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Isolation, characterisation, and NMR study of xyloglucan from enzymatically depectinised and non-depectinised apple pomace

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Abstract

Xyloglucan extracted with 4 M KOH from enzymically-depectinated and non-depectinated apple pomace was shown to be fucogalactoxyloglucan. The xyloglucan from the enzymically-depectinated apple pomace was found to be of lower molecular weight than that from the non-depectinated pomace. Xyloglucan from the non-depectinated pomace had an intrinsic viscosity of 244 ml/g. The oligosaccharides XXFG and XLFG were isolated from the 1,4-β-D-glucanase hydrolysate of the xyloglucan and their ^{13}C NMR spectra were obtained for the first time. Assignment of the anomeric ^{13}C NMR signals of these oligosaccharides permitted the unambiguous assignment of the anomeric region of the ^{13}C NMR spectra of the fucogalactoxyloglucan. In the spectra of the oligosaccharides, splitting was observed in the C-6 signal from the fucosyl residues, indicating their proximity to the reducing glucosyl residues and thus yielding information about the conformation of xyloglucan in solution. Integration of the anomeric signals of ^{1}H NMR spectra permits the rapid and non-destructive screening of the composition of xyloglucans from different plants. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Xyloglucan; Apple pomace; NMR spectroscopy; Plant cell walls

Abbreviations: AIR, alcohol insoluble residue; CDTA, *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid; CTAB, cetyltrimethylammonium bromide; CWM, cell wall material; DP, degree of polymerisation, HPSEC, high performance size exclusion chromatography; PAW, phenol–acetic acid–water (2:1:1); TFA, trifluoroacetic acid.

1. Introduction

Apple pomace is a low value waste from apple juice production. Amongst the possible uses of the pomace are its addition to food to increase dietary fibre and in the production of ethanol, butanol and citric acid by fermentation (Kennedy, 1994). Recently, apple pomace has been utilised as a source of potentially valuable phenolic antioxidants (Lu and Foo, 1997). Traditionally, pectin has been obtained from pomace (May, 1990). Beside pectin the other major components of pomace are the cell wall polysaccharides cellulose and xyloglucan. Our approach to utilising apple pomace was to seek uses for the xyloglucan component. After isolation from the pomace by alkaline extraction (Renard et al., 1995) xyloglucan could be derivatised into a range of compounds comparable to those that can be prepared from cellulose (e.g. methylcellulose, hydroxypropylcellulose, carboxymethylcellulose). Such derivatives might be useful as thickening agents or texture modifiers. Another potential use for xyloglucan is as a source of biologically active oligosaccharides (Ogawa et al., 1991; Aldington and Fry, 1993).

Xyloglucan itself is of interest because it is thought to play an important role in dicot cell wall expansion. It apparently cross-links cellulose microfibrils by hydrogen bonding to their surface cellulose molecules. The cell wall modifying enzymes may then act on the xyloglucan to break either the cross-links (Fry, 1989) or the hydrogen bonds (McQueen and Cosgrove, 1994) and thus allow the cell wall to expand.

Xyloglucan has been extracted from the cell walls of apple fruit by using 4 M NaOH (Renard et al., 1992). After purification the xyloglucan was hydrolysed into the oligosaccharides XXXG, XXFG and XLFG (Fig. 1) which were obtained in approximately equal proportions. For an explanation of xyloglucan oligosaccharide terminology the reader is referred to Fry et al. (1993). In other work xyloglucan from apple fruit was hydrolysed by endoglucanase and 80% of it was found to be composed of XXXG, XXFG, XLXG and XLFG (Vincken et al., 1996). Depectinated apple pomace has been extracted with hot acid and the

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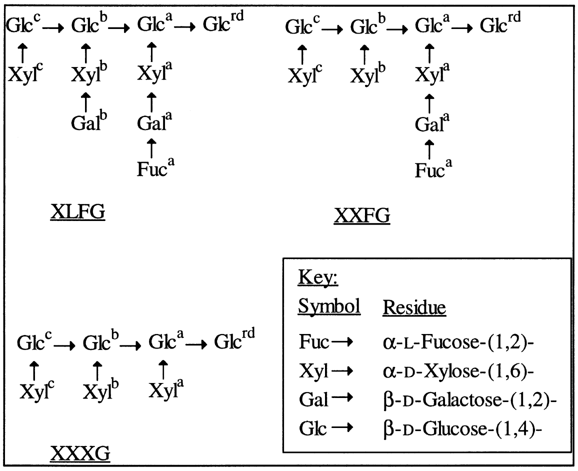


Fig. 1. The structures of xyloglucan oligosaccharides XLFG, XXFG and XXXG with the designation of the glycosyl residues.

resulting xyloglucan was found to have low viscosity and a relatively low fucose content (Renard et al., 1995). It was suggested the hot acid caused the hydrolysis of many of the fucosyl residues from the xyloglucan.

In the present paper we report the properties of xyloglucans that were extracted by 4 M KOH from commercial apple pomace with and without pectinase pretreatment. The ^{13}C and ^{1}H NMR and intrinsic viscosity of the xyloglucan were recorded. The component oligosaccharides XXXG, XXFG and XLFG were obtained and their ^{13}C NMR spectra were used to interpret the ^{13}C NMR spectra of xyloglucan. In addition, we have investigated the conformational transitions in xyloglucan at different temperatures.

2. Experimental

2.1. Materials

Apple pomace samples were obtained from the New Zealand Apple and Pear Marketing Board's factory operation at Nelson. The initial stage of all pomace preparations was maceration and pressing. No exogenous enzymes were employed in production of pomace samples 1–3. Pomace samples 2–4 were freeze-dried soon after production, and the freeze-dried material was kept at −20°C until used.

Pomace 1 contained the pressings from whole Granny Smith apples, and was not dried before extraction.

Pomace 2 contained the pressings from whole Granny Smith apples (85%), and Red Delicious apples (15%).

Pomace 3 contained the pressings from whole Braeburn apples, unlike pomace 1 and pomace 2 the pressings had been washed with water.

Pomace 4 was as for apple pomace 3 but with the additional treatment with a commercial pectolytic enzyme preparation (Pectinex Ultra SP™, at 80 g/MT).

2.2. Pomace 1 extraction

Pomace 1 was frozen with liquid nitrogen and ground to a fine powder in a cryo-mill and cell wall material (CWM) was prepared by extracting 1 part pomace with 2 parts phenol–acetic acid–water, 2:1:1 w/v/v (PAW) (Selvendran and Du Pont, 1980). The cell walls, and PAW-soluble extract were dialysed separately against distilled water and freeze-dried. The material solubilised by PAW contained material which became insoluble during dialysis and this was recovered separately.

The cell wall material was sequentially extracted. CWM (9.8 g) was extracted twice with 0.05 M CDTA (pH 6.5, 795 ml) containing 0.05% chlorbutol for 16 h at 25°C. The pellet from the CDTA extractions was extracted with 1 M KOH containing 26 mM NaBH$_4$ (950 ml) under nitrogen for 2 h at 20°C. The pellet was re-extracted twice with 4 M KOH containing 26 mM NaBH$_4$ (950 ml) under nitrogen for 2 h at 20°C. The soluble extracts were collected by centrifugation, neutralised if necessary and dialysed. Material solubilised during extraction but which became insoluble with dialysis was recovered separately. The final insoluble residue was dialysed and freeze-dried.

