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Rheological properties of xyloglucans from different plant species

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Abstract

The rheological properties of three xyloglucans (XGs) from the extracellular medium of suspension cultured *Nicotiana plumbaginifolia* cells, apple pomace and tamarind seeds, with different structural features and molecular weights have been studied. The molecular weights (weight average) of the *Nicotiana*, apple pomace and tamarind seed XGs determined by multi-angle laser light scattering were 129, 219 and 833 kDa, respectively. Tamarind seed XG had the highest viscosity and *Nicotiana* XG had the lowest viscosity, with that of apple pomace XG intermediate. The viscosity of apple pomace XG at 5% w/v was almost equivalent to that of tamarind seed XG at 2% w/v, but their behaviour at high shear rates differed; both XGs were non-Newtonian in their rheological properties, but that from tamarind seeds showed more pronounced shear-thinning. The viscosity of *Nicotiana* XG at 5% w/v was almost equivalent to tamarind seed XG at 0.5% w/v, displaying Newtonian behaviour. Modification of the molecular weight of the XGs and their degree of branching revealed that differences in viscosity between the molecules, and their shear-field behaviour, was due primarily to differences in molecular weight. Removal of fucose residues from apple pomace XG decreased the viscosity of solutions from 8 to 4 mPa·s, whereas removal of both fucose and galactose from apple pomace XG, resulted in precipitation from solution. Deacetylation of *Nicotiana* XG also resulted in precipitation from solution.

Keywords: Rheological properties; Xyloglucans; Plant species

1. Introduction

Xyloglucans (XGs) are a family of plant polysaccharides consisting of a backbone of 1,4-β-D-Glc*p* residues substituted at C(O)6 with side-chains of α-D-Xyl*p* (Darvill et al., 1980; McNeil et al., 1984; Bacic et al., 1988; Fry, 1989; Hayashi, 1989). Most XGs are composed of the heptasaccharide unit structure XXXG (using nomenclature of Fry et al., 1993). Tamarind seed (*Tamarindus indica*) XG is composed of Glc:Xyl:Gal:Ara in the ratio of 4.0:3.4:1.5:0.3 (Gidley et al., 1991). XGs from many species are composed of the same structural features as tamarind seed XG but also contain α-L-Fuc*p*-(1 → 2)-β-D-Gal*p* disaccharides attached to O-2 of some of the Xyl*p* residues; one such XG has been isolated from parenchymatous tissues of apples (*Malus domestica*; Ruperez et al., 1985; Renard et al., 1992) and from apple pomace which has been treated enzymically to remove pectin (Renard et al., 1995). Several features distinguish the XGs produced by solanaceous plants. An unusual XG which is branched at less than half of the backbone β-D-Glc*p* residues has been purified from cell walls of *Nicotiana tabacum* (Eda and Kato, 1978; Mori et al., 1979), the cell walls of potato (*Solanum tuberosum*; Vincken et al., 1996), the cell walls and extracellular polysaccharides (ECPs) of suspension cultures of *N. tabacum* (Eda et al., 1983; Akiyama and Kato, 1982; York et al., 1996) and the ECPs of *N. plumbaginifolia* (Sims and Bacic, 1995; Sims et al., 1996) and tomato (*Lycopersicon esculentum*; York et al., 1996). These XGs are highly arabinosylated and contain side-chains of α-D-Xyl*p* and α-L-Ara*f*-(1 → 2)-α-D-Xyl*p*; the tomato XG contains, in addition, β-Ara*f*-(1 → 3)-α-L-Ara*f*-(1 → 2)-α-D-Xyl*p* (York et al., 1996). In addition, *N. plumbaginifolia* XG bears *O*-acetyl groups on slightly less than half of the non-reducing 4-Glc*p* residues that are not xylosylated (Sims

