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Characterisation of extracellular polysaccharides from suspension cultures of apple (*Malus domestica*)

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Abstract

The polymers secreted by suspension-cultured apple cells were composed of 85% carbohydrate (76% neutral sugar and 9% uronic acid) and 15% w/w protein. The extracellular polysaccharides (ECPs) contain 23% XG and 59% AGPs. The monosaccharide composition of the ECPs consisted of Gal, Ara, Glc and Xyl, with smaller amounts of Rha, Fuc and Man. Fractionation of the ECPs by anion-exchange chromatography yielded an unbound neutral fraction and a bound acidic fraction. Monosaccharide and linkage compositions of each fraction were determined. The neutral fraction (48% recovered carbohydrate) was composed of xyloglucan (XG; $>$ 90 mol%) which was purified by selective precipitation with Fehling's solution to yield pure XG. The purified XG had a Glc:Xyl:Gal:Fuc ratio of 4.0:2.5:0.8:0.5; the XG was not *O*-acetylated. The structure of the secreted XG was similar to that extracted from apple-pomace. The acidic fraction (52% recovered carbohydrate) was composed primarily of arabinogalactan-proteins (AGPs) as detected by the β-glucosyl Yariv diffusion test. The AGP had a Gal:Ara ratio of 1.3: 1.0. Minor amounts of arabinan, xylan and mannan were also detected in the ECPs. This study is the first examination of the polysaccharides secreted by apple cells grown in suspension culture.

Keywords: Apple:*Malus domestica*; Cell-suspension cultures; Linkage analysis; Xyloglucan; Arabinogalactan-protein; Extracellular polysaccharide

1. Introduction

Plant primary cell walls of dicotyledons are composed of ca. 95% polysaccharides and 5% glycoprotein, with small amounts of methyl, acetyl and feruoloyl esters (Carpita & Gibeaut, 1993). Primary walls can be obtained from isolated plant tissues and from suspension-cultured cells, but are usually extracted with harsh chemical treatments (York, Darvill, McNeil, Stevenson & Albersheim, 1985). Suspension-cultured plant cells secrete into their culture medium polysaccharides that are similar to those present in the walls of intact plants (Takeuchi & Komamine, 1978) and hence polysaccharides isolated from the medium may serve as models for cell wall polysaccharides.

Xyloglucans (XGs) are a principal component of the primary cell walls of dicotyledons and make up approximately 20% of the wall. XGs are released into the medium during the growth of cells in suspension culture and secretion increases as cells age (McDougall & Fry, 1991). Joseleau, Cartier, Chambat, Faik and Ruel (1992) compared XG in the walls of blackberry cells harvested from cultures with that secreted into the culture medium. They found that the structure and composition of XG obtained by treating the cell walls with strong base (2.5 M NaOH) was very similar to the extracellular XG. Examination of the cell walls by electron microscopy, using an anti-XG polyclonal antibody suggested that extracellular polysaccharides (ECPs) are released progressively from the cell wall by a sloughing mechanism as a result of structural rearrangement within the cell wall.

Structural proteins inserted into the wall act as reinforcing elements in conjunction with the cellulose microfibrils (Bacic, Harris & Stone, 1988). Arabinogalactan-proteins (AGPs) are commonly secreted by plant cell suspension cultures and are typically composed of 95% carbohydrate, principally Gal*p* and Arap residues. The sugar groups are *O*-linked to hydroxy amino acids in the core protein which is usually rich in serine, alanine, glycine and hydroxyproline (Chasan, 1994). AGPs can be detected by staining with a synthetic phenylglycoside, β-glucosyl Yariv reagent,

