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Pectic polysaccharides from mature orange (*Citrus sinensis*) fruit albedo cell walls: Sequential extraction and chemical characterization

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ABSTRACT

The orange albedo alcohol insoluble solids (AIS) wall preparation, material prepared from the albedo tissue of mature orange fruit, contained 55 mol% pectic polysaccharides, including homogalacturonan (HG), rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II) together with Type I arabinogalactan (AG) and arabinan. It also contained cellulose (22 mol%) and other non-cellulosic polysaccharides (14 mol%), including xyloglucans (XGs) (10 mol%), heteromannans (2 mol%) and heteroxylans (2 mol%). Sequential extraction of the AIS performed with NaOAc, CDTA, Na_2CO_3, 1 M KOH and 4 M KOH produced soluble fractions (Frs.) with different relative proportions of these polysaccharides. The NaOAc and CDTA Frs. were composed predominantly of pectic polysaccharides (89 and 97 mol%, respectively) with only very low proportions of other non-cellulosic polysaccharides. Na_2CO_3 (50 mM) released highly ramified pectic polysaccharides, whereas stronger alkali (1 M and 4 M KOH) liberated mostly non-cellulosic polysaccharide, comprised predominantly of XG. The NaOAc, CDTA and Na_2CO_3 Frs. contain low levels of 2-*O*-methyl-Fuc and 2-*O*-methyl-Xyl, suggesting the presence of RG II. To obtain the RG II for structural characterization, the NaOAc fraction was digested with a combination of endo-polygalacturonase (endo-PG; PG I and PG II) and exo-PG, and the degradation product was fractionated by anion exchange and size exclusion chromatography. The isolated product (approx. 0.5% of orange albedo AIS) contained all six diagnostic sugars and has a glycosyl linkage composition consistent with the structural model of RG II.

1. Introduction

Since pectin was first described in the 19th century, the elucidation of its fine structure remains a considerable challenge for modern carbohydrate biochemists. To date, three major pectic polysaccharide components are recognized (Harholt, Suttangkakul, & Scheller, 2010; Ridley, O'Neill, & Mohnen, 2001; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995): homogalacturonan (HG) which is composed of linear α-1,4-linked D-galacturonic acid (GalA) with varying proportions of the carboxyl groups (C-6) present as methyl esters and in some species the GalA backbone can be substituted with xylose (Xyl) residues, primarily single, to form a xylogalacturonan, rhamnogalacturonan I (RG I), which contains GalA, rhamnose (Rha), as well as a variety of other sugars as branches of arabinan, arabinogalactan (AG) (Type I) and galactan on the RG dissacharide repeat backbone; and rhamnogalacturonan II (RG II) that contains an oligomeric GalA backbone to which a complex array of side chains containing several unusual monosaccharides are attached. Pectins are critical matrix components, particularly of primary cell walls of dicots, gymnosperm and most monocots, but are less significant in walls of the commelinoid monocots (Bacic, Harris, & Stone, 1988; Gibeaut & Carpita, 1994; Somerville et al., 2004). Pectins are also of immense industrial importance where they are used extensively as gums and stabilizers in the food industry.

Citrus fruits are particularly rich in pectin, especially in the albedo tissue (Liu, Shi, & Langrish, 2006), and the large quantities of citrus (lemon, lime, orange and grapefruit) waste generated by the fruit juice industry have become the most important raw material for the production of commercial pectin (May, 1999). Unlike pectins obtained from either lemon or lime that are reported to comprise predominantly HG (of variable degrees of methyl/acetyl esterification) and smaller quantities of RG I and RG II that are highly modified as a consequence of the harsh (acidic) extraction procedures that strip away the side chains, particularly RGI and arabinose containing polymers (Ralet & Thibault, 1994; Ros, Schols, & Voragen, 1996; Yapo, Lerouge, Thibault, & Ralet, 2007), there are

Abbreviations: AEC, anion exchange chromatography; AG, arabinogalactan; AIS, alcohol insoluble solids; CDTA, cyclohexane diamine tetraacetate; DA, degree of acetylation; DE, degree of methylesterification; endo-PG, endopolygalacturonase; Fr., fraction; HG, homogalacturonan; RG, rhamnogalacturonan; SEC, size exclusion chromatography; SEM, scanning electron microscopy; XG, xyloglucan.

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only a limited number of publications on the pectins from orange, especially with respect to their composition and structural details. We therefore undertook a study into both the spatial and temporal changes in pectins during orange fruit development and the physicochemical characteristics of mature orange pectin. In the present paper, sequential chemical extraction with NaOAc, CDTA, Na_2CO_3, 1 M KOH, and 4 M KOH, was carried out to obtain soluble fractions and a residue fraction from the alcohol insoluble solids (AIS) wall preparation of the albedo tissue of mature Valencia orange peel. Digestion with a mixture of endo-PGs (PG I and PG II) and exo-PG, followed by anion exchange and size exclusion chromatography of the NaOAc Fr. further yielded an RG II component. The AIS and its fractions were chemically characterized, the results of which are presented.

2. Materials and methods

2.1. Materials

Valencia oranges [*Citrus sinensis* (L.) Osbeck] were purchased from fruiterers at the Victoria Market, Melbourne, Victoria, Australia. Four different batches of fresh oranges were used for the preparation of AIS.

2.2. Preparation of AIS from albedo tissue

Orange albedo AIS was prepared by a modification of the methods from De Vries, Rombouts, Voragen, and Pilnik (1984) and Renard (2005). Each orange was cut and peeled manually with a sharp knife to remove the flavedo. The albedo tissue (100 g) was separated from the flesh, cut into small pieces of <1 cm^2, and immediately dropped into a mixture of ethanol (600 mL) and water (167 mL), pre-heated to boiling (~80 °C), and treated for 30 min to inactivate endogeneous enzymes (Christensen, Nielsen, Kreiberg, Rasmussen, & Mikkelsen, 1988). The albedo was then collected on a sieve (pore size approx. 16–40 μm), transferred to 70% ethanol (500 mL), and wet-milled with a Waring blender (2 min) followed by an Ultra Turrax (TP 18/2 Janke and Kunkel, KG, Germany; 4× 3 min). The ground mixture was stirred (30 min), and filtered on to a filter paper (Whatman No. 541). This extraction process was repeated (1×). The albedo was then solvent-exchanged in absolute ethanol (500 mL, 2×) and acetone (300 mL, 1×), and after collection by filtration, the solids were air dried and then dried in a vacuum oven at 40 °C overnight. The dried AIS was pulverized using a grinder (Breville) and passed through a 300-μm sieve. Four batches of AIS were blended together and stored at −20 °C.