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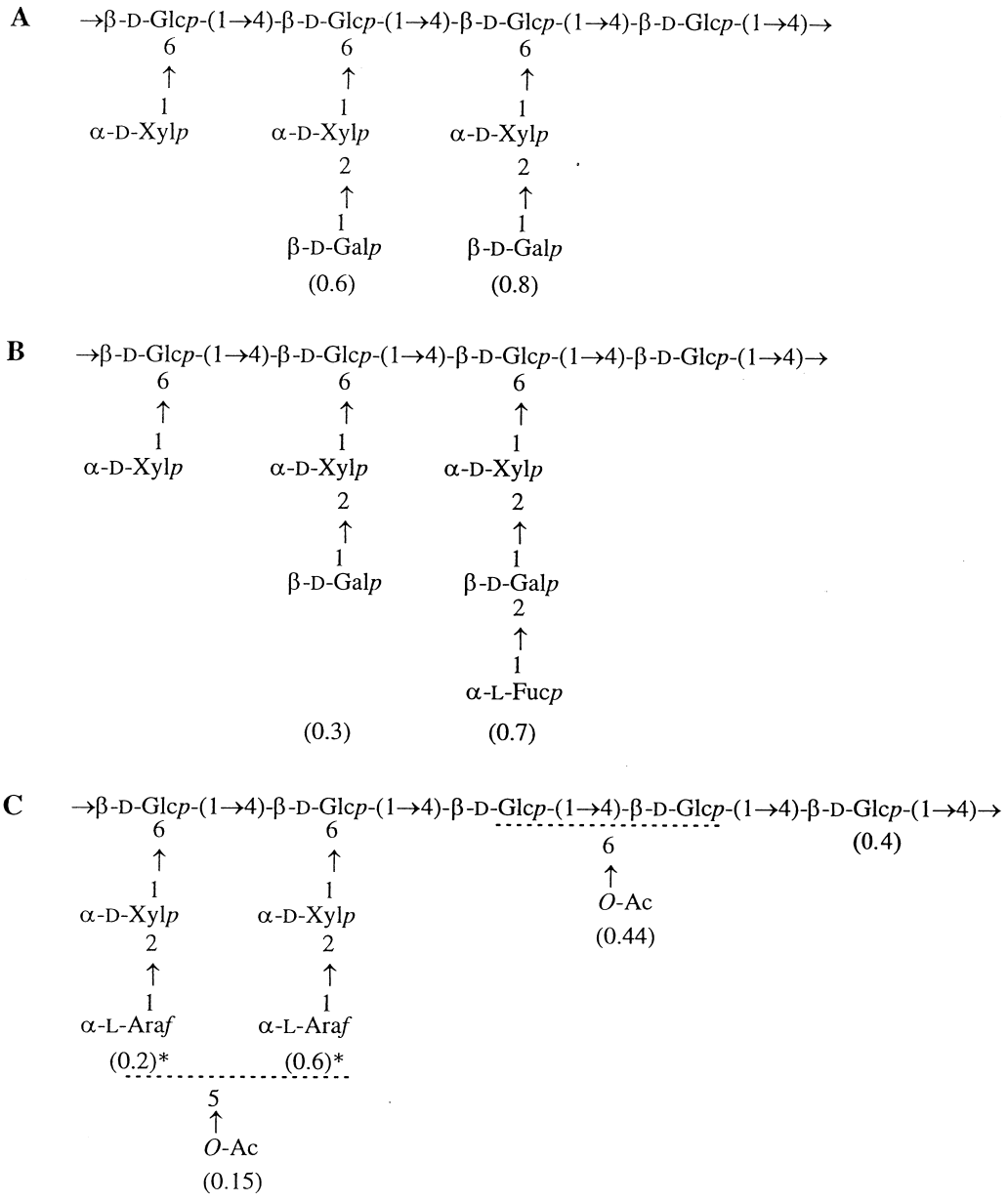

Fig. 1. Generalized repeat structures of XGs from (A) tamarind seeds (York et al., 1990); (B) apple pomace (Renard et al., 1992); and (C) *Nicotiana plumabinifolia* suspension cultures (Sims et al., 1996). Numbers in parentheses denote the approximate degree of substitution of each of the side-chains. *O*-Ac = *O*-acetyl substituents. *Precise residue with highest degree of α-L-Ara*f* substitution not determined.

et al., 1996); a feature of other solanaceous arabinosylated XGs (York et al., 1996).

Studies on the location of substituents on the backbone of XGs show that their distribution is predominantly regular (York et al., 1990; Renard et al., 1992; Sims et al., 1996; York et al., 1996). From these data, generalized repeat structures of the XGs from tamarind seeds, apple pomace and suspension cultures of *Nicotiana* have been deduced, showing the differences in their side-chain substituents (Fig. 1). Incubation of tamarind seed XG with endo-(1 → 4)-*β*-D-glucanase yields four oligosaccharides with the structures XXXG (13% of total digest), XLXG (9%), XXLG (28%) and XLLG (50%)(Fig. 1A; York et al., 1990). Renard et al. (1992) have shown that XG from apple fruits was composed predominantly of approximately equal proportions of XXXG, XXFG and XLFG (Fig. 1B). Comparison of the monosaccharide composition of apple fruit XG (Ruperez et al., 1985; Renard et al., 1992) with that of apple pomace XG (Renard et al., 1995) suggested that these XGs were composed of similar repeats. The major oligosaccharide subunits obtained from endo-(1 → 4)-*β*-D-glucanase digestion of *Nicotiana plumbaginifolia* XG are based on the subunits XXGG (34% of total digest) and XXGGG (27%). Almost 60% of these subunits are further substituted with one terminal Ara*f* residue, and almost 15% are substituted with two terminal Ara*f* residues attached to O-2 of the Xyl*p* residues (Fig. 1C; Sims et al., 1996). *O*-Acetyl groups are attached to C-6 of 44% of the non-reducing 4-Glc*p* residues

that are not xylosylated, and to C-5 of 15% of the terminal Ara*f* residues (Sims et al., 1996). Thus, it appears that in these XGs, blocks of unsubstituted 4-Glc*p* are short (up to four Glc*p* residues in *Nicotiana* XG), and are probably distributed regularly along the backbone.

