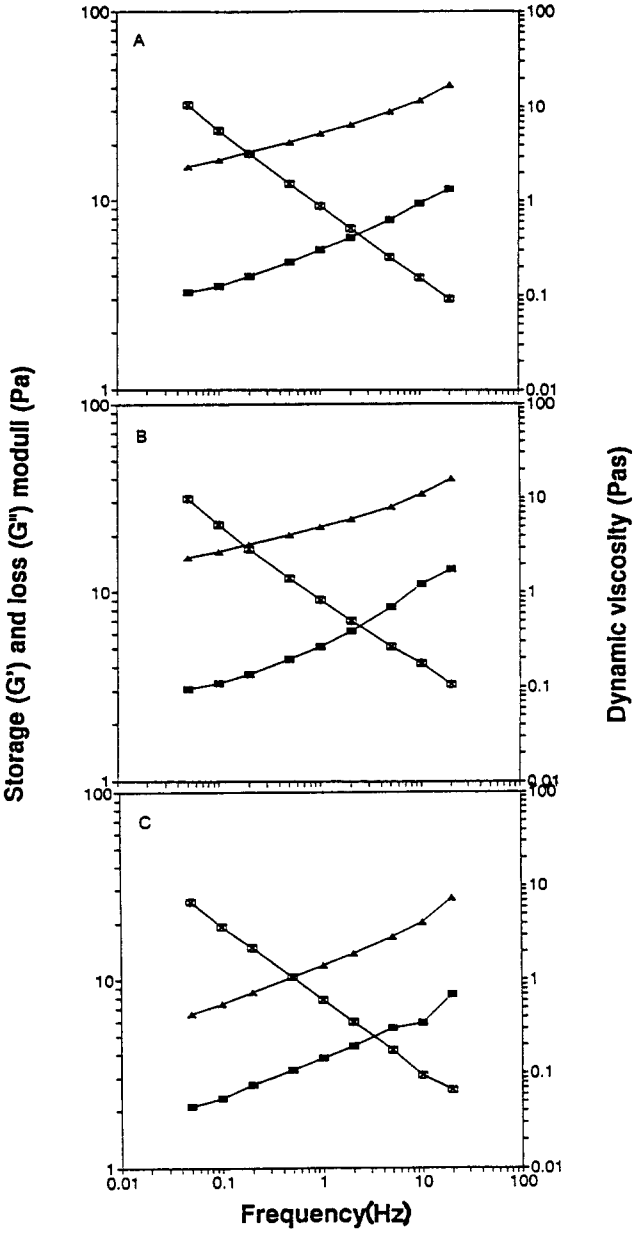

Fig. 5. Frequency dependence of storage (G', ▲) and loss (G'', ■) moduli, and dynamic viscosity (η', ⊠) of yellow mustard mucilage water-soluble fraction (WS, A) and its subfractions: CTAB-precipitated fraction (WSCP, B) and CTAB-soluble fraction (WSCS, C) at concentration 2·0%, 22·0 ± 0·1°C.

Table 5. n and K values[a] of the water-soluble fraction (WS) and its subfractions: CTAB-precipitated fraction (WSCP) and CTAB-soluble fraction (WSCS) at different concentrations (22°C)

Concentration (%)	WSCP		WSCS		WS	
	n	K(Pa s)	n	K(Pa s)	n	K(Pa s)
0·3	0·668	0·041	0·641	0·033	0·537	0·070
0·5	0·557	0·119	0·473	0·138	0·468	0·173
1·0	0·407	0·740	0·331	0·845	0·321	1·120
2·0	0·340	2·506	0·285	2·764	0·224	6·440

[a]Parameters n and K were calculated using the power-law model: $\eta = k\dot{\gamma}^{n-1}$, (Witcomb *et al.*, 1980).