2.3. Sequential extraction of polysaccharides from AIS

AIS (150 mg) was stirred in 100 mL sodium acetate buffer (50 mM, pH 5, 0.1% NaN_3) for 2 h at RT. The suspension was centrifuged (3373 × *g*, 5 min) and the supernatant was collected. This extraction was carried out three times. The supernatants were pooled, filtered through a sintered glass filter (Quickfit SF 3A33 with 16–40 μm pore size), and concentrated to 100 mL by diafiltration (Amicon 8400 with YM10 membrane, Millipore Corporation, Bedford, USA) and the polysaccharides precipitated with ethanol (5 vol., overnight at 4 °C) and centrifuged (3373 × *g*, 15 min). After solvent exchange with ethanol (3×) and acetone (1×), the NaOAc soluble Fr. was dried in a vacuum oven (40–50 °C, overnight), and kept in a freezer until use.

The NaOAc wall residue was extracted with 50 mM CDTA in sodium acetate buffer (100 mL, 50 mM, pH 6.5, 0.1% NaN_3) at RT overnight and again for 6 h. The sample was centrifuged (3373 × *g*, 5 min) pellets washed with water (2× 25 mL). The pooled supernatants (from two extractions and two water washes) were filtered on a sintered glass filter as above, dialyzed against 100 mM ammonium acetate (NH_4OAc pH 5.2, 0.1% NaN_3) for 2 days at 4 °C and then water for 2 days at 4 °C to remove CDTA and buffer salts. The dialyzed extract was concentrated to 50 mL using diafiltration, the polysaccharides precipitated with ethanol (5 vol.), and collected by centrifugation (21,860 × *g*, 20 min) and dried as above to give the CDTA Fr.

The wall residue (pellet) from above was suspended in 100 mL of 50 mM Na_2CO_3, 20 mM $NaBH_4$ and stirred at 4 °C overnight. The slurry was centrifuged (3373 × *g*, 5 min) and the supernatant was collected. The pellet was re-extracted with Na_2CO_3 containing 20 mM $NaBH_4$ for 6 h at RT followed by centrifugation. The pooled supernatant was filtered as above and neutralized with acetic acid to pH 6.8. After dialysis against water for 3 days at 4 °C (0.1% NaN_3 was added), the extract was concentrated to 50 mL using diafiltration, followed by precipitation of the polysaccharide with ethanol (5 vol.), centrifugation, washing, and drying as above to give the Na_2CO_3 Fr.

The residue from above was extracted in 1 M KOH (100 mL, supplemented with 20 mM $NaBH_4$) overnight at RT, then centrifuged (3373 × *g*, 5 min) to collect the supernatant. The pellet was re-extracted with 1 M KOH/20 mM $NaBH_4$ at RT for 6 h, and centrifuged to collect the supernatant. The procedure that followed to harvest the wall polymers in the supernatants was the same as described above for Na_2CO_3 Fr. The wall residue was then used for 4 M KOH/20 mM $NaBH_4$ extraction (2×) in the same way to produce a 4 M KOH Fr.

The pellet from the 4 M KOH extraction was suspended in 30 mL H_2O, neutralized (to pH 6.8) with 1 acetic acid, and centrifuged (3373 × *g*, 15 min). The pellet was further washed with water (50 mL, 4×), centrifuged and dried to give the residue fraction (Residue Fr.).

2.4. Isolation of RG II

The NaOAc Fr. (60 mg) was dissolved in water (4 mL) then NaOH (500 mM, 1 mL) was added and the mixture vortexed and incubated at 0–4 °C for 24 h to de-esterify the pectins. Acetic acid (0.1 M) was added to adjust the solution pH to ~6. The polysaccharides were precipitated by adding absolute ethanol (5 vol.; standing overnight at 4 °C) and centrifuged (21,860 × *g*; 15 min). After washing with 80% ethanol (3×), the precipitate was dried as described above.

To release the RG II, the de-esterified pectic polysaccharides dissolved in 5 mL of 50 mM NaOAc buffer, pH 4.5 were digested with a combination of polygalacturonases from *Aspergillus niger*; endo-PG I (15 μL, 7000 nkat/mL), endo-PG II (15 μL, 7000 nkat/mL), and exo-PG (10 μL, 7000 nkat/mL) at 35 °C for 24 h. Another addition of the three enzymes at the same dosage was made, and the sample then incubated for another 24 h at 35 °C. The treated sample was dialyzed (1 kDa MWCO) against water at RT for 24 h. The retentate was freeze-dried, and subjected to anion exchange and size exclusion chromatography.

2.4.1. Chromatographic separation of RG II

Anion exchange chromatography (AEC) was performed at RT on a DEAE-Sepharose Fast Flow (GE Healthcare, Uppsala, Sweden) column (22.5 cm × 3.3 cm) equilibrated with 50 mM Na-succinate buffer (pH 4.5) at a flow rate of 90 mL/h for 10 h. The de-esterified and PG-digested NaOAc Fr. (in 10 mL of 50 mM Na-succinate buffer, pH 4.5) was loaded onto the column and washed with 400 mL of 50 mM Na-succinate buffer. The bound material was eluted with a linear NaCl gradient (0–0.4 M) in 50 mM Na-succinate buffer (800 mL). Fractions (~9 mL each) were collected and analyzed for their total sugar and uronic acid contents. The fractions of interest were pooled, concentrated by rotary evaporation at 40 °C, extensively dialyzed (1 kDa MWCO) and freeze dried.

Size exclusion chromatography (SEC) was performed at RT on a Sephacryl S-200 (GE Healthcare, Uppsala, Sweden) column (90 cm × 1.3 cm) equilibrated with 50 mM Na-succinate buffer (pH 4.5). The sample (desalted fractions collected from AEC; dissolved in 15 mL of 50 mM Na-succinate buffer, pH 4.5) was loaded and eluted with the Na-succinate buffer at a flow rate of 0.4 mL/min (Ralet et al., 2005). Fractions (1.5 mL each) were collected and analyzed for their total sugar and uronic acid contents. The fractions of interest were pooled, concentrated, dialyzed and freeze dried as described above.

2.5. Scanning electron microscopy of AIS

To examine the physical state of the AIS, the sample powder was mounted on metal stubs using double-sided adhesive tape, coated with gold (Edwards Sputter Coater S150B) and then viewed with a Philips XL30 Field-Emission Scanning Electron Microscope (Eindhoven, The Netherlands). Digital images were captured using the Philips microscope control software.

2.6. Analytical procedures

2.6.1. General methods

All analyses were performed in duplicate unless otherwise specified. The starch content of AIS was determined using a total starch assay kit from Megazyme (Ireland) according to the manufacturer's instructions. The moisture content of AIS (1 g) was calculated as the loss in weight after drying in an oven at 110 °C for 2 h. The nitrogen content of AIS was determined using the Micro-Dumas combustion method of analysis on a PerkinElmer 2400 Series II CN Analyzer and calculated from the elemental nitrogen content using a multiplication factor of 6.25 (AOAC, 1984). The ash content (%, w/w) was determined by weighing AIS (200 mg) after incineration in a furnace (500–600 °C) overnight. Total carbohydrate content was determined by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), using D-galactose as the standard. To analyze the eluate fractions from AEC and SEC, the phenol–sulfuric acid method with a microplate reader was used (Fox & Robyt, 1991). The uronic acid content was determined by a colorimetric procedure (Filisetti-Cozzi & Carpita, 1991), using D-galacturonic acid (GalA) as the standard. The colorimetric procedure was further adapted for microtiter plate assay of uronic acid (van den Hoogen et al., 1998) of eluate fractions from AEC and SEC. The degree of acetylation (DA) was determined using HPLC (Voragen, Schols, & Pilnik, 1986) and the degree of methylesterification (DE) was calculated from methylation data. The orange albedo AIS wall preparation was hydrolyzed in 6 M HCl at 110 °C for 24 h, and the amino acids released were analyzed using the Waters AccQTag Ultra chemistry. Cysteine, tryptophan and hydroxyproline were not detected with this method.