Table 1
Yields, neutral sugar, uronic acid, protein and ash analysis of sequential extracts of pomace 1 from non-depectinated Granny Smith apples. All weights are milligram of anhydro-sugar per gram dry weight (mg/g) of each fraction

Fraction	Yield (% CWM)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acid	Protein	Ash
PAW soluble	5.8	5	—	39	6	5	30	92	370	60	10
PAW insoluble	7.8	2	2	14	2	4	15	18	86	310	12
CDTA Ext. 1 sol.	8.9	6	3	44	1	—	11	2	494	130	147
CDTA Ext. 1 insol.	1.3	—	—	30	7	8	14	330	156	100	19
CDTA Ext. 2 sol.	5.7	2	1	38	1	—	8	1	343	210	185
CDTA Ext. 2. insol.	0.5	1	—	23	5	1	10	432	83	230	28
1 M KOH sol.	5.4	3	26	27	168	13	73	287	38	100	33
1 M KOH insol.	5.8	4	1	54	16	6	17	74	85	320	29
4 M KOH Ext. 1 sol.	4.1	4	23	31	150	98	85	317	59	40	23
4 M KOH Ext. 1 insol.	2.5	2	1	28	22	3	13	280	45	300	6
4 M KOH Ext. 2 sol.	0.8	13	13	76	78	36	69	167	118	130	74
4 M KOH Ext. 2 insol.	1.0	5	1	43	8	3	14	75	136	140	25
Insoluble residue	58.6	7	—	132	31	13	57	351	188	6	17

Table 2
Yields of partially purified xyloglucan from apple pomace 2, pomace 3, and pomace 4

Sample description	Mass of pomace extracted (g)	Mass of AIR (g)	AIR taken for extraction (g)	Yield of 0.1 M KOH fraction (P1) (% AIR)	Mass of purified xyloglucan (H1) (g)	Yield of xyloglucan (% AIR)
Pomace 2	450.1	282.1	120.0	n.d.[a]	4.67	3.9
Pomace 3	42.1	28.0	28.0	16.4[b]	1.56	5.6
Pomace 4	100.0	88.5	30.0	9.5[b]	3.10	10.3

[a] n.d.: not determined.

[b] Calculated from an aliquot of the extract.

Table 3
Comparison of monosaccharide composition of partially purified xyloglucans from 85% Granny Smith and 15% Red Delicious apple pomace without enzyme treatment (pomace 2), Braeburn apple pomace without enzyme treatment (pomace 3) and Braeburn apple pomace treated with commercial pectinase (pomace 4). The results are given as milligram of anhydro-sugar per gram dry weight (mg/g) of each xyloglucan

Monosaccharide	Pomace 2	Pomace 3	Pomace 4
Rha	—[a]	—[a]	—[a]
Fuc	41	38	32
Ara	8	7	7
Xyl	176	176	173
Man	64	34	29
Gal	98	89	87
Glc	382	384	388
Uronic acid	31	20	23
Total (mg/g)	800	748	739

[a] —: not detected.

2.3. Extraction and partial purification of xyloglucan from pomace 2, pomace 3, and pomace 4

In order to obtain gram quantities of xyloglucan rapidly, the method of Voragen et al. (1986) was modified. Typically alcohol insoluble residue (AIR) (30 g) was prepared as described by Vries et al. (1981), and extracted under nitrogen, with 0.1 M KOH containing 8 mM $NaBH_4$ (2 l). The mixture was centrifuged and the pellet was then extracted with 4 M KOH (1 l) containing 26 mM $NaBH_4$ at 20°C under nitrogen for 2 h. The soluble material was separated by centrifugation, cooled on ice and adjusted to pH 5 by the addition of glacial acetic acid. The 4 M KOH-soluble material was dialysed and centrifuged to yield a solution of xyloglucan. The results of the duplicate extraction of pomace 2 (Table 2) agreed within ± 6%.

2.3.1. Further purification of xyloglucan

A solution of xyloglucan was stirred with an equal volume of aqueous CTAB (2%) containing 20 mM Na_2SO_4 (Scott, 1965). The mixture was held at 37°C overnight, centrifuged and filtered through a 10 mm bed of Celite on glass filter paper. To remove excess CTAB, a solution of 1.3 M potassium iodide (0.05% of the volume) was added and the bulk of the precipitate was removed by centrifugation. Subsequent filtration through sintered glass gave a clear solution, which was dialysed exhaustively and then freeze-dried.

The freeze-dried material was dissolved in water to give a 1% solution, and 0.17 volumes of Fehling's solution was added to precipitate the xyloglucan (Jones and Stoodley, 1965). After 2 h the mixture was centrifuged and the pellet blended with cold 0.2 M HCl. The resulting blue solutions were adjusted to pH 6 with aqueous 1 M ammonia, concentrated by rotary evaporation and dialysed exhaustively against distilled water. The dialysate was freeze-dried to give xyloglucan as an amorphous white solid.

2.4. Determination of sugars as alditol acetates

The polysaccharide extracts were hydrolysed with 2 M trifluoroacetic acid at 121°C for 1 h and the resulting monosaccharides reduced and acetylated (Albersheim et al., 1967). The alditol acetates were analysed by gas chromatography (Hewlett–Packard 5890 gas chromatograph equipped with a gas splitter). The column was a SGE BPX-70 (12 m × 0.33 mm, film thickness: 0.25 μm) held at 180°C for 1 min and then the temperature was increased at 3°C/min to 215°C. The injector temperature and flame ionisation detector temperature were 275°C. Inositol was used as an internal standard. The results (Table 3) are the average of duplicates and are reported as the anhydro-sugars.

2.5. Saeman hydrolysis and alditol acetate formation

Polysaccharide was hydrolysed in 72% H_2SO_4 (Selvendran et al., 1979) for 3 h at room temperature. The mixture was diluted to give 1 M H_2SO_4, held at 100°C for 2 h and then neutralised with barium carbonate. The monosaccharides were quantified as alditol acetates (see Section 2.4).

2.6. Uronic acid, protein and ash determinations

The uronic acid content of the extracts was determined by the method of Blumenkrantz and Asboe-Hansen (1973). The protein composition of the pomace extracts was calculated from the elemental nitrogen analyses using a conversion factor of 6.25. The nitrogen and ash contents were determined by the Micro-Analytical Laboratory of the University of Otago.

2.7. HPSEC

HPSEC was conducted using an analytical BioSep-Sec-S4000 column (300 × 7.8 mm, 5 μm particle size). The eluent was potassium acetate buffer (50 mM, pH 7.0) and the flow rate was 1 ml/min. Detection was by a Waters 410 differential refractometer. Samples of purified pomace 3 and 4 xyloglucans were dissolved in the mobile phase (5 mg/ml and filtered through a 0.45 μm Millipore filter prior to injection. Dextrans (obtained from Sigma Chemical Co.) were used as molar mass markers.

2.8. The intrinsic viscosity of apple pomace xyloglucan

The reduced viscosity of pomace 2 xyloglucan at concentrations between 0.025 and 0.13 g/100 ml was determined using a Ubbelohde dilution viscometer at 25.00 ± 0.05°C. The experiment was done in duplicate. The intrinsic viscosity was determined by extrapolation to zero concentration.

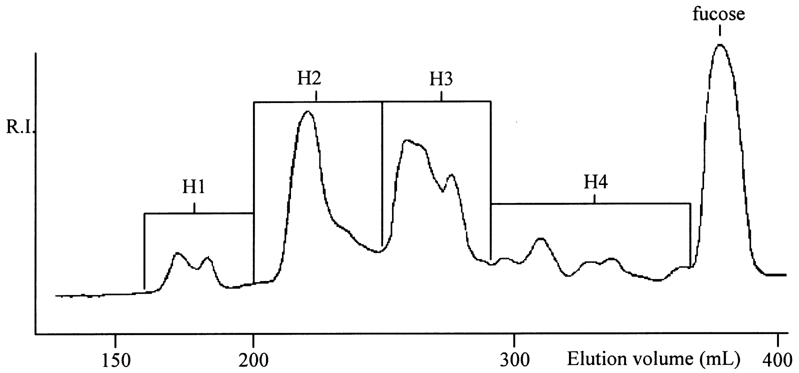


Fig. 2. Size exclusion chromatography of the products from the cellulase treatment of pomace 2 xyloglucan. RI; refractive index response.