The rheological properties of polysaccharides in solution are influenced by a number of parameters, including their molecular weight, and the presence, number and location of substituents (glycosyl and non-glycosyl) along the backbone. Hwang and Kokini (1991) have reviewed the effects of side branches on functional properties, including the effects of branching on viscosity, solubility, gelling, retrogradation, freeze–thaw stability and film formation. Izydorczyk and Biliaderis (1992a, b) have demonstrated that the viscosity of wheat arabinoxylan increases with increasing molecular weight. In addition, they showed that shear-thinning behaviour, a characteristic of non-Newtonian fluids, is most pronounced in fractions with the higher molecular weights, with lower molecular weight fractions exhibiting near-Newtonian behaviour (i.e, no shear-thinning) at low shear rates.

Tamarind seed gum, a crude extract of tamarind seeds, has been used as a replacement for starch in cotton sizing, and as a wet-end additive in the paper industry, where it replaces starches and galactomannans (Glicksman, 1986). In Japan, where it is a permitted food additive, refined tamarind seed XG is used as a thickening, stabilizing and gelling agent in the food industry (Glicksman, 1986; Gidley et al., 1991). Tamarind seed XG is a high-molecular-weight polysaccharide (880 kDa), which forms viscous solutions when dissolved in water; the solution properties of tamarind seed XG have been studied (Gidley et al., 1991). The removal of terminal β-D-galactopyranosyl residues from tamarind seed XG by β-D-galactosidase results in a gradual decrease in viscosity, followed by an increase and then gelation (Reid et al., 1988). The formation of the gel is a function of the molecular weight of the tamarind seed XG, and if the molecular weight is decreased by digestion with endo-(1 → 4)-β-D-glucanase during removal of terminal β-D-galactopyranosyl residues, then precipitation occurs rather then gel formation (Reid et al., 1988).

In this study we report on the solution properties of an arabinosylated XG isolated from ECPs of suspension-cultured *N. plumbaginifolia* cells (*Nicotiana* XG) and a fucosylated XG isolated from apple pomace (apple pomace XG) and compared them with those of commercially available tamarind seed XG. The changes in viscosity properties of apple pomace and *N. plumbaginifolia* XGs were also investigated following enzymic and chemical modification, and the role of the side-chain substituents discussed.

2. Materials and methods

2.1. Purification of XGs

Apple pomace which had been treated with commercial pectinases was extracted sequentially with MeOH:$CHCl_3$:-formic acid:H_2O (16:5:1:1; twice), 0.1 M KOAc (pH 6.5, adjusted with acetic acid; four times), and 6 M KOH containing 20 mM $NaBH_4$ (three times; under a blanket of nitrogen gas). Each extraction was for 1 h at 20°C. Soluble fractions were then dialysed extensively against deionized water and freeze-dried. The KOH-soluble fraction was neutralized with actetic acid and treated with Fehlings solution (Jones and Stoodley, 1965). The precipitated material was applied to a column of DEAE-Sepharose CL-6B (Sims and Bacic, 1995), and the unbound neutral XG was concentrated, dialysed extensively against deionized water and freeze-dried.

Nicotiana XG was purified from ECPs of suspension-cultured *N. plumbaginifolia* cells as described by Sims and Bacic (1995). Tamarind seed (*T. indica*) XG was obtained from Megazyme (Ireland). The ratio of Glc:Xyl:-Gal:Ara was reported by Megazyme as 45:36:16:3.

2.2. Modification of XGs

Nicotiana XG (0.55 g) was dissolved in 0.2 M NaOH (200 ml) and deacetylated by incubation at room temperature for 2 h. The deacetylated *Nicotiana* XG was concentrated, dialysed extensively against deionized water and freeze-dried.

Apple pomace XG (0.5 g) was dissolved in 20 mM ammonium acetate (50 ml, pH 4.5) and incubated with α-L-fucosidase from bovine kidney (2 U, 1.2 mg protein, Sigma) at 25°C for 20 days under a few drops of toluene, then heated in a boiling water bath for 10 min. The defucosylated XG was collected by precipitation with 80% (v/v) ethanol, redissolved in deionized water and freeze-dried. Defucosylated apple pomace XG (0.1 mg) was dissolved in 20 mM ammonium acetate (50 ml) and incubated with β-D-galactosidase from *Aspergillus niger* (8 U, 0.2 mg protein; Megazyme, Australia) for 24 h at 40°C. The reaction was stopped by boiling for 10 min, protein removed by centrifugation (1000 *g*, 20 min), and the products collected by precipitation with 80% (v/v) ethanol, redissolved in deionized water and freeze-dried.