2.6.2. Determination of microfibrillar cellulose content

The cellulose content of the AIS and Residue Fr. was determined using a modified acetic/nitric acid method (Updegraff, 1969). The dried samples (20 mg) were weighed into eppendorf tubes, acetic/nitric reagent (1.5 mL, prepared by mixing 80 mL acetic acid, 10 mL nitric acid, and 20 mL water) was added to each, and the tubes were sealed with microtube sealers and placed in an oven pre-heated to 100 °C. After heating for 90 min the tubes were centrifuged (1000 × *g*, 15 min, RT) to pellet the cellulose. The supernatant was carefully removed, and the pellet was washed/centrifuged sequentially with water (1×), 80% ethanol (4×), and acetone (1×). The pellet was dried in a vacuum oven overnight at 40 °C and weighed to calculate the percentage of microfibrillar cellulose.

2.6.3. Monosaccharide composition and linkage analysis

2.6.3.1. Sugar analyses by alditol acetates. AIS, extracted fractions and residue were carboxyl-reduced (Kim & Carpita, 1992), hydrolyzed [Saeman hydrolysis by dissolving samples in 72% (w/w) H_2SO_4 for 1 h and then diluting to 1 M H_2SO_4 at 100 °C for 3 h for AIS and residue, and 2.5 M trifluoroacetic acid (TFA) at 121 °C for 1.5 h for extracted fractions], followed by reduction with $NaBD_4$ and acetylation (Sims & Bacic, 1995). The resulting alditol acetates were analyzed in a Hewlett Packard gas chromatograph (6890 series) fitted with a CPSIL5 capillary column (Chrompack, Australia) and coupled to a 5973 mass selective detector (Zhu, Pettolino, Mau, & Bacic, 2005). The alditol acetates were identified based on their retention time and fragmentation patterns.

2.6.3.2. Linkage analysis. To determine the linkage of glycosyl residues, carboxyl-reduced AIS, extracted fractions and residue were methylated with CH_3I/NaOH (Ciucanu & Kerek, 1984; McConville, Homans, Thomas-Oates, Dell, & Bacic, 1990). The partially methylated alditol acetates (PMAAs) generated were separated by GC on a BPX70 capillary column (SGE, Australia) and analyzed as above.

2.6.3.3. Estimation of polysaccharide composition. The proportion of a certain polysaccharide present in the AIS, extracted factions or residue was estimated by summing up the mol% sugar linkages attributed to that polysaccharide (Shea, Gibeaut, & Carpita, 1989; Zhu et al., 2005).

2.6.3.4. Sugar analyses by methanolysis for the RG II. Methanolysis and trimethylsilylation of the resultant methyl glycosides were performed according to the procedures described previously (Chaplin, 1982; McConville & Bacic, 1989). *myo*-Inositol was used as an internal standard. RG II isolate of red wine was used as the standard to facilitate the identification and quantitation of unique sugars of orange albedo RG II.

3. Results and discussion

3.1. Preparation and microscopic examination of orange albedo AIS

To isolate walls from plant tissues with low amounts of starch, intra-cellular protein and polyphenols, the preparation of alcohol insoluble solids (AIS) from the tissues is usually performed (Fry, 1988). The purity of the AIS wall preparation obtained from the albedo tissue of mature orange peel was examined by SEM (Fig. 1), which revealed a mixture of parenchymatous wall fragments and some pieces of intact vascular tissue (Fig. 1A and C). The wall fragments were generally uniform in size, approx. <300 μm (Fig. 1A) and often found folded or clumped together (Fig. 1B), the latter probably an artifact of the drying process. The cells were mostly broken and the resulting walls (Fig. 1D) were generally free of adherent cytoplasmic material. The inner surface of the walls was uneven, showing the aligned microfibrils within the wall (Fig. 1D, indicated by an arrow).

3.2. Composition of orange albedo AIS

The composition data revealed that the orange albedo AIS wall preparation consisted mainly of carbohydrate (85%, w/w), similar to that of dried citrus peel (82%; a commercial sample most likely from lemon/lime; Yapo et al., 2007) but higher than the carbohydrate content found for lemon pulp (70%; Brillouet et al., 1988), commercial lemon dietary fibre (67%; Ralet & Thibault, 1994), and lemon albedo (59%; Ros et al., 1996). The difference presumably arises from the different methods used for preparation of walls