2.9. The viscosity–temperature relationship of apple pomace xyloglucan

The viscosities of pomace 2 and tamarind seed xyloglucans (0.530 g/100 ml) were determined at various temperatures using an Ostwald viscometer.

2.10. Rheology of apple pomace xyloglucan in 60% sucrose

Pomace 2 xyloglucan (0.30 g) was added to 15 ml of water containing 60% sucrose (w/v). The polysaccharide was dissolved by boiling the mixture for 20 min. Losses due to evaporation were compensated for by the addition of water. After 24 h at room temperature the solution was examined to determine if a gel had formed.

2.11. Optical rotation of xyloglucan

The optical rotation of pomace 2 xyloglucan (0.530 g/100 ml) was determined over a range of temperatures. The sample was placed in a water-jacketed polarimeter cell, (path length 10 dm, cell volume 1 ml) and the optical rotations were measured using a Perkin–Elmer 141 Polarimeter equipped with a sodium lamp.

2.12. Enzymic hydrolysis of xyloglucan

Cellulase [from *Trichoderma viride* (EC 3.2.1.4), Sigma Chemical Co.] was desalted by using a Pharmacia XK16 Fractogel HW-40s column (100 cm × 16 mm) eluted with water, and freeze-dried. The purified pomace 2 xyloglucan (212 mg) was dissolved in acetate buffer (20 mM, pH = 4.7, 21 ml) which contained L-fucose (40 mM) (Fry, 1989). Desalted cellulase (21 mg) was added and the solution was stirred for 4 h at 20°C. The reaction was terminated by heating to 100°C for 10 min. The hydrolysate was fractionated by preparative size exclusion chromatography using three Fractogel HW-40s (100 cm × 16 mm) columns in series, which were eluted with water. Detection was by a Waters R401 differential refractometer. Fractions were pooled as indicated in Fig. 2. Only fraction H3 (108 mg) was subjected to further fractionation.

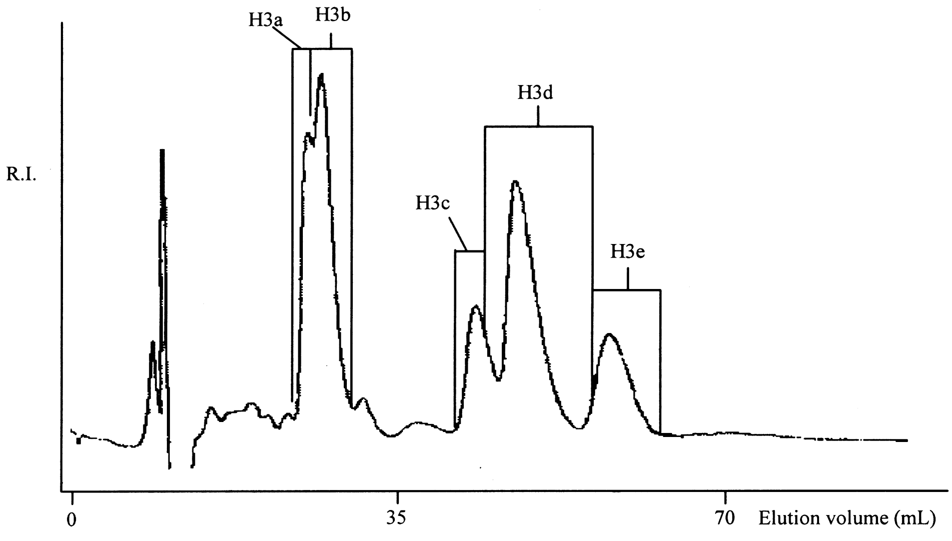


Fig. 3. Reverse phase HPLC of fraction H3 oligosaccharides from the cellulase treatment of pomace 2 xyloglucan. RI; refractive index response.

2.13. Reverse-phase HPLC chromatography of fraction H3 from cellulase treatment of xyloglucan

Fraction H3 (Fig. 2) was fractionated by preparative reverse-phase HPLC using an Activon ODS column packed with Exsil 100, 10 μm octadecylsilica (250 × 10 mm, 30 000 plates/m) and eluted with methanol–water (14:86) 2 ml/min. Fractions were pooled as shown in Fig. 3, concentrated by rotary evaporation, and dried in vacuo. Fraction H3d proved to be a mixture of XXFG and XLFG, and it was re-chromatographed to allow more of fractions H3c and H3e to be collected. Fractions H3c and H3e were purified by re-chromatography on the same HPLC column followed by preparative size-exclusion chromatography on a Fractogel HW-40s column, before being concentrated to dryness. The final yields of XXFG and XLFG were 11 and 4 mg, respectively. Small quantities of XXFG (3.5 mg) and XLFG (2 mg) were reduced with $NaBH_4$ using the standard procedure.

2.14. NMR spectroscopy

^{1}H and ^{13}C NMR were recorded on a Varian VXR 300 MHz spectrometer. Samples were dissolved in D_2O and methanol was used as a reference. For xyloglucan a 10 mm probe was used. Methanol was referenced relative to TMS. At 25°, methanol was referenced to δ 3.324 for the proton spectra and δ 50.17 for the ^{13}C spectra. At 75°, methanol was referenced at δ 3.357 for the proton spectra and δ 50.23 for the ^{13}C spectra. For proton NMR of xyloglucan oligosaccharides homonuclear irradiation was employed for the suppression of the HOD peak. For proton NMR of xyloglucan the 180–τ–90 pulse sequence was used for water suppression. The HETCOR spectrum of fraction H3 (244 mg) dissolved in D_2O (1 ml) was run with a carbon spectral window of 8400 Hz, acquiring 6700 data points.

3. Results and discussion

3.1. Composition of apple pomace

In order to compare apple pomace cell walls with published work on apple fruit cell walls, we isolated the pomace cell walls and subjected them to a standard sequential extraction procedure. Pomace from Granny Smith apples (pomace 1) treated with PAW yielded 10% cell wall material (CWM). The yield of water-soluble material obtained by two CDTA extractions of the CWM was 14.6% (w/w). This compares favourably with the 9.5% yield of CDTA-soluble material from the AIR of starch-free cider apples (Chapman et al., 1987), and the 10.6% extracted from the AIR of Golden Delicious apples (Renard and Thibault, 1993). The soluble material from the first CDTA extraction contained 49% uronic acid and 7% neutral sugars (Table 1). The neutral sugars were mainly arabinose (4.4%) and galactose (1%). These results are comparable with those of Renard and Thibault (1993) who found that their CDTA extract of Golden Delicious apples AIR contained 55% uronic acid, 3.5% arabinose, 4% galactose, 2% glucose and 11.2% protein. In contrast however, Chapman et al. (1987) extracted the AIR of cider apple cortex with CDTA and found it contained 78% uronic acid, 3% arabinose and 1% galactose.

In the present study when apple pomace 1 AIR was extracted twice with CDTA, the extracts on standing formed insoluble material which was separated to produce the CDTA-soluble and CDTA-insoluble fractions. The CDTA-insoluble fractions contained large amounts of glucose (33% and 43% for the first CDTA extraction (Ext. 1) and the second extraction (Ext. 2), respectively) and smaller amounts of uronic acid (16% and 8% for Ext. 1 and Ext. 2, respectively) which suggested that starch and pectin are present in these fractions. Freeze-dried extracts tested with a solution of KI and I_2 confirmed the presence of starch.