Tamarind seed XG (50 mg) was dissolved in 20 mM ammonium acetate (pH 4.5) and incubated with endo-(1 → 4)-β-D-glucanase from *Trichoderma viride* (1 U, 0.15 mg protein; Megazyme, Ireland) at room temperature for either 20 s or 30 s, then heated in a boiling water bath for 10 min. The depolymerized XG was precipitated with 80% v/v ethanol, collected by centrifugation and redissolved in deionized water. The depolymerized XG samples were chromatographed on a gel-filtration column of Toyopearl HW-65(S) (Toyo Soda Manufacturing Co. Ltd; 90 × 2.2 cm i.d.) using 6 M urea as eluant as described by Gane et al. (1995), and fractions assayed for total carbohydrate. Fractions corresponding in molecular weight (i.e., with the same elution positions) to unmodified apple pomace and *Nicotiana* XGs were collected, dialysed against deionized water (molecular weight cut-off 3500) and freeze-dried.

Table 1
Linkage composition of XGs from apple pomace, *Nicotiana* suspension cultures and tamarind seeds

Sugar	Deduced glycosidic linkage[a]	(mol %)[b]		
		Nicotiana[c]	Apple pomace	Tamarind seed
Glc*p*	4-	19	17	12
	4-, with 6-*O*-Ac	15	—	—
	4,6-	24	37	32
Xyl*p*	terminal	10	17	20
	2-	11	9	17
Gal*p*	terminal	1	2	16
	2-	—	4	—
Fuc*p*	terminal	—	7	—
Ara*f*	terminal	11	—	3
	terminal, with 5-*O*-Ac	2	—	—
Man*p*	4-	tr	6	—
Degree of acetlyation (% w/w)		10	—	—

[a]Terminal Ara*f* is deduced from 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylpentitol etc.
[b]Average of duplicate determinations.
[c]From Sims et al. (1996).
tr, Trace ($< 1\%$); —, not detected.

2.3. Linkage analysis

Methylation was performed following carboxyl reduction of uronic acids and esterified uronic acids using the NaOH method of Ciucanu and Kerek (1984), as described by Sims and Bacic (1995). Purified *Nicotiana* XG was also methylated first under neutral conditions and then resuspended in DMSO and remethylated with CD_3I, as described in Sims et al. (1996). The methylated polysaccharides were hydrolysed with 2.5 M trifluoroacetic acid for 4 h at 100°C, reduced with 1 M sodium borodeuteride in 2 M ammonium hydroxide (60°C, 60 min), and acetylated using perchloric acid as a catalyst (Harris et al., 1984). The partially methylated alditol acetates were analysed by GC-MS as described by Lau and Bacic (1993).

2.4. Analytical methods

Total carbohydrate was determined by the phenol–sulphuric acid method (Dubois et al., 1956) using glucose as a standard.

2.5. Determination of molecular weight

Weight average molecular weights were determined using multi-angle laser light scattering (MALLS). Measurements were made using a Malvern 4700 light scattering system (Malvern Instruments Ltd., Malvern, UK) with an Ar^+ ion laser operating at 488 nm. Zimm plots were constructed from measurements of solutions of XG dissolved in deionized water at five concentrations (0.05–0.25% w/v). Refractive index increments were determined using a Waters 410 differential refractive index system by integrating the differential refractive index output and comparing this with the injected polymer mass ($dn/dc = 0.135$).

2.6. Viscosity measurements

The viscosity of the XGs was measured on a Carri-Med CSL2 100 Constant Stress Rheometer (T.A. Instruments, New Castle, DE, USA) using a 6 cm diameter cone with a 2° angle and a gap of 52 μm. Steady-state viscosity measurements with increasing shear-stress were made on solutions of XG dissolved in deionized water at 0.5, 1.0, 2.0 and 5.0% (w/v) at 20°C, and on 1% (w/v) solutions at 10, 20, 30 and 50°C. The effect of pH on viscosity was determined by the addition of citrate and phosphate buffers to samples dissolved in deionized water; pH 3, 10 mM citric acid–trisodium citrate (4:1); pH 7, 10 mM sodium phosphate–disodium phosphate (1:1); pH 11, 10 mM trisodium phosphate. Samples were dissolved in deionized water (4.75 ml) and 200 mM buffer (0.25 ml) was added immediately prior to viscosity measurements to minimize the effects of possible chemical modifications (i.e., deacetylation) on viscosity.