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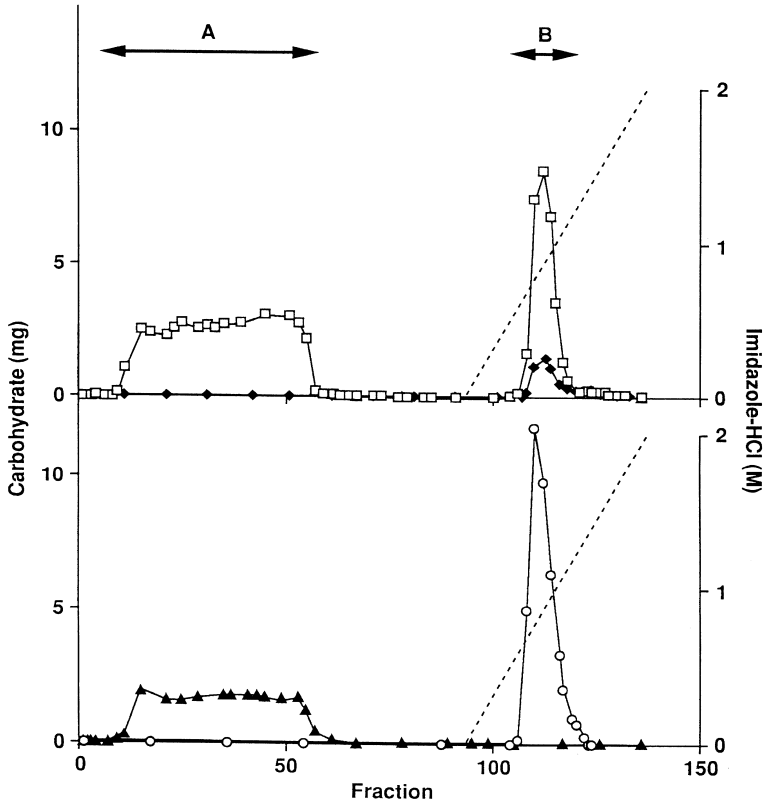

Fig. 1. . Anion-exchange chromatography of ECPs from suspension-cultured cells of apple (*Malus domestica*). ECPs were fractionated on a column of DEAE-Sepharose CL-6B with a gradient of imidazole-HCl (pH7) and fractions (5 ml) collected. Fractions were assayed for hexose using anthrone (□–□), uronic acid residues using 3-phenylphenol (◆–◆), AGP by radial diffusion using β-glucosyl Yariv reagent (○–○) and oxloglucan using iodine (▲–▲). The concentration of imidazole-HCl is indicated as –-.

whichbinds to and precipitates AGPs (Komalavilas, Zhu & Nothnagel, 1991).

In this paper we report the fractionation and characterisation of ECPs from cell-suspension cultures of apple fruit. The ECPs were fractionated by anion-exchange chromatography with a gradient of imidazole-HCl. Monosaccharide and methylation analyses were used to study the major polysaccharides present. The structure of the xyloglucan was compared with that of xyloglucan isolated from apple pomace.

2. Materials and methods

2.1. Growth of apple cell suspension cultures

Apple (*Malus domestica*, Borkh. cv Braeburn) cell suspension cultures were established by Dr Ann Percy (University of Otago, New Zealand) and supplied by Dr. Ian Ferguson (HortResearch, New Zealand). The suspension cultures were grown for 7–10 d in 500 ml conical flasks in 120 ml of cell proliferation medium, prepared according Codron, Latche, Pech, Nebie and Fallot (1979). Cultures were incubated on an orbital shaker (90 rpm) at 24°C, under continuous light (Sylvania GRO-LUX fluorescent lamps) at a photon flux density of 50 μmol m^{-2} sec^{-1}.

ECPs were harvested from the suspension cultures according to the method of Stevenson, McNeil, Darvill and Albersheim (1986). The cells were removed from the suspension cultures by filtration through a glass fibre filter (Whatman GF/A) and the filtrate was concentrated using a rotary evaporator (30°C). Four volumes of 96% ethanol were added to the filtrate and allowed to stand overnight at 4°C before centrifugation at 12,100 g for 15 min at 10°C. The precipitate was redissolved in water and freeze dried. The freeze dried material was dissolved in Na_2EDTA (1 mg ml^{-1}) containing NaN_3 (0.01% w/v) as a preservative and stirred overnight at room temperature. The solution was filtered under vacuum (Whatman GF/A glass fibre filter), dialysed extensively (molecular weight cut-off 12 kDa) against deionised water, concentrated using a rotary evaporator (30°C) and freeze dried. Samples were assayed for hexose, uronic acids, AGP, xyloglucan and protein (see below).