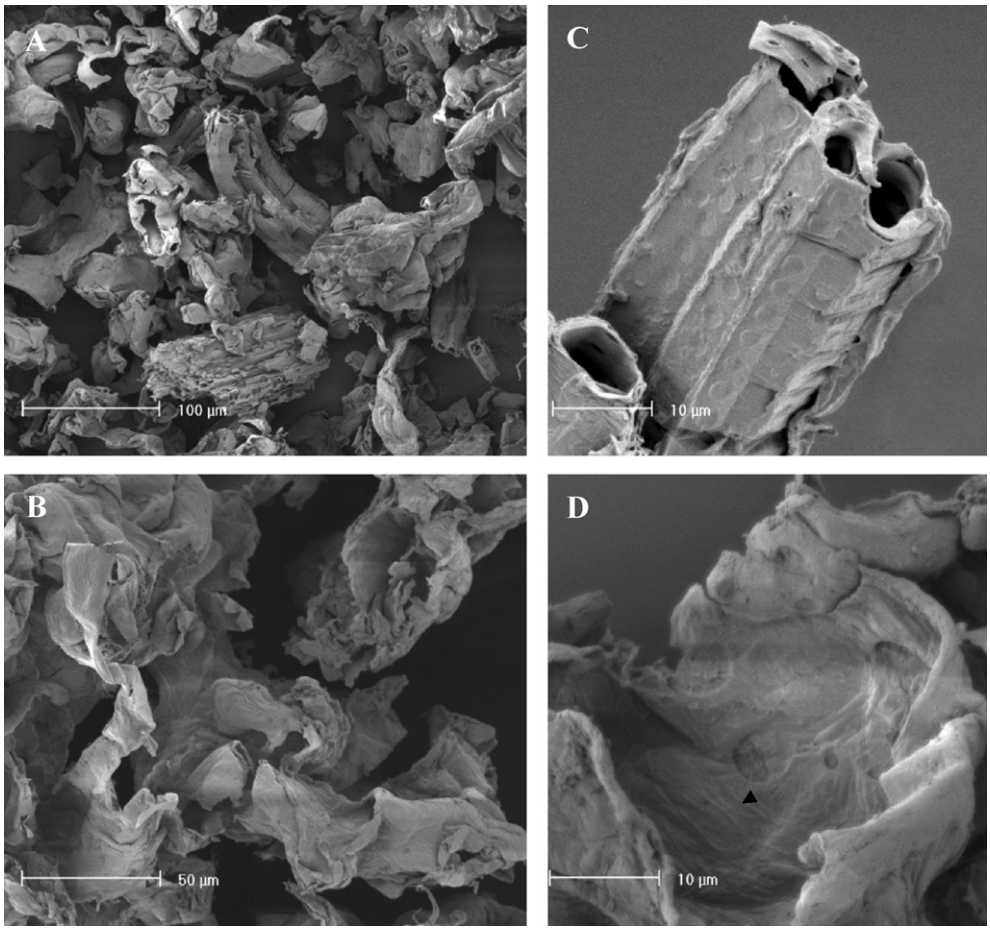

Fig. 1. Scanning electron micrographs of the alcohol insoluble solids (AIS) wall preparation of orange albedo. (A) Fragments of AIS. (B) Fragments of AIS were found folded and clumped together. (C) Close up of vascular tissue fragment found in the AIS. (D) The cells have been completely broken and the arrow shows microfibrils of the cell wall. The lack of adventitious deposits on the walls suggests the cellular contents were mostly removed in the isolation procedure.

and/or species-specific variation. No starch was detected in the AIS consistent with its reported absence in orange fruit (Spiegel-Roy & Goldschmidt, 1996). The microfibrillar cellulose content of AIS was 28% (w/w). The rest of the carbohydrate therefore arises from pectic and other non-cellulosic polysaccharides.

The AIS wall preparation also contained small amounts of protein, moisture and inorganic material (4%, 3% and 3%, w/w, respectively). The protein in the AIS wall preparation could arise from wall proteins and/or from co-precipitated intracellular proteins (Bacic et al., 1988), although the lack of adventitious cytoplasmic deposits observed by SEM (Fig. 1D) would suggest that the protein is mostly of wall origin. Primary walls of dicots generally contain <10% protein (Bacic et al., 1988). The protein of orange albedo AIS wall preparation comprised primarily of the amino acids aspartic acid, glutamic acid, glycine and leucine (11, 11, 10, and 10 mol%, respectively; Table 1), with alanine, lysine, valine and serine also present in slightly lower amounts. These amino acids are commonly found in various dicot walls (e.g. *Arabidopsis*) (Fry, 1988; Zablackis, Huang, Muller, Darvill, & Albersheim, 1995).

Table 1
Amino acid composition (mol%) of orange albedo alcohol insoluble solids (AIS) wall preparation and *Arabidopsis* leaf cell walls.

Amino acid	Orange albedo AIS	*Arabidopsis*[a]
Histidine	2	1
Serine	7	6
Arginine	4	5
Glycine	10	10
Aspartic acid	11	9
Glutamic acid	11	12
Threonine	5	5
Alanine	9	10
Proline	6	5
Lysine	7	–
Tyrosine	2	2
Methionine	tr	1
Valine	7	7
Isoleucine	5	5
Leucine	10	12
Phenylalanine	4	4
Hydroxyproline	ND	1

Values are the average of duplicate determinations; (–) not detected; ND, not determined; tr, trace (<0.5 mol%).
[a] Data from Zablackis et al. (1995).

3.3. Monosaccharide and linkage composition of orange albedo AIS wall preparation

Table 2 shows that orange albedo AIS is composed mainly of GalA (44 mol%) and Glc (27 mol%). In addition, the AIS also contained significant proportions of Ara, Gal and Xyl residues (11, 7 and 7 mol%, respectively) as well as small amounts of Rha and Man (2 mol% each). Using the linkage data derived from carboxyl reduced AIS (Table 3) and based on the characteristic linkage patterns established for various cell wall polysaccharides (Carpita & Gibeaut, 1993; Shea et al., 1989; Zhu et al., 2005), the polysaccharide composition of orange albedo AIS was estimated and summarized in Table 4. The wall of orange albedo was composed

Table 2
Yield and monosaccharide composition of carboxyl reduced AIS wall preparation, extracted fractions and residue obtained from orange albedo tissue.

	AIS	NaOAc Fr.	CDTA Fr.	Na_2CO_3 Fr.	1 M KOH Fr.	4 M KOH Fr.	Residue Fr.
Yield (%, w/w)[a]	100	16	14	10	6	8	35
Total carbohydrate (%, w/w)[b]	85	78	79	80	92	93	90
Sugar composition (mol%)[c]							
Rha	2	2	3	6	4	1	1
Ara	11	6	5	31	10	19	10
Fuc	tr	–	–	–	4	1	–
2-*O*-Methyl-L-Fuc[d]	–	tr (0.3)	tr (0.3)	tr (0.1)	–	–	–
Xyl	7	2	1	2	31	40	8
2-*O*-Methyl-D-Xyl[e]	–	tr (0.3)	tr (0.3)	tr (0.1)	–	–	–
Man	2	1	1	1	2	1	8
Glc	27	1	tr	2	34	14	56
GlcA	tr	1	tr	tr	3	2	2
Gal	7	4	5	33	10	14	10
GalA	44	83	85	25	2	8	5
DE[f] (%)	73	69	65	ND	ND	ND	ND
DA[g] (%)	8	2	2	ND	ND	ND	ND

Values were the average of duplicates; DE, degree of methyl esterification; DA, degree of acetylation; (–) not detected; ND, not determined; tr, trace (<0.5 mol%); values in brackets represent the actual values of two mono-*O*-methyl monosaccharides.

[a] Gram quantities per 100 g of AIS.
[b] Total carbohydrates were determined by phenol–sulfuric acid using Gal as a standard.
[c] Sugar composition was calculated from alditol acetates of carboxyl reduced samples.
[d] 2-*O*-Methyl-L-Fuc was detected by the presence of diagnostic fragment ions *m/z* 275, 173, 129, 118 and 113 from alditol acetates of carboxyl reduced samples.
[e] 2-*O*-Methyl-D-Xyl was detected by the presence of diagnostic fragment ions *m/z* 261, 159, 127 and 118 from alditol acetates of carboxyl reduced samples.
[f] DE calculated from methylation data in Table 3.
[g] DA derived from analysis with HPLC (Voragen et al., 1986).

mainly of pectic polysaccharides (55 mol%), including HG, together with some RG I, Type I AG and arabinans. HG was the predominant pectic polysaccharide (43 of 55 mol%, i.e. 78%; Table 4). This result is consistent with previous studies that the HG in the primary walls of dicots accounts for up to 60% of the pectic polysaccharides (O'Neill & York, 2003). Type II AG, a highly branched polysaccharide of β-Gal*p* residues joined by (1→3) and (1→6) linkages (Ridley et al., 2001; Voragen et al., 1995), was found in only a trace amount. Other components were cellulose (22 mol%; a value consistent with the 28% derived from acetic-nitric analyses) and other non-cellulosic polysaccharides (14 mol%), including xyloglucans (10 mol%), heteromannans (2 mol%) and heteroxylans (2 mol%) (Table 4). The polysaccharide composition of the orange albedo AIS wall preparation was therefore typical of most dicot walls.