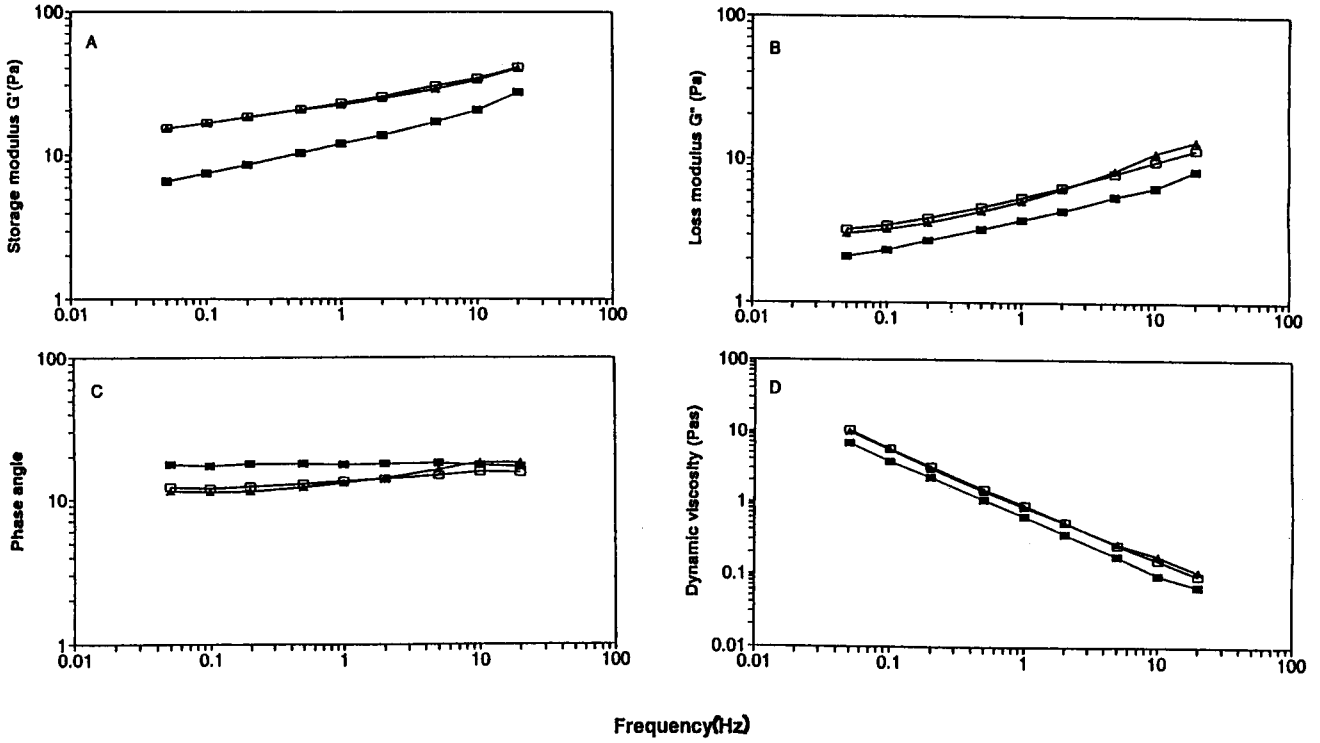

Fig. 6. Comparison of storage modulus (A), loss modulus (B), phase angle (C) and dynamic viscosity (D) as a function of frequency for the water-soluble fraction (WS, -⊟-) and its subfractions: CTAB-precipitated fraction (WSCP, -▲-) and CTAB-soluble fraction (WSCS, -■-) at concentration 2·0%, 22·0 ± 0·1°C.

$G'' \propto \omega^{0.25}$ for WSCP, $G' \propto \omega^{0.23}$ and $G'' \propto \omega^{0.22}$ for WSCS as compared to $G' \propto \omega^{0.16}$ and $G'' \propto \omega^{0.21}$ for WS.