3. Results

3.1. Structures of XGs

The linkage compositions of the purified *Nicotiana* and apple pomace XGs compared with tamarind seed XG are summarized in Table 1. All three XGs contained 4-Glc*p* and 4,6-Glc*p*, terminal Xyl*p* and 2-Xyl*p*, and terminal Gal*p* in varying proportions (Table 1). *Nicotiana* XG contained terminal Ara*f* (11 mol%), together with 4-Glc*p* and 4,6-Glc*p*, terminal Xyl*p* and 2-Xyl*p*, and a small amount of terminal Gal*p* (Sims et al., 1996). Methylation under neutral conditions showed that *O*-acetyl groups were present at C(O)6 of 44% of the 4-Glc*p* residues (15 mol% 4-Glc*p*, with 6-*O*-Ac), and at C(O)5 of 15% of the terminal Ara*f*

Table 2
Radius of gyration and molecular weight of XGs from apple pomace, *Nicotiana* suspension cultures and tamarind seeds determined by MALLS

	Nicotiana	Apple pomace	Tamarind seed
Static light scattering			
Radius of gyration (nm)	34	70	136
Molecular weight (kDa)	129	219	833

residues (2 mol% terminal Ara*f*, with 5-*O*-Ac) (Sims et al., 1996). In addition to the linkages common to all three XGs, apple pomace XG also contained 2-Gal*p* (4 mol%), terminal Fuc*p* (7%) and 4-Man*p* (6%); this latter residue was considered to represent contaminating mannan (Renard et al., 1995). Tamarind seed XG contained only terminal Ara*f* (3 mol%) in addition to the other linkages present. Gidley et al. (1991) have shown that tamarind seed XG contains a small amount of arabinan with a 5-Ara*f* backbone and side-chains of 2-Ara*f* and 3-Ara*f*, although only terminal Ara*f* residues were detected in this preparation. *O*-Acetyl groups were not detected on either apple pomace or tamarind seed XG.

3.2. Molecular weight of XGs

The radius of gyration determined by MALLS of *Nicotiana*, apple pomace and tamarind seed XGs was 34, 70 and 136 nm, respectively (Table 2). The calculated molecular weights were 129, 219 and 833 kDa, for *Nicotiana*, apple pomace and tamarind seed XGs, respectively (Table 2).