3.4. Sequential chemical extraction of orange albedo AIS wall preparation

In order to have a better understanding of the nature and distribution of the pectic and other non-cellulosic polysaccharides, sequential chemical extraction and structural analysis were performed. Sequential extraction of orange albedo AIS wall preparation resulted in five soluble fractions (NaOAC Fr., CDTA Fr., Na_2CO_3 Fr., 1 M KOH Fr., 4 M KOH Fr.) with yields in the range of 6–16% (w/w), together with 35% for the Residue Fr. The total recovery was 89% (Table 2), being comparable with those reported for lemon albedo (87%; Ros et al., 1996) and dried citrus peel (85.8%; Yapo et al., 2007).

The NaOAc Fr. was comprised predominantly of GalA (83 mol%; Table 2), similar to that of NaOAc fraction obtained from apple cell walls, where GalA was the major glycosyl moiety (80 mol%; Schols, Vierhuis, Bakx, & Voragen, 1995). Small proportions of neutral sugars, including Ara (6 mol%), Gal (4 mol%), and Rha and Xyl (2 mol% each) were also present. Based on the estimated relative proportions of polysaccharides (Table 4), derived from the linkage data (Table 3), pectic polysaccharides were the dominant components of the NaOAc Fr. (89 mol%), of which HG accounted for 83 mol% with minor amounts of Type I AG (4%), arabinan (1%), Type II AG (1%) and only traces of RG I and II. Other non-cellulosic polysaccharides were only minor components. The pectic component of NaOAc Fr. had a high DE, with 69% of the carboxyl groups of GalA being methylesterified (57 of 83 mol%; Table 3). The high DE for pectin obtained from buffer extraction was also reported in previous studies (De Vries, Voragen, Rombouts, & Pilnik, 1981; Schols et al., 1995). The DA of the NaOAc Fr. was low (2%), similar to that reported for NaOAc fraction of apple (3%; Schols et al., 1995), but lower than that of water-extracted fraction from dried citrus peel (5.5%; Yapo et al., 2007).

Pectic polysaccharides solubilized by chelating agents are believed to be complexed into the wall mainly with Ca^{2+} (Fry, 1988). Extraction of orange albedo AIS with CDTA produced a fraction in 14% yield. The combined yield of wall polymers extracted from orange albedo AIS using buffer and chelating agent in separate steps (NaOAc Fr. + CDTA Fr.) was 30% (Table 2) and this value is comparable with previous studies using extraction protocols without the buffer step but directly with chelating agents for lemon albedo (33.4%; Ros et al., 1996) and apple (30%; Schols et al., 1995). The CDTA Fr. contained 79% total carbohydrate, and had a monosaccharide composition rich in GalA (85 mol%). The estimated polysaccharide composition revealed that pectic polysaccharides were the dominant components (97 mol%) in the CDTA fraction, with more hairy components (RG I plus neutral polysaccharide side chains) than those in the NaOAc Fr. (14 mol% vs 6 mol%; Table 4). Similar results were reported for apple walls where pectic polysaccharides extracted with chelating agent were more branched with neutral sugars than the buffer-extracted fraction (Schols et al., 1995). The DE of pectin in CDTA Fr. was 65%, since 56 of 86 mol% carboxyl groups were methyl-esterified (see Table 2). Notably, higher DE values were found in chelator extracted fractions of lemon albedo (79%; Ralet & Thibault, 1994) and dried citrus peel (73.7%; Yapo et al., 2007). The DA of pectin in CDTA Fr. was found to be 2% (Table 2), similar to that of chelator-extracted fraction from lemon albedo (2%; Ralet & Thibault, 1994) and dried citrus peel (3%; Yapo et al., 2007).

The Na_2CO_3 Fr. was obtained in 10% yield and its total carbohydrate (80%) was similar to those of the NaOAc Fr. and CDTA Fr. (Table 2). However, the monosaccharide composition of the Na_2CO_3 Fr. differed from both the NaOAc and CDTA fractions, with the for-

Table 3
Linkage composition (mol%) of carboxyl reduced AIS, extracted fractions and residue obtained from orange albedo tissue.

Sugar	Linkage[a]	AIS	NaOAc Fr.	CDTA Fr.	Na_2CO_3 Fr.	1 M KOH Fr.	4 M KOH Fr.	Residue Fr.
Rha*p*	1,2-	1	tr	2	4	tr	tr	1
	1,2,4-	1	tr	1	2	2	5	–
	Total	2	tr	3	6	2	5	1
Ara*f*	t-	4	1	tr	8	4	5	3
	1,5-	5	2	1	14	4	3	4
	1,2,5-	–	–	–	1	1	4	–
	1,3,5-	–	–	–	1	–	–	–
	Total	9	3	1	24	9	12	7
Fuc*p*	t-	tr	–	–	–	4	1	–
Total		tr	–	–	–	4	1	–
Xyl*p*	t-	3	1	–	–	13	11	–
	1,2-	3	1	tr	1	8	10	5
	1,4-	tr	1	tr	2	8	11	3
	1,2,4-	1	–	–	–	1	3	2
	1,3,4-	1	–	–	–	–	–	–
	1,2,3,4-	tr	tr	tr	tr	tr	tr	tr
	Total	8	3	tr	3	30	35	10
Man*p*	1,4-	2	1	1	1	3	2	6
	Total	2	1	1	1	3	2	6
Glc*p*	t-	1	–	–	–	2	1	–
	1,4-	25	2	1	2	10	4	57
	1,4,6-	2	–	–	–	23	16	1
	Total	28	2	1	2	35	21	58
Glc*p*A	t-	–	–	–	–	–	–	tr
	1,4-	1	tr	tr	1	1	tr	1
	Total	1	tr	tr	1	1	tr	1
Gal*p*	t-	2	2	2	1	5	7	1
	1,3-	–	tr	–	1	tr	1	–
	1,4-	3	4	5	30	7	7	9
	1,6-	tr	tr	–	–	–	–	–
	1,3,4-	tr	tr	2	1	–	–	–
	1,3,6-	tr	2	tr	–	–	1	–
	Total	5	8	9	33	12	16	10
Gal*p*A	1,4-	41 (30)	83 (57)	86 (56)	29	4	8	7
	1,3,4	2	tr	tr	1	–	–	–
	1,2,4-	2	tr	tr	–	–	–	–
	Total	45	83	86	30	4	8	7
Uronic acids[b]		46	83	86	31	5	8	8

Values are the average of duplicates; (–) not detected; tr, trace (<0.5 mol%). Values in brackets represent the mol% of methyl esterified 4-Gal*A*p.
[a] 1,2-Rha*p* is derived from 1,2,5-tri-*O*-acetyl-6-deoxy-3,4-di-*O*-methyl hexitol, etc.
[b] Uronic acids = total of all Gal*p*A and Glc*p*A residues.