Comparisons of storage and loss moduli, phase angle (δ) and dynamic viscosity (η') *versus* frequency (f) of WSCP and WSCS to those of WS are illustrated in Fig. 6. WSCP was found to be much more similar to WS over WSCS in terms of storage and loss moduli, phase angle (δ) and dynamic viscosity (η'), although phase-angle deviation was observed for WSCP from that of WS in the higher frequency range (Fig. 6(C)). The storage modulus and dynamic viscosity (η') of WSCP are superimposable with that of WS, while the G' and η' of WSCS were far below (Fig. 6(A) and (D)). In Fig. 6(B), the loss modulus G'' of WSCP generally resembles that of WS, although G'' of WSCP was slightly lower at low frequencies and slightly higher at high frequencies than that of WS, with a cross point at 3 Hz. Similar to G', the G'' of WSCS is also lower than that of WS, as shown in Fig. 6(B). The phase angle (δ) describes the extent of departure of a viscoelastic system from an ideal elastic system. Figure 6(C) illustrates the changes of δ as a function of frequency. It was observed that WSCP exhibited the lowest phase-angle values at low frequencies and the highest at higher frequencies. In contrast to WSCP, the phase angle of WSCS remained constant over the frequency investigated. Figure 7 shows the changes of G' as a function of polymer concentration. Compared to WSCP, whose G' coincides with that of WS, the G' of

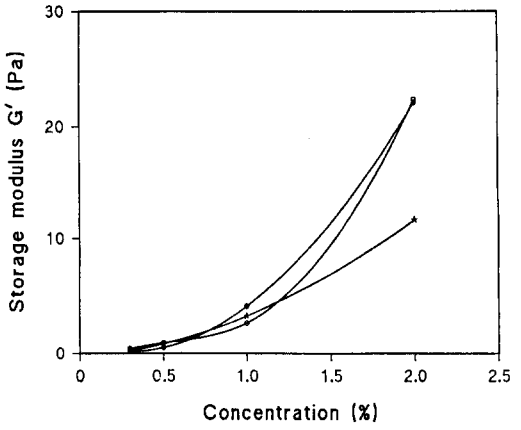

Fig. 7. Increase of storage modulus as a function of concentration of the water-soluble (WS, O—O) fraction of yellow mustard mucilage and its subfractions: CTAB-precipitated fraction (WSCP, ●—●) and CTAB-soluble fraction (WSCS, ☆—☆).

WSCS appeared similar to that of WS only at low concentrations, and departed from that of WS at higher concentrations (e.g. 2·0%). The non-linear increase of G' of WSCP and WS may reflect that a more ordered network structure develops in WS and WSCP solutions at higher concentrations.

The relationship of apparent viscosity (η) and complex viscosity (η^*, defined as $\eta^* = [G'^2 + G''^2]^{0.5}/\omega$) can be used diagnostically to distinguish normal polysaccharide solutions from 'weak gels'. For a 'weak

gel' system, the complex viscosity $\eta^*(\omega)$ is substantially higher than the apparent viscosity $\eta(\dot{\gamma})$ at equivalent values of frequency and shear rate. In contrast, the two viscosities coincide in a normal polysaccharide solution; this is known as the Cox-Merz rule (Cox & Merz, 1958; Morris, 1990; Navarini *et al.*, 1992). The apparent viscosity $\eta(\dot{\gamma})$ and complex viscosity $\eta^*(\omega)$ of mustard mucilage samples are presented in Fig. 8. The complex viscosity $\eta^*(\omega)$ of WS is substantially higher than the apparent viscosity $\eta(\dot{\gamma})$ at equivalent values of frequency and shear rate, thus exhibiting the typical behaviour of a 'weak gel' (Fig. 8(A)). The extent of $\eta^*(\omega)$ over $\eta(\dot{\gamma})$ for WSCP is greater than that for WS, indicating a more ordered 'weak gel' structure or more elastic character for the WSCP (Fig. 8(B)). This is in agreement with the results of Fig. 6(C) as WSCP exhibited the smallest phase angle. The Cox-Merz rule was also tested for the WSCS over the whole range of frequencies and shear rates investigated; again, $\eta^*(\omega)$ and $\eta(\dot{\gamma})$ were not superimposable, as shown in Fig. 8(C). The degree of departure of $\eta^*(\omega)$ from $\eta(\dot{\gamma})$, however, was much smaller for WSCS compared to WSCP. Such a departure from the Cox-Merz superimposability has been attributed to the making and breaking of non-covalent (hydrogen) bonds (Morris *et al.*, 1981). The rheological properties discussed above suggested that both WSCP and WSCS contribute to the rheological properties of WS. Being the major fraction, WSCP contributes to both the shear thinning and the 'weak gel' properties of WS, while WSCS contributes more to the viscous properties of WS solutions.