2.2. Fractionation of ECPs

ECPs were fractionated according to the method of Sims and Bacic (1995). Solutions of ECPs (250 mg) in 20 mM

Table 1
Carbohydrate composition of apple suspension culture ECPs and fractions from anion-exchange chromatography

Component	Total carbohydrate(% w/w)[a]				
	ECPs	A			B
			A-1	A-2	
Neutral sugar	76	100	n.d.[b]	n.d.[b]	84
Uronic acid	9	—[d]	n.d.[b]	n.d.[b]	16
Xyloglucan	23	63	n.d.[b]	n.d.[b]	—[d]
AGPs	59	—[d]	n.d.[b]	n.d.[b]	100
Monosaccharides	% weight[d]				
Rha	4	—[d]	—[d]	—[d]	3
Fuc	2	8	8	2	tr[c]
Ara	23	8	tr[c]	62	36
Xyl	12	34	35	12	6
Man	2	3	2	4	2
Gal	44	15	15	8	51
Glc	13	32	40	12	2

[a] Average of duplicate determinations.
[b] n.d. not determined
[c] tr. Trace (< 1%).
[d] — Not detected.

imidazole-HCl buffer (250 ml, pH 7.0) were applied to a column (20 x 3 cm) of DEAE-Sepharose CL-6B equilibrated in the same buffer, and eluted at 20 ml h^{-1} until no carbohydrate could be detected in the eluate by the colorimetric assay for neutral hexose. Material which bound to the column was then eluted by a linear gradient (0.02–2 M) of imidazole-HCl over 400 ml. Fractions (5 ml) were assayed for hexose, uronic acids, xyloglucan and AGP. Appropriate fractions were pooled to give fractions A and B (see Fig. 1), concentrated, dialysed extensively against deionised water and freeze-dried.

Fraction A from anion-exchange chromatography was treated with Fehling's solution according to the method of Jones and Stoodley (1965). The fraction was dissolved in deionised water (10 mg ml^{-1}) and a half volume of Fehling's solution was added. The mixture was allowed to stand at room temperature for 2 h and centrifuged at 10 000 g for 20 min at 15°C. The pellet was macerated in chilled ethanol which contained 5% v/v conc. HCl and then centrifuged, and the supernatant removed. Maceration of the pellet in ethanol was repeated until no more chloride could be detected. The combined supernatants were neutralised with glacial acetic acid, dialysed extensively against deionised water and freeze dried.

2.3. Purification of apple-pomace XG

Apple-pomace (a mixture of 70% Gala and 30% Braeburn apples), which had been treated with commercial pectinases, was obtained from ENZA (New Zealand). Pomace was extracted sequentially two times with MeOH:$CHCl_3$:HCOOH:H_2O (16:5:1:1), followed by four times with 0.1 M KOAc (pH 6.5, adjusted with acetic acid) to remove protein and arabinans, and then three times with 6 M KOH containing 20 mM $NaBH_4$ (under nitrogen) to remove XG. Each extraction lasted 1 h at room temperature. Soluble fractions were then dialysed extensively against deionised water and freeze-dried. Prior to dialysis, the fraction solubilised by KOH was neutralised with acetic acid. The KOH-soluble fraction was treated with Fehling's solution (see above), and the material precipitated applied to a column of DEAE-Sepharose CL-6B as described above. The unbound neutral fraction was concentrated, dialysed extensively against deionised water and freeze-dried.

2.4. Analytical methods

Total carbohydrate was determined by the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956) using glucose (10–80 μg) as a standard. Uronic acid was determined by the method of Filisetti-Cozzi and Carpita (1991) using galacturonic acid (5–40 μg) as a standard. Hexoses were determined by the anthrone method (Dische, 1962) using glucose (10–100 μg) as a standard. Xyloglucan was determined by the method Nishitani and Masuda (1981), using tamarind xyloglucan (20–400 μg, Megazyme) as the standard. Arabinogalactan-protein (AGP) was determined by radial diffusion against β-glucosyl Yariv reagent (Van Holst & Clarke, 1985) using gum arabic (Sigma; 0.25–3.0 μg) as a standard. Total protein was determined using the Bio-Rad protein micro-assay, with bovine serum albumin (BSA, 2–20 μg) as the standard (Bradford, 1976). The degree of acetylation was determined by saponification of native xyloglucan and measurement of released acetic acid by HPLC (Voragen, Schols & Pilnik, 1986).