mer having a relatively low proportion of GalA (25 mol%) compared to the latter two (83 and 85 mol%, respectively). In addition, the Na_2CO_3 Fr. contained high proportions of Ara (31 mol%), and Gal (33 mol%). The two unique mono-*O*-methyl sugars of RG II, 2-*O*-methyl-Fuc and 2-*O*-methyl-Xyl were also found in the Na_2CO_3 Fr., but at a lower level (approx. 0.1 mol%) than the two prior fractions (Table 2). Like the two prior fractions, the Na_2CO_3 Fr. also contained a relatively high proportion (83 mol%) of pectic polysaccharides (Table 4). However, in contrast to NaOAc and CDTA fractions, it was enriched in hairy segments comprised of RG I (12 mol%), Type I AG (31 mol%) and arabinan (18 mol%), and contained a relatively low proportion of HG (21 mol% vs 83 mol% in the two prior fractions). A previous study also showed that 50 mM Na_2CO_3 solubilized more ramified pectic polysaccharides from squash walls than that using chelating agent (Ratnayake, Melton, & Hurst, 2003). The proportion of other non-cellulosic polysaccharides in Na_2CO_3 Fr. was 5 mol%, with heteroxylans, XGs and heteromannans (2, 2 and 1 mol%, respectively; see Table 4).

Compared with the three prior fractions, especially the NaOAc and CDTA Frs. rich in GalA residues, the yields of alkali-soluble fractions were lower, 6% for the 1 M KOH Fr. and 8% for the 4 M KOH Fr. (Table 2). The major sugars of the 1 M KOH Fr. were Glc (34 mol%) and Xyl (31 mol%), followed by considerable amounts of Ara and Gal (10 mol% each), and minor proportions of Rha, Fuc, GlcA, Man and GalA. Similar to the 1 M KOH Fr., the 4 M KOH Fr. was comprised mainly of Xyl (40 mol%), Ara (19 mol%), Gal (14 mol%), and Glc (14 mol%) (Table 2). As expected, the relative proportions of polysaccharides (Table 4) revealed that the

Table 4
Estimated relative proportions of polysaccharides (mol%) present in alcohol insoluble solids (AIS), extracted fractions and residue obtained from orange albedo tissue.

Polysaccharides	AIS	NaOAc Fr.	CDTA Fr.	Na_2CO_3 Fr.	1 M KOH Fr.	4 M KOH Fr.	Residue Fr.
Yield (%, w/w)[a]	100	16	14	10	6	8	35
Pectic polysaccharides							
HG	43	83	83	21	2	3	6
RG I	4	tr	6	12	4	10	2
RG II[b]	–	tr	tr	tr	–	–	–
Type I AG	3	4	7	32	7	7	9
Arabinan	5	1	1	18	9	11	4
Type II arabinogalactan	tr	1	tr	1	tr	1	–
Sub-total	55	89	97	84	22	32	21
Other non-cellulosic polysaccharides							
XG	10	5	tr	2	63	50	6
Heteromannans	2	1	1	1	3	2	6
Heteroxylans	2	2	2	2	9	14	8
Sub-total	14	8	3	5	75	66	20
Cellulose	22	–	–	–	–	–	57
Undefined	9	3	–	11	3	2	2

The proportion of polysaccharide in AIS and factions were calculated based on mol% sugar linkage (Table 3) attributed to that polysaccharide (Shea et al., 1989; Sims & Bacic, 1995); tr, trace (<0.5 mol%); (–) not detected.

[a] Gram quantities per 100 g of AIS.

[b] The presence of RG II was indicated by the detection of two diagnostic mono-*O*-methyl monosaccharides, 2-*O*-methyl-L-Fuc and 2-*O*-methyl-D-Xyl from alditol acetates of carboxyl reduced samples.

1 M and 4 M KOH fractions were composed mainly of other non-cellulosic polysaccharides (75 and 66 mol%, respectively) with XG as the dominant component (63 and 50 mol%, respectively) with some heteroxylans (9 and 14 mol%, respectively) and heteromannans (3 and 2 mol%, respectively). Despite the dominance of other non-cellulosic polysaccharides, the 1 M KOH Fr. and 4 M KOH Fr. still contained significant amounts of pectic polysaccharides (22 and 32 mol%, respectively) mainly as hairy segments, with the proportion of HG (2 and 3 mol%, respectively) only accounting for approx. 9% of the pectic polysaccharides in both fractions (see Table 4). This is consistent with previous studies showing that extraction of dicot walls with strong alkali produces predominantly other non-cellulosic polysaccharides (XGs and acidic xylans), together with lower levels of pectic polysaccharides (Fry, 1988; Zablackis et al., 1995).

After five sequential extractions, approx. 35% (w/w) of the starting material (AIS) remained and it was predominantly (90%) carbohydrate (Table 2). Glc was the most abundant sugar (56 mol%), together with significant amounts of Gal, Ara, Xyl and Man (8–10 mol% each), as well as other minor sugars. Compared with the AIS, the residue was greatly reduced in GalA. The Residue Fr. is composed mainly of cellulose (57 mol%), with pectic and other non-cellulosic polysaccharides essentially accounting for the rest and occurring in about equal proportions (21 and 20 mol%, respectively) (Table 4). The incomplete solubilization of matrix phase cell wall polysaccharides is common (e.g. Fry, 1988; Ratnayake et al., 2003; Zablackis et al., 1995). This is presumably caused by the heterogeneity in the bonding of the polysaccharides to other polymers in the wall (Ratnayake et al., 2003) or physical entrapment during the assembly of the wall.

3.5. Isolation of RG II from AIS wall preparation

The NaOAc Fr. was used as the source material to isolate RG II since it was found to contain the diagnostic mono-*O*-methyl monosaccharides, 2-*O*-methyl-Fuc and 2-*O*-methyl-Xyl and would be expected to be enriched in RG II. The release of RG II, after digestion of the abundant HG component with endo-PG, has been demonstrated for wall preparations from angiosperms (Ishii & Matsunaga, 2001), and gymnosperms (O'Neill, Ishii, Albersheim, & Darvill, 2004). Both endo-PG (PG I and PG II) and exo-PG were used in the present study to achieve effective release of RG II. Since PG can only hydrolyze the glycosydic linkages adjacent to GalA residues with free carboxyl groups (Voragen et al., 1995), the NaOAc Fr. was first de-esterified before subjecting it to enzyme digestion.

Based on dry weight, approx. 50% of the NaOAc Fr. (approx. 30 mg) was recovered after the enzyme digestion and desalting processes similar to that reported for a sugar beet wall fraction (Ishii & Matsunaga, 2001). The enzyme-digested NaOAc Fr., containing a mixture of oligogalacturonides, RG II and rhamnogalacturonides with neutral sugar chains attached, was fractionated by AEC (Fig. 2). The overall recovery of total sugar and uronic acid for all fractions combined was 92% and 96%, respectively. The unbound fractions were presumed to contain mono-, di- and tri-mers of GalA as reported previously (Ralet et al., 2005) and discarded. The pooled fraction (corresponding to elution volume 700–1400 mL), accounting for approx. 71% total carbohydrate and 65% uronic acid of the loaded sample, was subjected to SEC with Sephacryl S-200 column. SEC yielded three peaks (I, II, and III; Fig. 3) representing approx. 40%, 18% and 30%, respectively, of the material loaded onto the column. Peak III, containing mainly oligogalacturonides resulting from hydrolysis of HG as previously reported for a citrus pectin treated in a similar manner (Yapo et al., 2007), had essentially no material retained after dialysis and was thus unable to be further analyzed. Peaks I and II were subjected to methanolysis and trimethylsilylation for their monosaccharide composition, and methylation for neutral sugar linkage.