The yield of water-soluble material from the 1 M KOH extraction of pomace was 5.4%. The composition of this extract is reported in Table 1. Previous studies on apple cell walls (Stevens and Selvendran, 1984; Ruperez et al., 1985; Voragen et al., 1986) suggest this fraction can be seen to contain small amounts of arabinans, arabinogalactans and galactans which are likely to be bound to fragments of pectic backbone. The presence of mannose indicates that a small amount of mannan is also present, and the fucose and xylose content indicates that xyloglucan is a large proportion of the extract.

The yield of the water-soluble material extracted with the first 4 M KOH extraction was 4.1%. The monosaccharide composition is shown in Table 1. The monosaccharide composition is similar to those of reported extractions of apple cortex cell walls (Stevens and Selvendran, 1984; Ruperez et al., 1985; Renard et al., 1991b). Comparison with literature data indicates the presence of xyloglucan, mannan and some pectic substances.

During the juice extraction process, the apple cell walls are subject to the action of enzymes liberated from the ruptured cells. In addition polyphenols are formed which precipitate on the cell wall matrix (Renard et al., 1995) and these may interfere with the extraction of polysaccharides. In spite of this, it is apparent that the cell wall composition of apple pomace which has not been depectinated remains similar to that of the cortex of whole apples.

3.2. Preparation and properties of xyloglucan extracted from AIR of apple pomace

A direct route was sought for the extraction of large amounts of xyloglucan from apple pomace. The method decided upon for extraction was similar to that which Voragen et al. (1986) used for the extraction of xyloglucan from apple cortex. The major difference was the time of 4 M KOH extraction, which was reduced to 2 h. To purify the

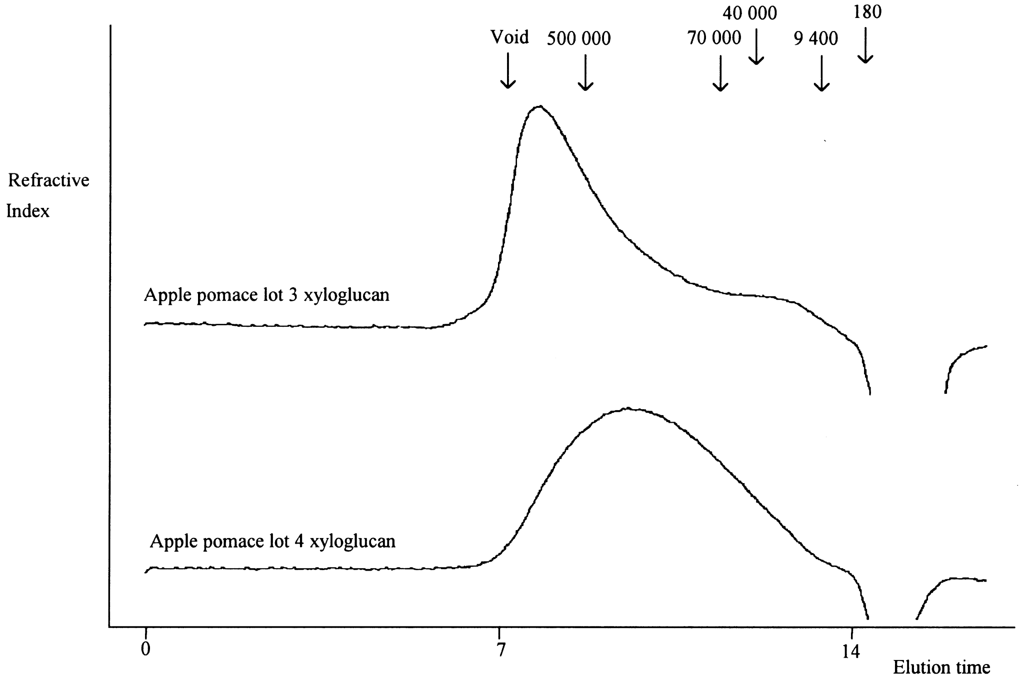


Fig. 4. HPSEC of xyloglucans from pomace 3 (no pectinase treatment) and pomace 4 (after pectinase treatment), The diagrams have been offset for clarity. The molar mass peaks of standard dextrans are indicated.

extract, pectin was removed by precipitation with a quaternary ammonium salt (Scott, 1965), and xyloglucan was then precipitated with Fehling's (Jones and Stoodley, 1965).

For unwashed and non-depectinated Granny Smith and Red Delicious apple pomace 2, the average yield of partially purified xyloglucan was 3.9% on a dry weight basis (Table 2). The yield from the washed, non-depectinated Braeburn apple pomace was 5.6%, and the washed and enzyme depectinated Braeburn pomace gave a 10.3% yield. The yield of xyloglucan based on the sum of the xylose and glucose content was 5.1% for the enzyme depectinated Braeburn pomace. This is comparable with the yield of 4.8% obtained by Renard et al. (1995) from acid-depectinated apple pomace for a 16 h extraction with 4 M NaOH at 40°C.

Pomace 4 was treated with Pectinex Ultra SP™, which is a pectolytic enzyme preparation that has been shown to hydrolyse rhamnogalacturonan, arabinans and β-1,4-galactan but not β-1,3-, β-1,6-galactans in type II arabinogalactans, which have been found in apple juice (Will and Dietrich, 1992). Some cellulase was also present in the enzyme preparation. The yield of xyloglucan from pomace 4 was significantly higher than from the other two types of pomace. A synergistic effect is observed when pectinases and endo-glucanases are used to extract polysaccharides from the cell walls of the apple (Renard et al., 1991a) which indicates some intermeshing of pectin and xyloglucan. Given this result, one would expect a higher yield of xyloglucan following the hydrolysis of some of the pectin, and this is indeed was found with pomace 4. HPSEC (Fig. 4) showed that the molar mass of the purified hemicellulose from pomace 4 is considerably smaller than that from pomace 3, and thus it is apparent that the enzyme treatment has depolymerised the xyloglucan probably due to the presence of cellulase in the commercial pectinase. Remarkably, all purified xyloglucan preparations had very similar neutral sugar compositions (Table 3), with little variation due to the apple variety or the type of pomace used for extraction. However, there were some small differences. For example, the mannose contents (which is presumably due to a mannan contaminant) of the preparation from pomace 3 and pomace 4 were less than that from pomace 2.

Table 4
Neutral sugar and uronic acid composition (mol%) of purified pomace 2 xyloglucan and comparison with literature data

Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acid
Pomace 2 xyloglucan (purified from non-depectinated pomace)							
—	5.6	1.2	25.3	6.9	12	46.2	2.8
Xyloglucan from hot acid treated apple pomace (Renard et al., 1995)							
0.5	1.8	1.4	34.1	2.5	14.1	44.8	0.8

3.3. Viscosity study of partially purified apple pomace xyloglucan

The intrinsic viscosity of purified apple pomace xyloglucan in water was found to be 244 ml/g which agrees with the value of 240 ml/g for xyloglucan from hot acid treated pomace (Renard et al., 1995). This result is unexpected as different methods of extraction were used. Hot acid treatment for pectin extraction might be expected to cause a small amount of hydrolysis of the xyloglucan backbone which would lower the molecular weight and thus the

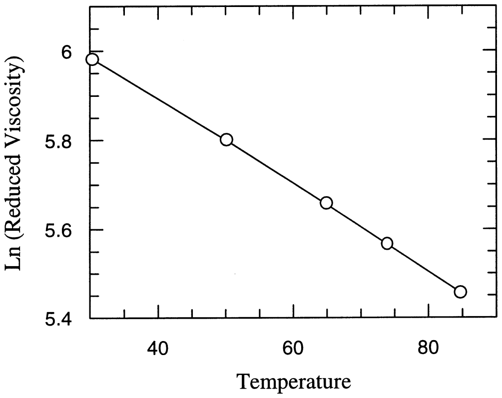


Fig. 5. The log of the reduced viscosity versus temperature for apple pomace 2 xyloglucan (0.530 g/l).

intrinsic viscosity. However, the depolymerisation appears to have been minimal, or alternatively, differences in monosaccharide composition compensate for the depolymerisation. The neutral sugar composition of the two preparations are compared in Table 4. The xyloglucan preparation described by Renard et al. (1995) contained similar amounts of glucose and galactose to that from pomace 2, however it contained a larger proportion of xylose, and a smaller proportion of fucose. Tamarind seed xyloglucan contains no fucose and its intrinsic viscosity (600 ml/g; Gidley et al., 1991) is considerably higher than that of apple pomace xyloglucan.