2.5. Monosaccharide analysis

Monosaccharides were determined by GC of alditol acetates. Samples (1 mg) were hydrolysed with 2.5 M trifluoroacetic acid (TFA, 0.5 ml), reduced with 1.0 M $NaBD_4$ in 2.0 M ammonia (1.0 ml) for 1 h at 60°C and acetylated for 10 min at room temperature in ethyl acetate (1.0 ml), acetic anhydride (3.0 ml) and 70% perchloric acid (0.1 ml) (Harris, Henry, Blakeney & Stone, 1984). The alditol acetates were separated on a capillary column (BPX70, 12 m × 0.33 mm internal diameter, film thickness 0.25 μm, SGE, Australia), and the GC oven programmed from 180°C (held for 2 min) to 220°C at a rate of 3°C min^{-1}.

2.6. Linkage analysis

Methylation was performed using the NaOH method of Ciucanu and Kerek (1984) as described by McConville, Homans, Thomas-Oates, Dell and Bacic (1990). The methylated polysaccharides were hydrolysed with 2.5 M trifluoroacetic acid (4 h, 100°C). After hydrolysis, partially methylated alditol acetates were prepared as described for monosaccharide analysis (see above). The partially methylated alditol acetates were separated on a BPX70 column

Table 2
Linkage composition of apple suspension culture total ECPs and fractions from anion-exchange chromatography

Sugar	Deduced glycosidic linkage[a]	Linkage composition (mol %)[b]				
		ECPs	A	A-1	A-2	B
Rha*p*	Terminal	1	—[d]	—[d]	—[d]	3
Fuc*p*	Terminal	2	3	6	—[d]	—[d]
Ara*f*	Terminal	13	1	—[d]	23	26
	Terminal*p*	1	—[d]	—[d]	6	3
	3-	1	—[d]	—[d]	6	3
	5-	5	1	—[d]	17	3
	2,5-	tr[c]	tr[c]	—[d]	4	—[d]
	3,5-	3	tr[c]	—[d]	5	2
	2,3,5-	—[d]	tr[c]	—[d]	6	1
Xyl*p*	Terminal	13	31	22	8	2
	2-	6	12	10	3	—[d]
	4-	2	—[d]	—[d]	2	5
	2,4-	2	—[d]	—[d]	—[d]	1
Man*p*	2-	1	—[d]		—[d]	—[d]
	4-	1	2	—[d]	1	—[d]
Gal*p*	Terminal	3	6	4	3	10
	2-	1	3	6	2	—[d]
	3-	—[d]	—[d]	—[d]	—[d]	4
	6-	4	1	—[d]	1	9
	3,6-	8	—[d]	—[d]	1	28
Glc*p*	Terminal	2	1	—[d]	1	—[d]
	4-	7	12	14	5	—[d]
	4,6-	18	25	38	8	—[d]

[a] Terminal Rha*p* is deduced from 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methyl-hexitol etc.
[b] Average of duplicate determinations.
[c] tr: Trace ($<$ 1%).
[d] — Not detected.

(25 m $\times$ 0.22 mm i.d.) on a Finnigan MAT 1020B GC-MS. Identifications were based on peak retention times and by comparison of electron impact mass spectra with published spectra.

3. Results and discussion

3.1. Composition of ECPs

The polymers secreted by suspension-cultured apple cells were composed of ca. 85% w/w carbohydrate (76% neutral sugar and 9% uronic acid) and 15% w/w protein. The polymers contained ca. 23% w/w XG, determined colorimetrically, and ca. 59% w/w AGPs, determined by the β-glucosyl Yariv diffusion assay (Table 1). The nature of the protein in the extracellular material was not investigated.

The monosaccharide composition of the ECPs from apple suspension cultures contained predominantly Gal, Ara, Glc and Xyl, with smaller amounts of Rha, Fuc and Man (Table 1). The presence of these sugars was consistent with the presence of fucosylated XG which is composed of Glc, Xyl, Gal and Fuc (Fry, 1989; Hayashi, 1989), and AGPs which contain predominantly Ara and Gal together with smaller amounts of Rha, Xyl, Man and Glc. The uronic acid sugars detected colorimetrically were not identified.