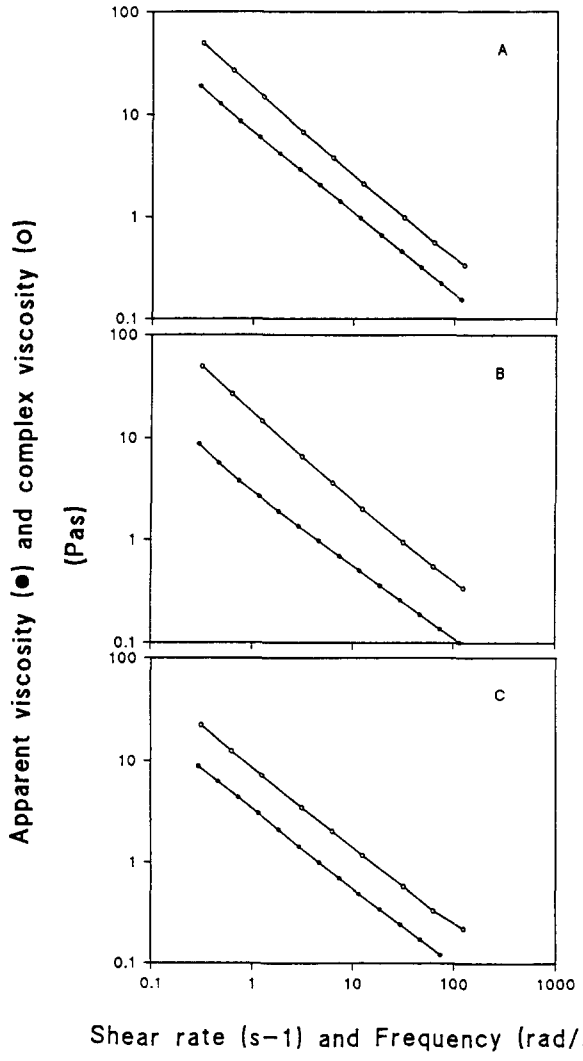

Fig. 8. Cox-Merz plot for 2% solutions or dispersions of the water-soluble (WS, A) fraction of yellow mustard mucilage and its subfractions: CTAB-precipitated fraction (WSCP, B) and CTAB-soluble fraction (WSCS, C) at 22·0°C.

The effect of temperature, pH and co-solutes on 'apparent viscosity' is shown in Fig. 9. The effect of temperature on the viscosity of WSCP and WSCS obeyed the expected trend of decreasing viscosity with increasing temperature (Fig. 9(A)). The influence of pH on viscosity of WSCP and WSCS are in agreement with previous reports (Weber *et al.*, 1974; Cui *et al.*, 1993); the lowest viscosity was observed between pH 3 and 7, and the highest viscosity at the low pH region (Fig. 9(B)). The substantial increase in viscosity at the low pH region could be attributed to the reduction of repulsion forces between polymer chains, which allows interchain associations between polymer molecules and thereby increasing viscosity. Addition of sucrose resulted in enhanced viscosity for both WSCP and WSCS, as shown in Fig. 9(C). It appeared that the initial addition of sucrose brought about a faster increase in viscosity for WSCP with a possible turning point at ~0·4 M sugar concentration; above this concentration, the rate of increase in viscosity became smaller. In contrast to WSCP, sucrose caused an almost linear increase in viscosity for WSCS. The effect of sucrose on the apparent viscosity of polysaccharide solutions can be attributed to a concentrating effect as well as improved polymer-solvent interactions (Dea *et al.*, 1977). The effect on apparent viscosity of WSCP and WSCS on addition of salt are different, as shown in Fig. 9(D). The initial addition of NaCl resulted in a reduction in viscosity for the WSCP solutions up to 0·25 M salt concentration. Further addition of NaCl resulted in a rapid recovery of viscosity, with the increase of viscosity being more pronounced at a higher concentration range (~1·0 M). The initial reduction in viscosity on addition of NaCl is attributed to the progressive suppression of intramolecular charge-charge repulsion and consequent contraction of the polysaccharide molecules (Morris, 1990). As the salt concentration increased beyond a certain point, intermolecular charge-charge repulsions are suppressed and intermolecular associations could occur which result in the recovery of viscosity. Further increase in viscosity at higher salt concentrations could also be due to a concentrating effect in addition to charge suppression. In contrast to WSCP, WSCS exhibited fairly stable