The monosaccharide composition of Peak I showed that Ara was the predominant sugar (67 mol%), followed by Gal (10.8 mol%), GalA (8.3 mol%) and Rha (7.2 mol%) (Table 5). The molar ratio of GalA/Rha was ~1.1, suggesting that the polysaccharide(s) in Peak I corresponded to RG I (Table 5) similar to that reported for RG I isolated from a dried citrus peel sample (Yapo et al., 2007). In addition, the presence of Ara at a high level and Gal in a significant amount suggests that Peak I contained RG I with side chains much richer in arabinans than in galactans, similar to that of RG I from *Argania spinosa* fruit pulp (Aboughe-Angone et al., 2008). Xyl and Glc were found in small amounts (3.6 and 1 mol%, respectively; Table 5),

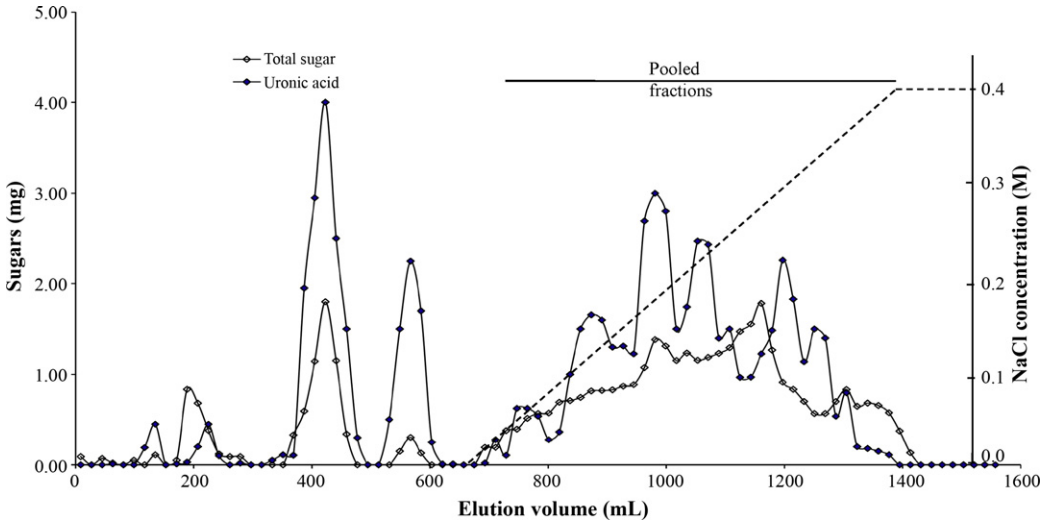

Fig. 2. Anion exchange chromatography of de-esterified and polygalacturonase (a combination of endo- and exo-PG) treated NaOAc fraction of orange albedo AIS. Fractions (~9 mL each) were collected and analyzed for their total sugar (Dubois et al., 1956) and uronic acid contents (Filisetti-Cozzi & Carpita, 1991). Uronic acid (♦); total sugar (◊); NaCl gradient (- - -).

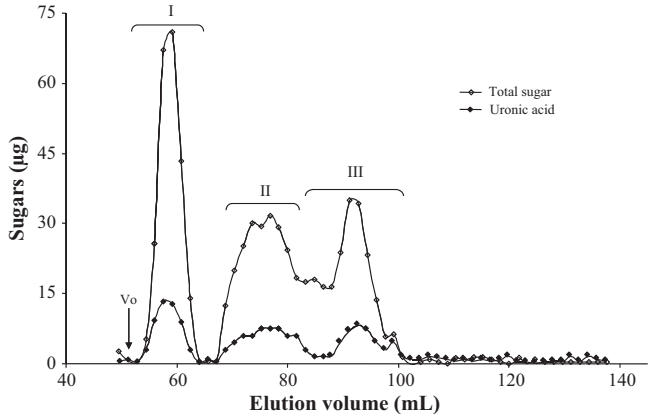

Fig. 3. Size exclusion chromatography of the pooled fractions from anion exchange chromatography (see Fig. 2) of de-esterified and polygalacturonase treated NaOAc Fr. Uronic acid (♦); total sugar (◊). I, II and III are the pooled fractions used for structural studies.

probably originating from non-cellulosic polysaccharides. Overall, the compositional data indicated that RG I was the dominant component of Peak I. To confirm these conclusions a linkage analysis was performed.

Unlike methanolysis from which 7.2 mol% of Rha residues was revealed for Peak I (Table 5), linkage analysis of neutral sugars without prior carboxyl reduction showed, as expected, only trace amounts of 2- and 2,4-Rha*p* for the same fraction (Table 6) and no GalA residues as the glycosidic linkages between galactosyluronic and rhamnosyl residues are known to be relatively resistant to acid hydrolysis (Voragen et al., 1995). 5-Ara*f* was the most abundant neutral sugar linkage (36 mol%; Table 6) and together with 3,5- and 2,5-Ara*f* (5 and 1 mol%, respectively) and some associated t-Ara*f*, these residues indicated the abundance of arabinan, presumably as side branches of RG I. The detection of 4-Gal*p* (6 mol%) and 3,4-Gal*p* (<0.5 mol%), in association with some t-Ara*f*, reflected the presence of Type I AG, presumably also as side branches of RG I but in an amount much lower than arabinan. The glycosyl linkages 3,6-, 3- and 6-Gal*p* (6, 1 and 1 mol%, respectively) are indicative of the presence of a Type II AG. In addition to the dominant pectic

Table 5
Glycosyl composition (mol%, from methanolysis) of Peak I and Peak II fractions (labeled in Fig. 3 corresponding to RG I and RG II, respectively) obtained from NaOAc Fr. of orange albedo AIS after enzyme digestion (PG) followed by separation with anion exchange and size exclusion chromatography. Glycosyl composition expected for the structural model of RG II derived from studies of several plant species (O'Neill et al., 2004; Ridley et al., 2001) is included for comparison.

Glycosyl residue	Peak I	Peak II	Structural model of RG II
Ara	67.0	16.5	10.3
Rha	7.2	15.7	17.2
Gal	10.8	5.8	6.8
Glc	1.0	ND	–
Fuc	–	2.6	3.5
Xyl	3.6	ND	–
Man	tr	ND	–
GalA	8.3	36.4	34.4
GlcA	2.1	2.7	3.5
Aceric acid[a]	–	1.4	3.5
Apiose	–	6.7	6.8
2-*O*-Methyl-Fuc	–	2.8	3.5
2-*O*-Methyl-Xyl	–	2.6	3.5
Dha[b]	–	3.3	3.5
KDO[c]	–	3.5	3.5

(–) not detected; tr, trace amount (<0.5 mol%); ND, not determined (components believed to be from contaminants and not included in compositional calculation).
[a] Aceric acid = 3-*C*-carboxy-5-deoxy-L-xylose.
[b] Dha = 3-deoxy-D-*lyxo*-heptulosaric acid.
[c] KDO = 3-deoxy-D-*manno*-octulosonic acid.