Linkage analysis of apple ECPs showed they contained mostly 4-Glc*p* (7 mol%) and 4,6-Glc*p* (18%), terminal Ara*f* (13%), terminal Xyl*p* (13%) and 2-Xyl*p* (6%), and terminal Gal*p* (9%) and 3,6-Gal*p* (8%) (Table 2). Using linkage structures characteristic of individual polysaccharides from apple (Vincken, Beldman & Voragen, 1994; Renard, Lomax & Boon, 1992; Ruperez, Selvendran & Stevens, 1985) and other dicotyledons (Bacic et al., 1988), the amounts of different polysaccharides present were estimated as the summed mol% of their individual glycosyl residues. The apple ECPs were thus deduced to contain ca. 50% XG, calculated from the sum of the 4,6-Glc*p* (18 mol%) and 4-Glc*p* (6%) equal to one-third of the 4,6-Glc*p*, terminal Xylp (13%) and 2-Xyl*p* (6%), terminal Gal*p* (4%) and 2-Gal*p* (1%), and terminal Fuc*p* (2%) (Table 2). The amount of XG determined from the linkage analysis was twice that estimated colorimetrically (Table 1), suggesting that the amount of XG was underestimated by the colorimetric assay.

Type II arabinogalactans (AGPs), calculated from the sum of 6-Gal*p* (4%) and 3,6-Gal*p* (8%) and terminal Ara*f* equal to 3,6-Gal*p* (8%), comprised ca. 20% of the ECPs. The β-glucosyl Yariv diffusion assay estimated that the ECPs contained 59% AGP; this linkage analysis contains other linkages which are often present in AGPs (e.g. terminal Rha*p* and 5-Ara*f*), and does not detect other components

Table 3
Linkage composition of cell walls and fractions obtained from apple-pomace

Sugar	Deduced glycosidic linkage[a]	Linkage composition (mol %) [b]			
		Pomace	KOH-soluble	Fehling's ppt.	Pure XG
Rha*p*	2,4-	1	—[d]	—[d]	—[d]
Fuc*p*	Terminal	1	5	5	7
Ara*f*	Terminal	1	5	1	—[d]
	3-	tr[c]	tr[c]	tr[c]	Nd[d]
	5-	4	7	3	—[d]
	3,5-	—[d]	1	—[d]	—[d]
Xyl*p*	Terminal	6	22	20	17
	2-	2	9	8	9
	4-	4	6	4	—[d]
	2,3-	1	—[d]	—[d]	—[d]
Man*p*	4-	3	tr[c]	1	6
Gal*p*	Terminal	3	6	2	2
	2-	1	5	4	4
Glc*p*	Terminal	1	1	—[d]	—[d]
	4-	52	8	14	17
	2,4-	1	—[d]	—[d]	—[d]
	3,4-	1	—[d]	—[d]	—[d]
	4,6-	17	24	37	38

[a] 2,4-Rha*p* is deduced from 1,2,4,5-tetra-*O*-acetyl-6-deoxy-3-*O*-methylthexitol etc.
[b] Average of duplicate determinations.
[d] — Not detected.
[c] tr. Trace (< 1%).

of AGPs such as uronic acid sugars and protein. Thus, the amount of AGPs in the apple ECPs was probably underestimated by this linkage analysis. Arabinans, calculated from the sum of 3-Ara*f* (1%), 5-Ara*f* (5%) and 3,5-Ara*f* (3%), and terminal Ara*f* equal to 3,5-Ara*f* (3%), comprised 12% of the apple ECPs. The presence of 4-Xyl*p* (2%) and 4-Man*p* (1%) indicated the presence of a small amount of xylan and mannan, respectively.

3.2. Anion-exchange chromatography of ECPs

Polysaccharides in the apple ECPs were fractionated to confirm the earlier deductions, and to determine the amounts of the individual polysaccharides. Fractionation of the ECPs by anion-exchange chromatography on DEAE-Sepharose CL-6B with a gradient of imidazole-HCl buffer (pH 7.0) gave an unbound, neutral fraction (A) and a bound fraction (B; Fig. 1). There was complete recovery of carbohydrate (from the sum of neutral hexose and uronic acid) from the anion exchange column (Table 1). Fraction A accounted for 48% w/w of the recovered carbohydrate, and contained only neutral carbohydrate; AGP was not detected in this fraction. Fraction B accounted for 52% w/w of the recovered carbohydrate and contained uronic acid (16% w/w total carbohydrate); the β-glucosyl Yariv diffusion assay suggested that all of the carbohydrate in this fraction was AGP.