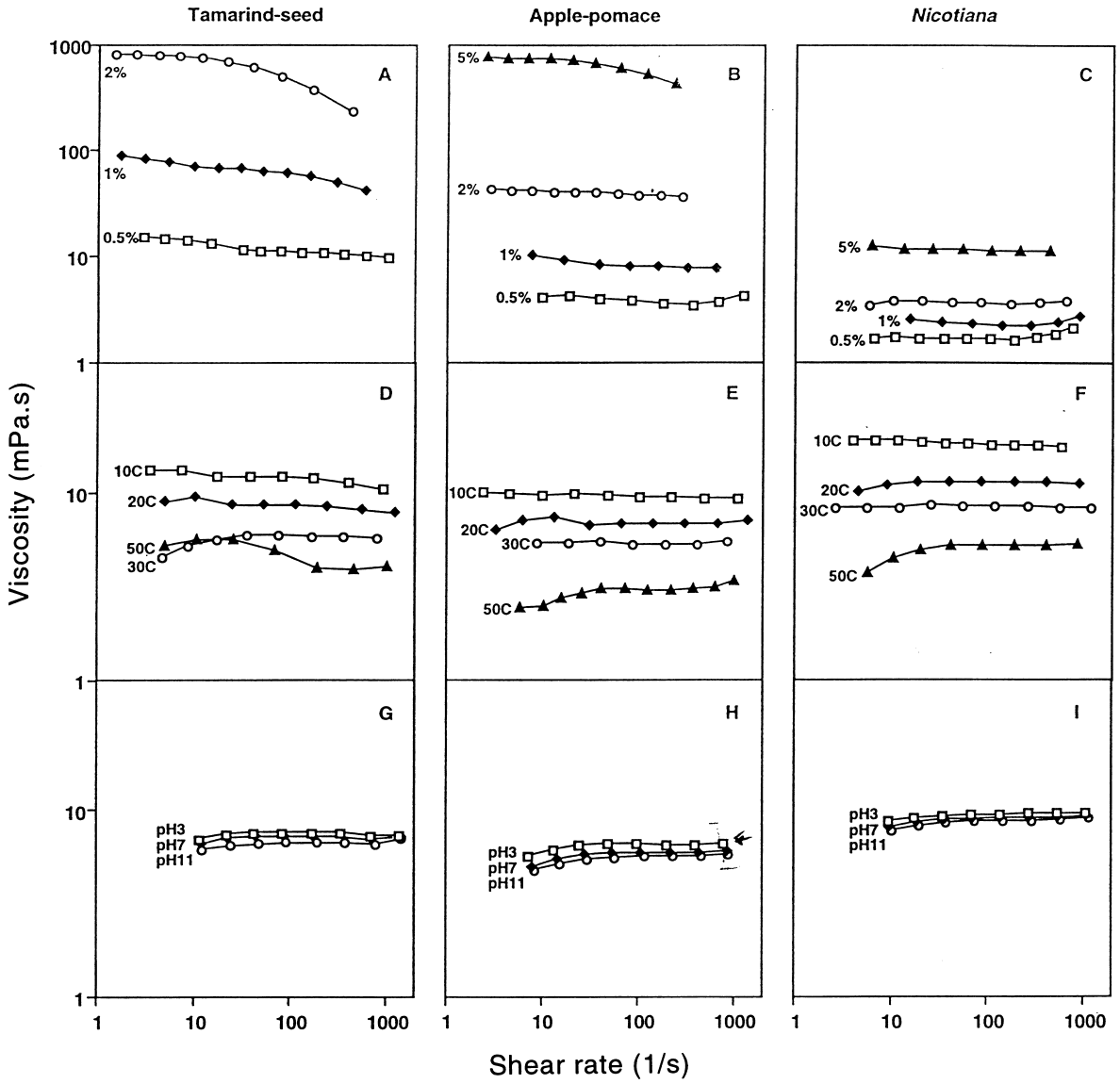

Fig. 2. Steady-state viscosity with increasing shear rate of XGs from tamarind seeds (A, D and G), apple pomace (B, E and H) and ECPs of *Nicotiana* suspension cultures (C, F and I). A, B and C, effect of concentration (% w/w) on viscosity; D, E and F, effect of temperature on viscosity of 0.5% w/v tamarind seed XG, 1% w/v apple pomace and 5% w/v *Nicotiana* XG; G, H and I, effect of pH on viscosity of 0.5% w/v tamarind seed XG, 1% w/v apple pomace and 5% w/v *Nicotiana* XG.

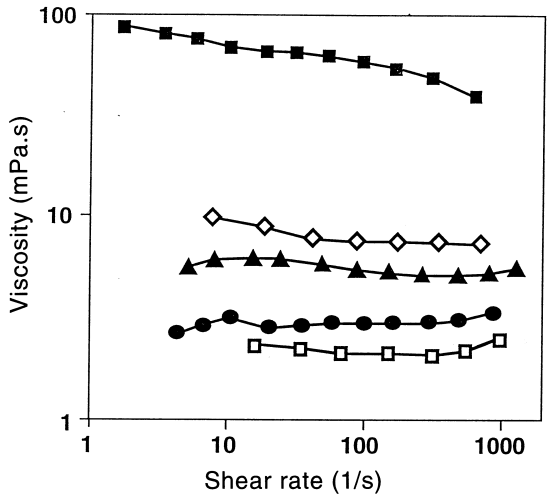

Fig. 3. Steady-state viscosity with increasing shear rate of XGs 1% w/v; unmodified tamarind seed XG (■–■), depolymerized to approximately the same molecular weight as apple pomace XG (▲–▲) and *Nicotiana* XG (●–●), respectively; unmodified apple pomace XG (◇–◇); unmodified *Nicotiana* XG (□–□).

3.3. Viscosity of XGs

Fig. 2 shows the viscosity of the XGs at different concentrations (Fig. 2A, B and C), temperatures (Fig. 2D, E and F) and pHs (Fig. 2G, H, I) of 0.5% (w/v) tamarind seed, 1% (w/v) apple pomace and 5% (w/v) *Nicotiana* XGs. Tamarind seed XG produced viscous solutions which displayed non-Newtonian behaviour (shear-thinning) at all concentrations tested, although the effect was most pronounced at 2% (Fig. 2A). At a shear-rate of $5\ s^{-1}$, 0.5 and 2% solutions had viscosities of approximately 15 and 800 mPa·s, respectively, while at $400\ s^{-1}$ their viscosities were 10 and 240 mPa·s, respectively. Tamarind seed XG solutions at 5% had a viscosity of 35 000 mPa·s at a shear-rate of $5\ s^{-1}$ and of 19 000 mPa·s at $10\ s^{-1}$ (data not shown). Solutions of apple pomace XG at 5% (w/v) were also shear-thinning (Fig. 2B), and showed viscosity properties similar to tamarind XG at 2% (Fig. 2A). Apple pomace XG at 5% and tamarind seed XG at 2% had a viscosity of approximately 800 mPa·s at $1\ s^{-1}$, but tamarind seed XG was more shear-thinning and at higher shear rates tamarind seed XG was less viscous than apple pomace XG. At 0.5, 1.0 and 2%, apple pomace XG had viscosities of approximately 4, 8 and 70 mPa·s, respectively, and displayed near Newtonian behaviour (Fig. 2B). The increase in viscosity observed at high shear-rates ($\geq 1000\ s^{-1}$) for 0.5% apple pomace XG was probably due to secondary flow (turbulence) effects resulting from the high shear rate. *Nicotiana* XG gave solutions which were considerably less viscous than either tamarind seed or apple pomace XGs (Fig. 2C). The solutions displayed near-Newtonian behaviour and had viscosities of approximately 1.5, 2, 4 and 12 mPa·s at 0.5, 1.0, 2.0 and 5.0%, respectively. Similar to apple pomace XG, increases in viscosity at high shear-rates for 0.5 and 1% *Nicotiana* XG are likely to be due to secondary flow effects. Solutions of *Nicotiana* XG at 5% (Fig. 2C) had a similar viscosity to tamarind seed xyloglucan at 0.5% (Fig. 2A), although tamarind seed XG displayed slight shear-thinning, while *Nicotiana* XG was Newtonian.