In addition, the reduced viscosities of xyloglucans from tamarind seed and apple pomace were found to be 2080 and 400 ml/g, respectively. Hence it is apparent that tamarind seed xyloglucan is considerably more viscous than the apple pomace xyloglucan in infinitely dilute, as well as in more concentrated solutions. Indeed, tamarind seed xyloglucan forms a gel in concentrated sucrose solutions and therefore can be used to form pectin-like gels in products such as jams and preserves (Glicksman, 1986). We were unable to form a gel from apple pomace xyloglucan in concentrated sucrose solutions.

In order to detect any major conformational changes which may occur in solutions of apple pomace hemicellulose upon heating, the reduced viscosity was determined over the temperature range of 30–85°C. A plot of the log of reduced viscosity versus temperature gave a relatively straight line up to 85°C (Fig. 5) showing no discontinuity which would be indicative of aggregation or dissociation of the molecules.

3.4. Optical rotation study

The optical rotation of a solution of apple pomace xyloglucan showed no change with increasing temperature (Table 5). Changes in polysaccharide conformation are frequently accompanied by large changes in optical activity (Dea et al., 1977) and so the results of the present study suggest no major conformational change has occurred between 20 and 77°C. The optical rotation of +41.1° ($c = 0.53$, H_2O) is comparable with +43° ($c = 0.14$, H_2O) previously reported (Aspinall and Fanous, 1984) for xyloglucan extracted with 1 M KOH from apple cortex.

Table 5
Optical rotation of partially purified apple pomace 2 xyloglucan

Temperature (°C)	20.0	29.6	50.0	56.5	64.5	72	77
$[\alpha]_D$	41.1	41.5	41.3	41.4	41.5	41.0	41.4

3.5. Isolation of XXXG, XXFG and XLFG

Apple pomace xyloglucan was treated with cellulase and the resulting hydrolysate was fractionated by size exclusion chromatography (Fig. 2). Fraction H3, which NMR (see Section 3.6) indicated contained mainly XXXG, XXFG and XLFG was subjected to reverse-phase HPLC (Fig. 3). NMR was used to identify the oligosaccharides in each fraction. XXXG, XXFG and XLFG each eluted as two peaks, presumably due to their anomers. Fractions H3a and H3b each consisted largely of XXXG. Fraction H3c contained XLFG, fraction H3e contained XXFG, while fraction H3d contained a mixture of XLFG and XXFG. Fractions H3c and H3e were further purified by repeating the reverse-phase HPLC followed by size exclusion chromatography to give XLFG and XXFG respectively.

3.6. NMR spectroscopy of XXXG, XXFG and XLFG

Comparing the published ^{1}H NMR and ^{13}C NMR spectra of XXXG (Sakai et al., 1990; York et al., 1990) to the spectra of fractions H3a and H3b (Tables 6 and 7), it was apparent that H3a and H3b contain XXXG as a major component. Minor signals in the NMR of these fractions are not reported here and could not be assigned unambiguously, but are consistent with the presence of the octasaccharides XLXG or XXLG in combination with a small amount of a fucose containing oligosaccharide.

To help assign the ^{13}C NMR spectra of XXFG and XLFG, a HETCOR experiment was performed on fraction H3 (Fig. 2) which indicated it was a mixture of XXXG, XXFG and XLFG. The HETCOR (Fig. 6) showed that the signal at δ 100.69 ppm corresponds to the C-1 of fucose as it is correlated to the proton signal at δ 5.26 ppm. This information is necessary for the assignment of the fucose anomeric carbon signal in the spectra of XXFG, XLFG and the xyloglucan polysaccharide. The HETCOR experiment showed that the signals in the ^{13}C NMR spectra of XXFG and XLFG at δ 104.75 and 104.56, respectively, may be attributed to Gal^a, as the signal in the HETCOR experiment at δ 104.59 is correlated to the Gal^a proton at δ 4.62. The HETCOR spectra also revealed that the proton signal at δ 5.15, which originates from Xyl^b in XLFG correlates to the carbon signal at δ 100.03 ppm, this allows the signal at δ 100.04 in the carbon spectrum of XLFG to be assigned unambiguously to Xyl^b C-1.

The ^{1}H NMR spectrum of XXFG (fraction H3e) (Table 6) was very similar to that of the published spectrum (Sakai

Table 6
Selected ^{1}H NMR signals and coupling constants for oligosaccharides from apple pomace xyloglucan

Fraction	Glcrd (H-1)	Glc^{a} (H-1)	Glc^{b} (H-1)	Glc^{c} (H-1)	Xyl^{a} (H-1)	Xyl^{b} (H-1)	Xyl^{c} (H-1)	Gal^{a} (H-1)	Gal^{b} (H-1)	Fuc^{a} (H-1)	Fuc^{a} (H-6)
H3a,b (XXXG)	α 5.20(3.5)[a] β 4.64(8)	4.49–4.58 (m)[b]	4.49–4.58 (m)[b]	4.49–4.58 (m)[b]	4.90–4.95 (m)[b]	4.90–4.95 (m)[b]	4.90–4.95 (m)[b]				
H3c (XLFG)	α 5.20(3.5)[a] β 4.64(8)	4.47–4.55 (m)[b]	4.47–4.55 (m)[b]	4.47–4.55 (m)[b]	5.11(3)	5.16(3.5)	4.92(3.5)	4.59(7.5)	4.47–4.55 (n.d.)[c]	5.25(4)	1.24(6.5)
H3e (XXFG)	α 5.20(3.5)[a] β4.64(8)	4.52–4.58 (m)[b]	4.52–4.58 (m)[b]	4.52–4.58 (m)[b]	5.13 (3.5)	4.93 (apparent triplet)	4.93 (apparent triplet)	4.59(7.5)		5.25(4)	1.26(7)

[a] Coupling constants in hertz.
[b] Multiplet.
[c] Coupling constant not determined because of overlap.

Table 7
Selected ^{13}C NMR signals for oligosaccharides from apple pomace xyloglucan

Fraction	Glcrd (C-1)	Glc^{a} (C-1)^{a}	Glc^{b} (C-1)^{a}	Glc^{c} (C-1)^{a}	Xyl^{a} (C-1)	Xyl^{b} (C-1)	Xyl^{c} (C-1)	Gal^{a} (C-1)	Gal^{b} (C-1)	Fuc^{a} (C-1)	Fuc^{a} (C-6)
H3a,b (XXXG)	α 93.11, β 97.01	103.79	103.79	104.20	100.17	100.17	99.58				
H3c (XLFG)	α 93.09, β 97.00	103.71	103.98	104.36	100.19	100.04	99.58	104.56	105.82	100.66	β 17.22, α 17.27
H3e (XXFG)	α 93.11, β 97.04	103.75	104.07	104.23	100.25	100.25	99.60	104.75		100.73	β 17.25, α 17.30

[a] Tentative assignments.