Fraction A was composed predominantly of the monosaccharides Glc, Xyl and Gal, together with smaller amounts of Fuc, Ara and Man (Table 1). The linkage composition of this fraction (Table 2) consisted predominantly of 4-Glc*p*, 4,6-Glc*p*, terminal Xyl*p*, 2-Xyl*p* and terminal Gal*p* in the approximate ratio 2:4:5:1:1, with smaller amounts of terminal Fuc*p*, terminal Ara*f*, 5-Ara*f*, 4-Man*p*, 2-Gal*p* and 6-Gal*p*. These linkages suggested that fraction A contained more than 90% XG (from the sum of 4-Glc*p*, 4,6-Glc*p*, terminal Xyl*p*, 2-Xyl*p*, terminal Gal*p*, 2-Gal*p*, and terminal Fuc*p*).

The XG present in fraction A was purified further by treatment with Fehling's solution (Jones & Stoodley, 1965). The fractions insoluble (A-1) and soluble (A-2) in Fehling's solution accounted for approximately 92% and 8% (w/w) of the recovered carbohydrate, respectively. Monosaccharide analysis of fraction A-1 detected Glc, Xyl, Gal and Fuc in the ratio of 4.0:3.5:1.5:0.8 (Table 1); the ratio was 4.0:2.5:0.8:0.5 when calculated from the sum of the different linkages (Table 2), and suggested that the amount of Glc determined by the monosaccharide analysis may be underestimated. Linkage analysis showed that fraction A-1 contained mostly 4-Glc*p*, 4,6-Glc*p*, terminal Xyl*p*, 2-Xyl*p*, terminal Gal*p*, 2-Gal*p*, and terminal Fuc*p*, and was calculated to contain 98% fucosylated XG (Table 2).

The occurrence of *O*-acetyl groups has been reported on XGs from ECPs of *Nicotiana plumbaginifolia* (Sims, Munro, Currie, Craik & Bacic, 1996), *Mentha* hybrid (Maruyama et al., 1996) and *Acer pseudoplatanus* (sycamore) cell-suspension cultures (York, Darvill & Albersheim, 1984). The possible *O*acetyl groups on XG purified from ECPs of apple suspension cultures was determined by saponification and measurement of released acetic acid by

Table 4
Comparison of monosaccharide composition (mol %) of apple XGs from cell suspension cultures, pomace and fruit tissue

Monosaccharide	ECPs		Pomace	Fruit tissue		
	Monosacch.	Methylation	Methylation	Monosacch.		
				A[c]	B[d]	C[e]
Fuc	8	6	7	8	6	7
Ara	tr[a]	—[b]	—[b]	2	—[b]	1
Xyl	35	32	26	30	29	30
Man	2	—[b]	6	2	6	1
Gal	15	10	6	12	13	11
Glc	40	52	50	48	44	50

[a] tr. trace
[b] — not detected
[c] A Golden Delicious (Renard, Voragen, Thibault & Pilnik, 1991)
[d] B Golden Delicious (Renard et al., 1992)
[e] C Cox's Orange Pippin (XG3) (Ruperez et al., 1985)

HPLC; none were detected. Similarly, XG oligosaccharides extracted from the cell walls of apple fruit by digestion (1 → 4)-β-endoglucanase were not acetylated (Renard et al., 1992). Using (1 → 4)-β-endoglucanase avoided de-*O*-acetylation by strongly alkaline conditions commonly employed to extract XG.

Fraction A-2 contained predominantly Ara, with smaller amounts of Glc, Xyl, Gal, Man and Fuc (Table 1). Linkage analysis showed that this fraction contained predominantly terminal Ara*f*, 2-Ara*f*, 3-Ara*f*, 5-Ara*f*, 2,5-Ara*f*, 3,5-Ara*f* and 2,3,5-Ara*f* (sum total of 67 mol%), consistent with the presence of a high proportion of arabinan (Bacic et al., 1988). The presence of the same linkages detected in fraction A-1 (see above) suggested that fraction A-2 also contained XG (ca. 30%) that was not precipitated by Fehling's solution.

Fraction B, which bound to the anion-exchange column, contained mostly Ara and Gal, and low levels of Xyl, Rha, Glc and Man; this fraction also contained 16% w/w uronic acid (Table 1). Linkage analysis showed that there was a high proportion of terminal Ara*f*, terminal Gal*p*, 6-Gal*p* and 3,6-Gal*p*, together with smaller amounts of terminal Rha*p*, terminal Ara*p*, 3-Ara*f*, 5-Ara*f*, 3,5-Ara*f* and 2,3,5-Ara*f*, and 3-Gal*p* (Table 2), consistent with fraction B containing 92% type II arabinogalactans (AGs) (Saulnier, Brillouet, Moutounet, du Penhoat & Michon, 1992; Gane, Craik, Munro, Howlett, Clarke & Bacic, 1995), probably present as AGPs. Glucuronic acid is a common component of AGPs, and thus the uronic acid detected in fraction B was probably a component of AGPs from apple suspension cultures. The linkage composition suggested that the other polysaccharide present in fraction B was a xylan (8%) composed of terminal Xyl*p*, 4-Xyl*p* and 2,4-Xyl*p* (Bacic et al., 1988).