The effects of temperature and pH on viscosity were tested on tamarind seed (Fig. 2D and G), apple pomace (Fig. 2E and H) and *Nicotiana* (Fig. 2F and I) XGs at 0.5, 1.0 and 5.0% (w/v), respectively. In nearly all cases the solutions displayed near-Newtonian behaviour. The effect of temperature on viscosity shows that, in general, between 10 and 50°C the viscosity decreased as the temperature increased (Fig. 2D, E and F). The changes in viscosity were similar for all three XGs, although at lower shear rates ($5–25\ s^{-1}$) the viscosity of tamarind seed XG at 50°C was similar to that at 30°C (Fig. 2D). There was almost no effect of pH on viscosity over the range tested, with the viscosity decreasing only slightly as the pH increased (Fig. 2G, H and I).

3.4. Viscosity of modified XGs

The viscosities of tamarind seed XG following depolymerization with endo-(1 → 4)-β-D-glucanase are shown in Fig. 3. When the molecular weight of tamarind seed XG was reduced to that of unmodified apple pomace XG, the viscosity was reduced from approximately 85 to 7 mPa·s at 1% (w/v); unmodified apple pomace XG had a viscosity of 8 mPa·s when tested at the same concentration. Similarly, when the molecular weight of tamarind seed XG was reduced to that of unmodified *Nicotiana* XG, the viscosity was reduced from approximately 85 to 3 mPa·s at 1% (w/v), compared with 2 mPa·s for unmodified *Nicotiana* XG tested at the same concentration.

Apple pomace XG at 1% (w/v) gave solutions which displayed near Newtonian behaviour and had a viscosity of approximately 8 mPa·s (Fig. 2B). Following enzymic removal of terminal Fuc*p* residues (from 7 to 1 mol%), the viscosity at 1% (w/v) was approximately 4 mPa·s, similar to apple pomace XG at 0.5% (w/v). Following enzymic removal of terminal Gal*p* residues (from 6 to 1 mol%) the solution became turbid. MALLS gave large molecular weights (>1000 kDa) for defucosylated, degalactosylated apple pomace XG suspension, indicative of significant aggregation in this state. The viscosity of this defucosylated, degalactosylated apple pomace XG suspension (1% w/v) was approximately 5 mPa·s.

Native *Nicotiana* XG at 1% (w/v) gave clear solutions, which displayed near Newtonian behaviour and had a viscosity of approximately 2 mPa·s (Fig. 2C). Following deacetylation, the *Nicotiana* XG solution also became turbid and the viscosity (1.5 mPa·s at 1% w/v) was essentially unaltered. Similar to apple pomace XG following removal of Fuc*p* and the Gal*p* residues, MALLS gave large molecular weights of the order of 1000 kDa for deacetylated *Nicotiana* XG suspended in water, indicative of significant aggregation.

4. Discussion

Investigation of the solution properties of *Nicotiana*, apple pomace and tamarind seed XGs shows that their viscosity differs by several orders of magnitude. The viscosity of the solutions at the same concentration increases with increasing molecular weight (*Nicotiana* < apple pomace < tamarind seed). Furthermore, depolymerization of tamarind seed XG results in a reduction in its viscosity, and at molecular weights similar to *Nicotiana* and apple pomace XGs the viscosity of tamarind seed XG was close to that of the unmodified *Nicotiana* and apple pomace XGs (see Table 2). The branching patterns and structure of the three XGs is similar (see Fig. 1), and thus, as the molecular weight increases, the hydrodynamic volume and the radius of gyration also increases (Table 1). The larger radius of gyration and hence hydrodynamic radius results in higher effective volume fractions and hence increased viscosity. Thus, *Nicotiana* XG has the lowest viscosity (2 mPa·s at 1% w/v and 5 s^{-1}) and tamarind seed XG has the highest viscosity (95 mpa·s), with that of apple XG intermediate (8 mPa·s). The greater radius of gyration of tamarind seed XG compared with *Nicotiana* and apple pomace XGs also influences the shear-rate dependence of their solution viscosity. Thus, 5% w/v apple pomace XG and 2% tamarind seed XG have similar viscosities at low shear rates (800 mPa·s at 5 s^{-1}), but tamarind seed XG shows greater shear-thinning behaviour, owing to the greater influence of chain interactions and possible distortions in the higher molecular weight polysaccharide. This effect can also be observed when tamarind seed and *Nicotiana* XGs are compared, but the differences are less apparent.

The effect of temperature on viscosity was similar in all three XGs, suggesting that differences in the side-chain structures were not differentially altered by increasing temperature. In addition, there was almost no effect of pH on viscosity of any of the XGs over the range tested. Surprisingly, *Nicotiana* XG, which bears *O*-acetyl groups, did not show any change in viscosity at different pHs; at pH 3 *O*-acetyl groups will be ionized and expected to influence solution properties. However, the location of the *O*-acetyls and their conformation may be such that they do not provide any steric hindrance for intermolecular interactions (Fig. 1C). The pH stability of apple pomace and tamarind seed XGs, which are not *O*-acetylated, is expected; tamarind seed XG has been shown previously to have excellent stability over the acid pH range (Glicksman, 1986).