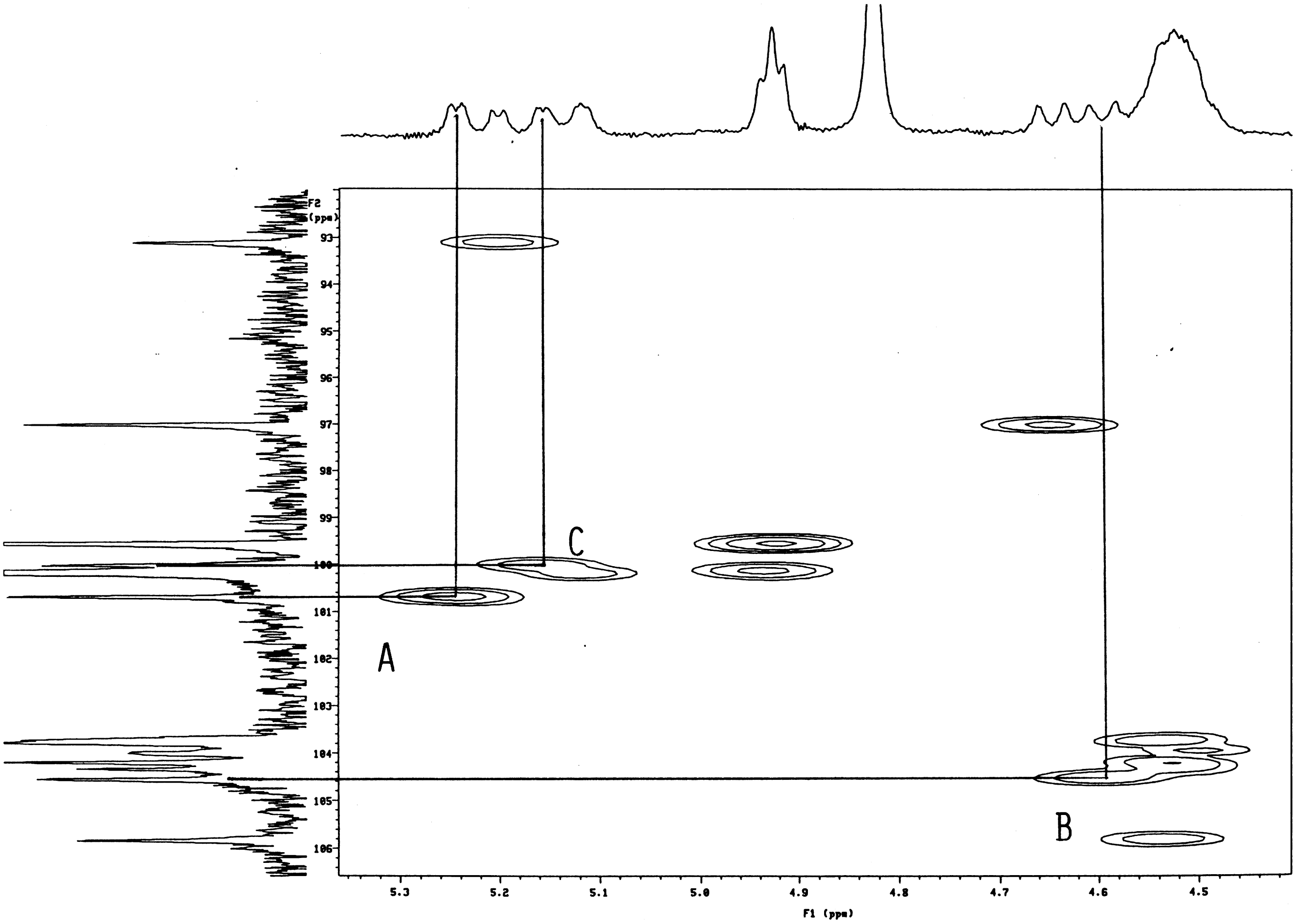


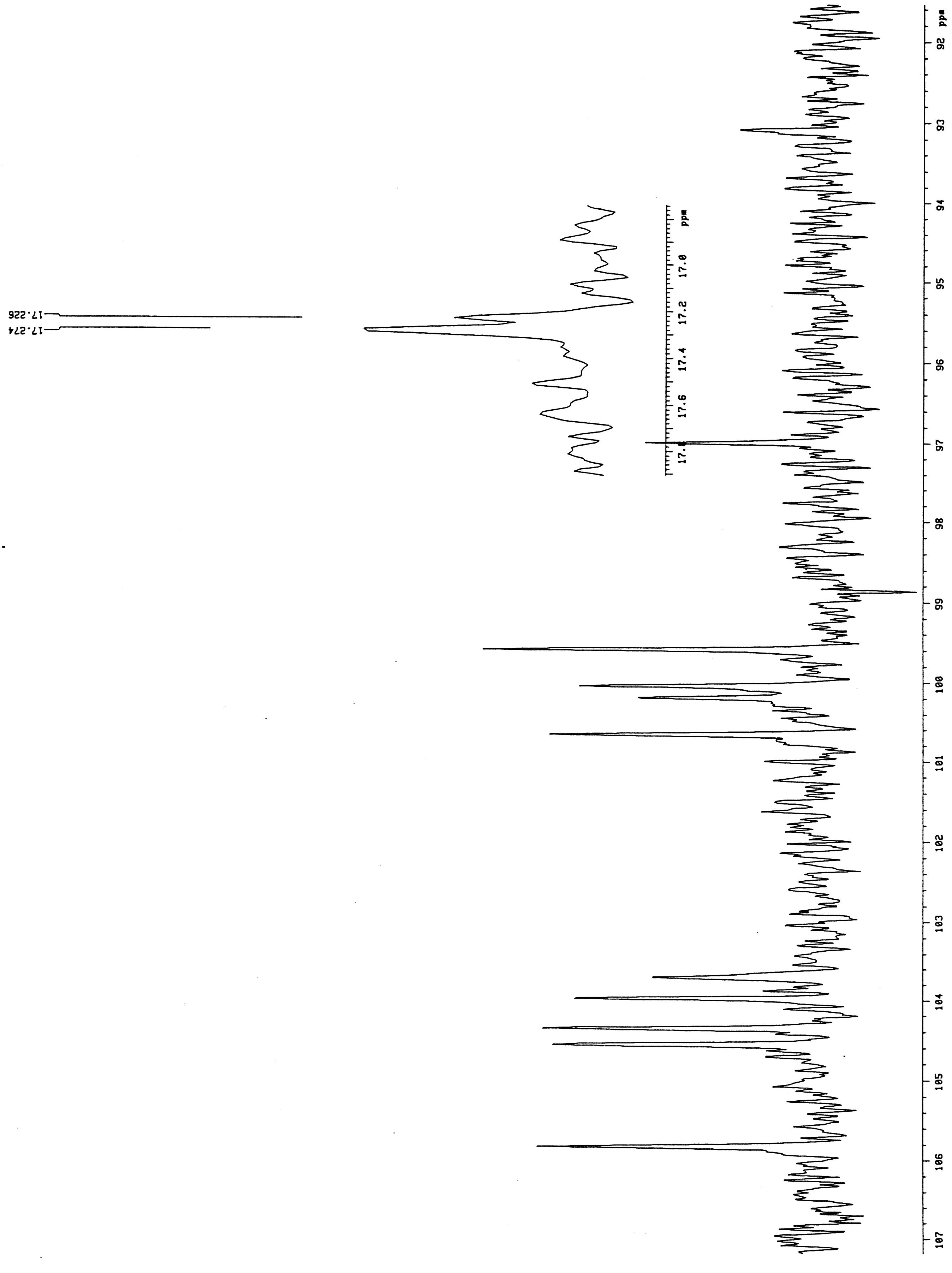
Fig. 6. HETCOR NMR spectra of fraction H3, a mixture of oligosaccharides XXXG, XXFG and XLFG, obtained from the size exclusion chromatography of the cellulase treated pomace 2 xyloglucan. Correlations are indicated between the (A) H-1 and C-1 of the fucosyl residues, (B) the H-1 and C-1 of the 2-O-substituted galactosyl moieties and (C) the H-1 and C-1 of the galactosylated xylosyl residue (Xyl^b).

et al., 1990). In the published spectrum the anomeric signals from the three glucose residues Glc^a, Glc^b and Glc^c are reported as one doublet, and a signal from the H-5 of fucose is not reported. Despite these anomalies the spectra are thought to be of the same compound. ^{1}H NMR spectra of the reduced oligosaccharide confirmed this, as apart from the differences in resolution due to a difference in field strength, the reduced H3e had a spectrum identical to that reported for XXFGol.