The compositional and linkage analyses showed that the ECPs from suspension-cultured cells of apple contained mainly two types of polysaccharides, namely XG and AGPs. Separation by anion-exchange chromatography, and selective precipitation suggested that the ECPs contained ca. 44% fucosylated XG and ca. 48% AGPs; the remaining material comprised neutral arabinan (3%) and acidic xylan (4%).

3.3. Purification of XG from apple-pomace

The predominant linkage types present in apple-pomace was 4-Glc*p* and 4,6, Glc*p* (Table 3). The pomace also contained 5-Ara*f*, terminal Xyl*p*, 2-Xyl*p* and 4-Xyl*p*, 4-Man*p* and terminal Gal*p*, together with linkages present in smaller amounts (Table 3). From the summed mol% of their individual glycosyl residues (see above) the pomace was deduced to contain 36% XG (from the sum of 4,6-Glc*p*, terminal Xyl*p* and 2Xyl*p*, terminal Gal*p* and 2-Gal*p* and terminal Fuc*p* and 4-Glc*p* equal to one-third that of the 4,6-Glc*p*) and 46% cellulose (4-Glc*p* not assigned to XG), together with smaller amounts of arabinan (5-Ara*f*, 4%), xylan (4-Xyl*p*, 4%) and mannan (4-Man*p*, 3%). Linkage types corresponding to AGPs in ECPs from apple suspension cultures (see above) were not detected in apple-pomace. AGPs are generally considered as secreted into the extracellular space (Gane et al., 1995) and are not commonly found in plant cell walls.

Linkage analysis of the KOH-soluble fraction of the pomace showed that this material was composed of mostly 4-Glc*p* and 4,6-Glc*p*, terminal Xyl*p* and 2-Xyl*p*, terminal Gal*p* and 2-Gal*p* and terminal Fuc*p* and was thus deduced to contain 79 mol% XG (Table 3). The linkage composition indicated that this material also contained 9 mol% arabinan (from the sum of 5-Ara*f* and 3,5-Ara*f* and terminal Ara*f* equal to 3,5 Ara*f*) and 6 mol% xylan (4-Xyl*p*). The same linkages were present in the Fehling's precipitated material, consistent with the presence of 90% XG, 3% arabinan and 4% xylan. Following anion-exchange chromatography, linkage analysis was consistent with the presence of 94 mol% XG; the analysis also contained 6 mol% 4-Man*p*

which suggested that a small amount of mannan was also present.

XG purified from apple-pomace contained Glc, Xyl, Gal and Fuc in the ratio 4.0:1.9:0.5:0.5 (Table 4; from the sum of different linkages in Table 3), compared with the ratio of 4.0:2.3:0.7:0.5 from linkage analysis of XG from apple ECPs (Table 2). The linkage compositions of these two XGs (see Tables 2 and 3) were very similar, indicating that XG secreted into the medium of suspension-cultured apple cells was essentially identical to that from apple pomace. Comparison of the monosaccharide compositions of XG from apple pomace and ECPs of suspension-cultured cells with those of XGs purified from apple cell walls (Renard et al., 1991; Renard et al., 1992; Ruperez et al., 1985) is shown in Table 4. The monosaccharide compositions are similar in each case, although the amount of Glc detected in XG from ECPs by monosaccharide analysis was lower than in every other sample.

This study represents the first examination of the polysaccharides secreted by suspension-cultured apple cells. The ECPs contained predominantly two types of polysaccharides, XG and AGPs. Interestingly, no pectic polysaccharides were detected in ECPs from suspension-cultured apple cells. XG is a component of apple cell walls (Renard et al., 1991, 1992; Ruperez et al., 1985) and can be isolated from apple-pomace with strong base; it can be purified from the growth medium of apple suspension cultures without such harsh chemical treatments, making it attractive for further structural analysis. The secreted AGPs can be obtained in significant amounts, making them readily available for further research.

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