Chemical and enzymic modification of XGs from apple pomace and *Nicotiana* suspension cultures all gave solutions with lowered viscosities, although the extent of the change in viscosity depended on the type of modification. Removing terminal Fuc*p* residues from apple XG gave the greatest relative reduction in viscosity (from 8 to 4 mPa·s), suggesting that the presence of Fuc*p* on the side-chains has a substantial effect on the conformation and flexibility of apple pomace XG. Using energy calculations of the static and dynamic equilibrium conformation of model XG heptadecasaccharide fragments, Levy et al. (1991) have determined that the fucosylated side-chains play a significant role in stabilizing the conformation of XG. The Fuc*p* residue interacts primarily with the preceding backbone Glc*p* residue (i.e., on the non-reducing side of the site of side-chain attachment), and interacts significantly with the Xyl*p* residue through which the Fuc*p* residue is attached, maintaining a tight folding of the trisaccharide side-chain. Thus, removing the Fuc*p* residues from apple XG probably reduces the stability of the backbone, increasing its flexibility and leading to the lower viscosity observed. Removing both the Fuc*p* and the Gal*p* residues from apple pomace XG reduced the viscosity of the solution from 8 to 5 mPa·s, but also resulted in the formation of large aggregates and precipitation of the XG from solution. Similar changes in viscosity and water solubility have been observed during enzymic removal of Gal*p* residues from tamarind seed XG with β-D-galactosidase; as Gal*p* was released, the viscosity of the solution gradually decreased, and then increased greatly until gel formation (Reid et al., 1988). If the chain length of tamarind seed XG was reduced by the addition of endo-β-D-glucanase together with the β-D-galactosidase, gel formation was suppressed and precipitation occurred. It can be speculated that defucosylated, degalactosylated apple pomace XG is more likely to undergo transitions to the flat conformation, making XG–XG self-associations more likely, resulting in precipitation (Levy et al., 1991).

Deacetylation of *Nicotiana* XG had little effect on viscosity, although there was a slight reduction from 2 to 1 mPa·s. However, the turbidity of the solution showed that deacetylated *Nicotiana* XG was no longer completely water soluble. The anomalous molecular weight determined by MALLS (> 1000 kDa) suggested that deacetylated *Nicotiana* XG forms large aggregates in water. Thus, it appears that *O*-acetylation of *Nicotiana* XG prevents self-association of molecules and maintains its solubility. In apple pomace and tamarind seed XGs, which are not O-acetylated, aggregation of molecules is supressed by the degree and nature of the glycosyl side-chains, as demonstrated by the effects of removing Fuc*p* and the Gal*p* residues apple pomace XG.

There has been much work focused on the solution properties of galactomannans and glucomannans, and the influence of the degree of substitution of the backbone (both glycosyl and non-glycosyl). The backbones of mannans and of XGs are similar, with both 1,4-β-D-Glc*p* and 1,4-β-D-Man*p* giving rise to polysaccharide chains with a 2-fold screw-axis. Precipitation of galactomannans, by self-association, occurs at degrees of substitution below 11% (Reid and Edwards, 1995), and gelation of tamarind seed XG occurs at a similar degree of Gal*p* substitution of the Xyl*p* branches on the glucan backbone (Reid et al., 1988). Deacetylation of *Nicotiana* XG reduces the degree of substitution of the backbone from 67 to 40% (Sims et al., 1996), suggesting that *O*-acetylation plays an important role in

maintaining solubility. Millane et al. (1992) suggest that the *O*-acetyl substitution of konjac glucomannan prevents self-association of the mannan chains, but that following deacetylation, chain interactions become more energetically favourable. Millane and Wang (1992) suggest that the *O*-acetyl groups on the side-chains of xanthan, which like XG has a 1,4-β-D-Glcp backbone, have no critical involvement in the ordered structure of the molecule, although Foster and Morris (1994) show that removal of *O*-acetyls decreases the order–disorder transition temperature of xanthan and its polytetramer variant, indicating that *O*-acetyl substituents do contribute to its stability (Dea, 1987; Hwang and Kokini, 1991; Lopes et al., 1992). Thus, *O*-acetylation probably plays an important role in stabilizing the conformation of *Nicotiana* XG, reducing the likelihood of conformational transitions and self-association.

This study demonstrates that molecular weight is the major factor influencing the solution properties of three structurally related XGs with similar degrees of substitution of the backbone, and similar distribution of backbone substituents. Differences in the types of side-chains between the XGs are only significant at given molecular weights. Modification of the side-chains alters the degree of aggregation of the XGs dramatically, and thus their solubility.

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