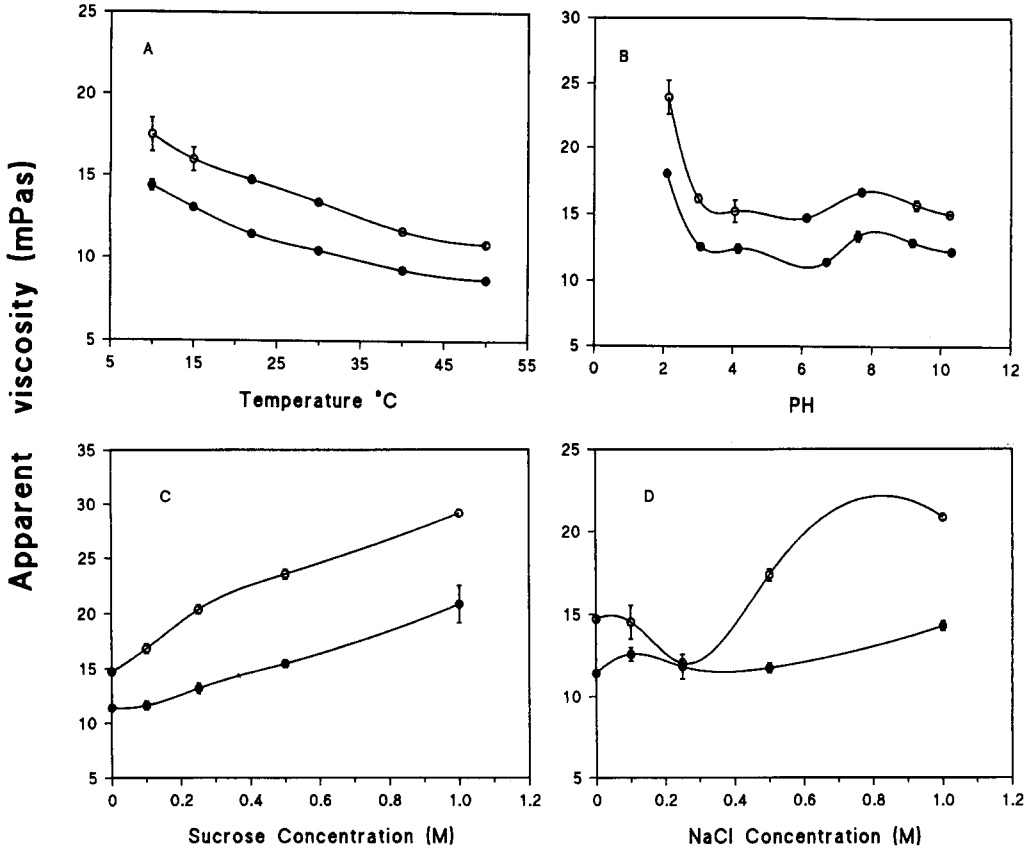

Fig. 9. Effect of temperature (A), pH (B), sucrose (C) and salt (D) concentrations on the apparent viscosity of water-soluble yellow mustard mucilage subfractions: CTAB-precipitated fraction (WSCP, O—O) and CTAB-soluble fraction (WSCS, ●—●) at 0·5% (w/w) polymer concentration, 22·0°C, shear rate 92·32 s^{-1}.

viscosity upon addition of salt. The initial addition of salt even increased the viscosity slightly. This is possibly due to the non-reducing end glucuronic acid which is attached as a side residue on the polysaccharide chain. The initial addition of NaCl would suppress the charge-induced intermolecular repulsions, and thereby enhance the intermolecular interactions between the polymer chains. Nevertheless, the overall response of WSCS solutions to added NaCl is minor compared to the WSCP and this may reflect the relatively fewer acidic groups present in WSCS.

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