The anomeric region of the ^{13}C NMR spectrum of XXFG (fraction H3e) is shown in Table 7. The anomeric signals of Xyl^c, Fuc^a, and Gal^a were assigned using the HETCOR spectrum. The C-1 signals of Xyl^a and Xyl^b appear as one peak at δ 100.25. Comparison of the spectrum with the published spectra of XXXGol and XXLGol (York et al., 1993) allowed the signals at δ 103.75, 104.07 and 104.23 to be tentatively assigned to Glc^a, Glc^b and Glc^c, respectively. The signal at δ 104.75 is assigned to Gal^a on the basis of the HETCOR experiment.

The ^{1}H NMR of XLFG (fraction H3c) may be readily assigned by comparison with the published spectrum of XLFGol. The ^{13}C NMR spectrum of XLFG is shown in Fig. 7. Two signals for the C-6 of fucose were observed due to its proximity to the Glcrd residue. This result will be discussed below. The anomeric region of the spectrum was assigned in the following way: Xyl^c (δ 99.58) was assigned by comparison with XXFG (δ 99.60), Fuc^a and Xyl^b were assigned using the HETCOR experiment and Xyl^a (δ 100.19) was assigned by comparison with XXFG (δ 100.25). Gal^a was assigned using the HETCOR experiment. Gal^b (δ 105.82) was assigned on the basis of its appearance in the spectra remote from any signals in the spectrum XXFG and as for XXFG the order of the assignments of the three non-reducing glucose signals is tentative.

In the ^{13}C NMR spectrum of XLFG two signals were observed for the fucose C-6 (δ 17.22 and 17.27) which were separated by 0.05 ppm. These were in a proportion which was consistent with them resulting from the β- and α-anomers of the oligosaccharide (ratio 3:2 by peak height). The ^{13}C NMR spectra of XXFG also gave two signals for the fucose C-6 (δ 17.25 and 17.30). These results are consistent


17.226
17.274
ppm
17.0
17.2
17.4
17.6
17.8
ppm
92
93
94
95
96
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98
99
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103
104
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106
107

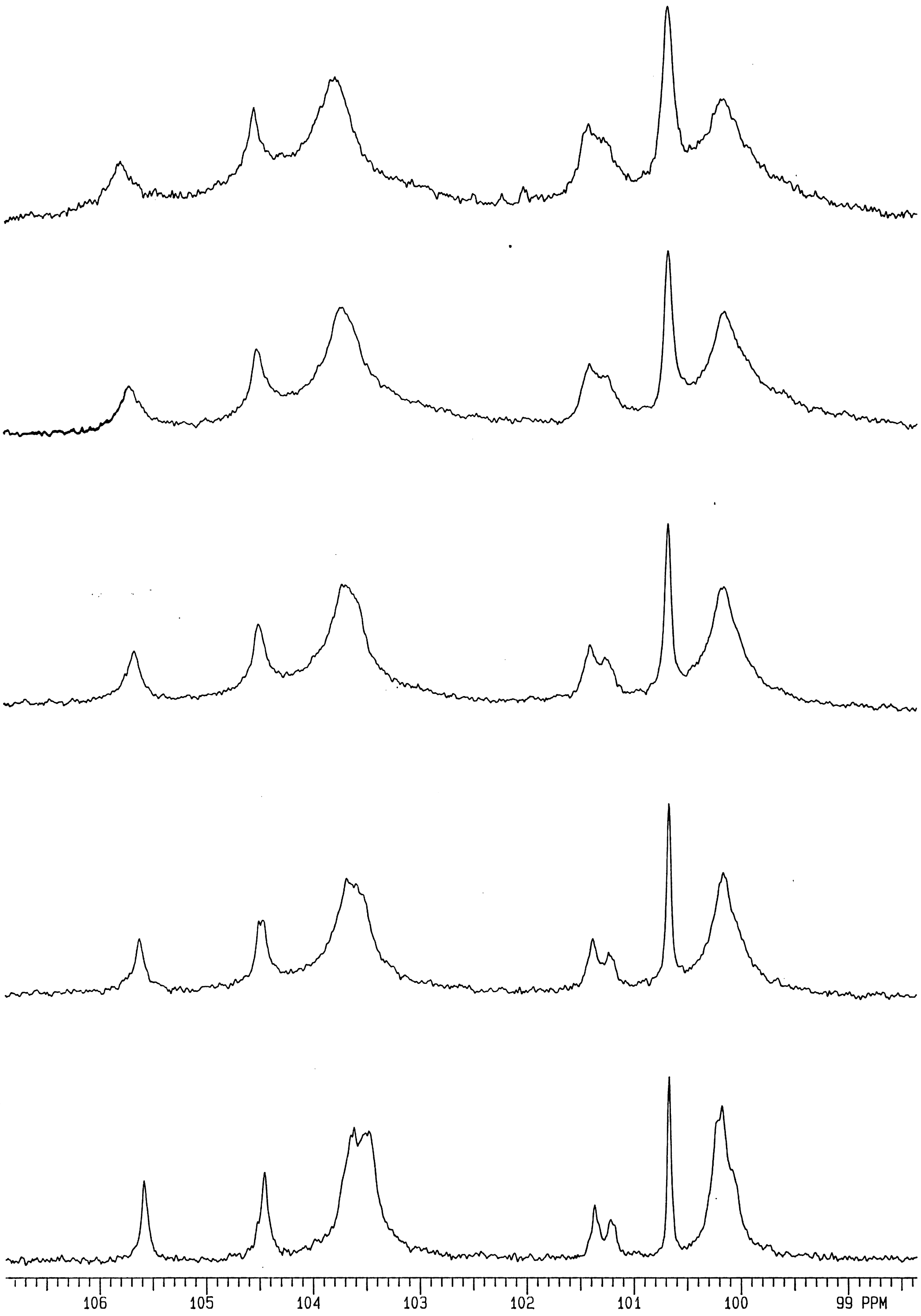

Fig. 8. The anomeric region of the ^{13}C NMR spectra of pomace 2 xyloglucan at various temperatures (25, 43, 60, 75, and 90°C).

with the hypothesis that the fucose residue is close in space to the Glcrd group in XXFG and XLFG and thus support the molecular modelling and ^{1}H NMR studies of xyloglucan oligosaccharides reported by Levy et al. (1991).

3.7. NMR of apple pomace xyloglucan

In the ^{13}C NMR of purified apple pomace xyloglucan, anomeric signals were observed at δ 100.25 (broad),

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Fig. 7. Selected regions of the ^{13}C NMR spectrum of fraction H3c (XLFG).

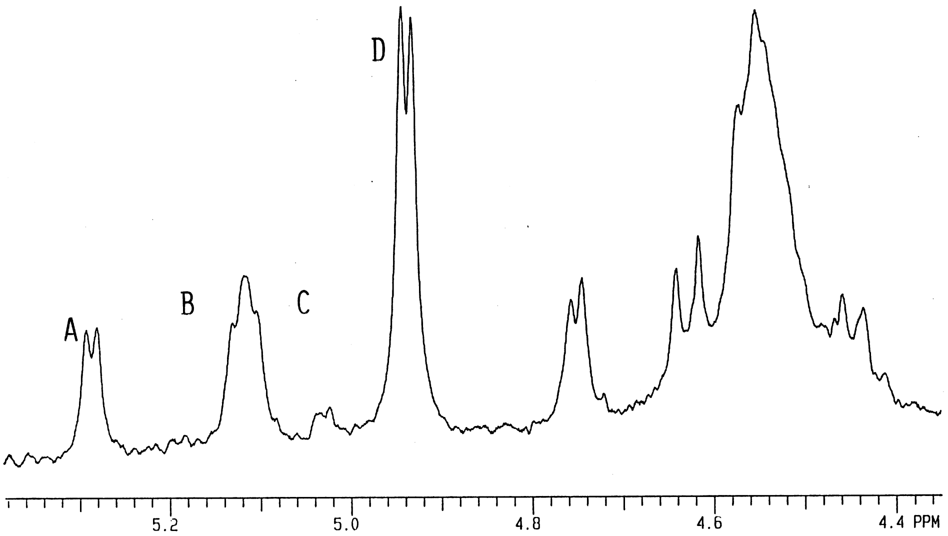

Fig. 9. The anomeric region of the ^{1}H NMR spectrum of pomace 2 xyloglucan at 90°C. (A) fucosyl residues, (B) xylosyl residues substituted by galactose, (C) xylosyl residues substituted by fucosyl 2-*O*-galactose, (D) terminal xylosyl residues.

100.74, 101.29, 101.42, 103.59 (broad), 103.69 (broad), 104.53, 105.66 (Fig. 8). Many of these signals can be assigned unambiguously to fucogalactoxyloglucan by comparison with the spectrum of XXFG and XLFG. Other workers (Ryden and Selvendran, 1990) have successfully assigned the anomeric region of the ^{13}C NMR of fucogalactoxylglucan by comparing it with other polysaccharides or oligosaccharides. However, until now it was not possible to do so unambiguously. The broad signal at δ 100.25 can be assigned to the xylosyl residues by comparison with XLFG [100.04 (C-1 Xyl^{b}), 100.19 (C-1 Xyl^{a})]. The signal at δ 100.74 is due to the α-L-fucopyranosyl residues [XLFG, δ 100.66 (C-1 Fuc^{a})]. The broad signals at δ 103.59 and 103.69 are attributed to the 1,4-β-D-glucopyranosyl backbone [XXFG, δ 103.75 (C-1 Glc^{a}), δ 104.07 (C-1 Glc^{b})]. The signal at δ 104.53 is due to the fucosylated galactosyl residues [XLFG, 104.56 (C-1 Gal^{a})], and the signal at δ 105.66 is due to the terminal galactosyl residues [cf. XLFG, δ 105.82 (C-1 Gal^{b})].

The signals in the spectrum of the xyloglucan at δ 101.44 and 101.29 are attributed to the small amount of mannan in the preparation (cf. galactoglucomannan, δ 101.3, Kubacková et al., 1992).

Like the ^{13}C NMR spectrum, the ^{1}H NMR spectrum of purified apple pomace xyloglucan (Fig. 9) is consistent with the presence of a high percentage of fucogalactoxyloglucan. To the authors' knowledge this is the first time that the ^{1}H NMR of intact fucogalactoxyloglucan has been reported. The doublet at δ 5.28 (J 4 Hz) can be attributed to terminal α-L-fucosyl residues [cf. XLFG, δ 5.25 (d, 1H, J 4 Hz, H-1 Fuc^{a})]. The three signals at δ 5.10, 5.11, and 5.13 are consistent with the presence of two overlapping doublets. The signal at δ 5.12 (J ca. 4 Hz) results from α-D-xylosyl residues substituted at the 2 position with terminal galactose [XLFG, δ 5.16 (d, 1H, J 3.5 Hz, H-1 Xyl^{b})], and that at δ 5.11 (J ca. 3.5 Hz) may be interpreted as resulting from α-D-xylosyl residues substituted at the 2 position with fucosylated galactose [XLFG, δ 5.11 (d, 1H, J 3 Hz, H-1 Xyl^{a})], The large doublet at δ 4.94 (J 3.5 Hz) is attributed to unsubstituted terminal 1,6-α-D-xylosyl residues, [XXFG, δ 4.940 (d, J 4.0 Hz, H-1 Xyl^{b}), (Sakai et al., 1990)]. Interestingly, the different chemical shifts seen in xyloglucan oligosaccharides for terminal xylosyl residues in different positions relative to the unbranched glucosyl residue are not observed in the polysaccharide. This difference may be attributed to the end-effects of the oligosaccharides. The doublet which appears at δ 4.63 (J 7.5 Hz) is attributed to β-D-galactosyl residues which are substituted by α-L-fucopyranosyl residues [compare XLFG, 4.59 (d, 1H, J 7.5 Hz, H-1 Gal^{a})], and the doublet at δ 1.26 (J 6 Hz) is due to the C-6 of the fucosyl residues. Weak signals which occurred at δ 4.74 and 4.76 are thought to be due to mannan associated with the xyloglucan, but they cannot be assigned unambiguously.

The signals for the anomeric protons in the NMR spectrum of purified apple pomace xyloglucan (Fig. 9) have been integrated. The anomeric protons of the fucosyl residues (A), xylosyl residues substituted with galactose (B), the xylosyl residues substituted with fucosyl-2-*O*-galactose (C) and the terminal xylosyl residues (D) (Fig. 9) gave a ratio of 0.56:1.00:1.94 for the fucosyl, substituted xylosyl and terminal xylosyl residues, respectively. The result is in good agreement with the corresponding ratio of 0.66:1.00:2.00 that can be calculated for oligosaccharides from xyloglucan oligosaccharides isolated from apples (Renard et al., 1992). This is an important outcome of our paper as it allows the rapid compositional screening of xyloglucans from different sources without recourse to detailed chemical analysis.

3.8. ^{13}C NMR spectral features as a function of temperature

The NMR of apple pomace xyloglucan shows a gradual improvement in linewidth from 25 to 90°C indicating a gradual increase in spin–spin relaxation time (T_2) and in agreement with the viscosity versus temperature study, no major discontinuity is observed. Thus, the ^{13}C NMR of fucogalactoxyglucan shows a normal polymer spectrum at all temperatures which is indicative of normal coil conformation, with no evidence of the polymer adopting a specific, restricted conformation.

In an attempt to differentiate between the effects of greater T_2 due to molecular motion, or a breakdown of hydrogen bonding between 75 and 90°C, the spectrum was acquired at 75°C in 8 M urea. No improvement in linewidth was observed indicating that the improvement in the spectral resolution from 75 to 90°C is not likely to be due to a breakdown in intermolecular hydrogen bonding.

4. Conclusion

Fucogalactoxyloglucan was obtained from non-depectinated apple pomace and from apple pomace which was enzymatically depectinated. The molecular weight of the xyloglucan was reduced by the depectination process. The yields of xyloglucan from depectinated and non-depectinated apple pomace were moderate and its viscosity was relatively low. The fucogalactoxyloglucan did not form a gel at high sucrose concentration, unlike non-fucosylated xyloglucan from tamarind seed.

The ^{13}C NMR spectra of XXFG and XLFG showed an interaction between the fucosyl residue and the reducing glucosyl residue. This observation supports the contention that in intact fucogalactoxyloglucan, the fucosyl residue is close in space to the glucan backbone, and this is consistent with the polymer adopting a ‘twisted’ conformation which does not lend itself to backbone–backbone intermolecular associations (Levy et al., 1991).

Optical rotation, viscometric and NMR studies of apple pomace fucogalactoxyloglucan failed to show any evidence of intermolecular interaction. This finding is of significance in the understanding of the structure of the dicot primary plant-cell wall of which xyloglucan is a major component.

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