Table 6
Neutral sugar linkage composition (mol%)[a] of Peak I and Peak II (labeled in Fig. 3, corresponding to RG I and RG II, respectively) obtained from NaOAc Fr. of orange albedo AIS after enzyme digestion (PG) followed by separation with anion exchange and size exclusion chromatography.

Sugar	Linkages	Peak I	Peak II
Rha*p*	Terminal	–	14
	2-	tr	2
	3-	–	3
	2,4-	tr	tr
	2,3,4-	–	4
Ara*f*	Terminal	29	12
	2-	3	–
	3-	1	–
	5-	36	5
	3,5-	5	–
	2,5-	1	–
Ara*p*	Terminal	–	6
	2-	–	tr
Api*f*	3′-	–	9
Xyl*p*	Terminal	5	9
	2-	2	1
	4-	2	2
Fuc*p*	Terminal	–	9
	3,4-	–	4
Man*p*	4-	1	1
Gal*p*	Terminal	–	11
	3-	1	–
	4-	6	4
	6-	1	–
	2,4-	–	4
	3,4-	tr	–
	3,6-	7	–
	4,6-	tr	–

(–) not detected; tr, trace amount (<0.5 mol%).
[a] Samples analyzed without prior carboxyl reduction.

polysaccharides, the presence of terminal, 2-, and 4-Xyl*p* in small amounts indicated that some other non-cellulosic polysaccharides were also present (Table 6).

Methanolysis was used to quantify the monosaccharide composition of Peak II using the red wine RG II isolate as the reference material. Both preparations contained small amounts of Man, Glc and Xyl residues. Man and Glc appeared to be originating from contaminants, which were also reported for RG II isolated from sugar beet (Strasser & Amadó, 2002) and red wine (Doco, O'Neill, & Pellerin, 2001). Xyl was presumed to originate from either xylogalacturonans (Zandleven, Beldman, Bosveld, Schols, & Voragen, 2006) or other non-cellulosic polysaccharides. These monosaccharides were thus excluded from the calculation of monosaccharide composition of the orange fruit albedo Peak II. Table 5 shows that Peak II is composed mainly of GalA (36.4 mol%) followed by significant amounts of Ara (16.5 mol%), Rha (15.7 mol%) and Gal (5.8 mol%). Fuc and GlcA were detected in small amounts (2.6 and 2.7 mol%, respectively). The mol% of glycosyl residues, including the six unique sugars are generally in agreement with the values expected for the structural model of RG II derived from studies of several plant species (see Table 5). Ara was found at a level much higher than expected and could indicate variations within side chain B of RG II. Apiose was found at 6.7 mol%, corresponding to the presence of two Api residues in the orange RG II. The lower than expected recovery of aceric acid (1.4 mol%), could be due to the known acid-lability of this sugar (Spellman, McNeil, Darvill, & Albersheim, 1983).

Linkage analyses for neutral sugars of Peak II (Table 6) revealed that the linkage type and composition were consistent with the structural model of RG II (O'Neill et al., 2004; Ridley et al., 2001). Several types of Rha residues (t-, 2-, 3- and 2,3,4-Rha*p*) are expected in RG II. In addition to the most abundant t-Rha*p* (14 mol%), 2-, 3- and 2,3,4-Rha*p* were also detected (2, 3 and 4 mol%, respectively).

The structure of RG II is known to be highly conserved, although minor differences in the glycosyl residue composition of RG II derived from different sources have been found (Harholt et al., 2010; O'Neill et al., 2004; Ridley et al., 2001). In the structural model of RG II, Ara occurs in both furanose and pyranose forms. It has been suggested that the presence or absence of substituents at O-2 and/or O-3 of the Ara*p* residue would represent structural variation in the side chain B of RG II (O'Neill et al., 2004). For instance, a Rha*p* residue was found attached to O-2 of the Ara*p* in the side chain B of RG II from *Arabidopsis* (Glushka et al., 2003), a disaccharide Ara*f*-(1→2)-Rha*p*-(1→) was linked to O-2 of the Ara*p* residue in the side chain B of sycamore RG II (Whitcombe, O'Neill, Steffan, Albersheim, & Darvill, 1995), and the Ara*p* was not substituted in the side chain B of RG II from sugar beet pulp (Ishii & Kaneko, 1998). In the Peak II fraction of the current study, t-Ara*p* was found in an unexpectedly high amount (6 mol% for neutral sugar linkage composition; see Table 6). It was also found (at approx. 2.5 mol% for carboxyl reduced samples) in the RG II isolated from sugar beet pectin (Ishii & Matsunaga, 1996; Strasser & Amadó, 2002), and was suggested to represent variations within the aceric acid containing side chain B of RG II. Api was detected as 3′-Api*f*. There was evidence that O-2 and O-3 of the apiosyl residue of side chain A in each monomeric RG II is cross-linked by borate (Ishii et al., 1999). No 2,3,3′-Api*f* was detected in orange RG II, although it has been found in apple, carrot and tomato juice (Doco, Williams, Vidal, & Pellerin, 1997). 3,4-Fuc*p* is expected to be a constituent glycosyl residue of RG II and a small amount (4 mol%; Table 6) was detected in orange Peak II, similar to that found in RG II isolated from red beet (Strasser & Amadó, 2002), apple, carrot and tomato juice (Doco et al., 1997). Terminal (t-) and 2,4-Gal*p* are typical residues of RG II, and both were

detected in Peak II of orange. There have been reports of the presence of 3,4-Gal*p* in RG II isolated from sugar beet (Pellerin et al., 1996), representing an additional component of some RG IIs. However this residue was not found in Peak II of orange. Small amounts of 2- and 4-Xyl*p* (1 and 2 mol%, respectively), presumably derived from either xylogalacturonans (Zandleven et al., 2006) or other non-cellulosic polysaccharides rather than RG II, were detected in Peak II of orange. Man is not thought to be a component of RG II. The small amount of 4-Man*p* (1 mol%) detected in Peak II is most likely a contaminant from the dialysis tubing. This glycosyl residue was also found in RG II preparations obtained from red beet (Strasser & Amadó, 2002) and red wine (Doco et al., 2001).

RG II has been reported from various plant sources such as onion (De Vries et al., 1984), kiwi fruit (Redgwell, Melton, Brasch, & Coddington, 1992), *Bupleurum falcatum* roots (Yamada, Hirano, & Kiyohara, 1991), and *Arabidopsis* (Zablackis et al., 1995). Notably, it is present in red wine at relatively abundant levels (approx. 20% of ethanol-precipitable polysaccharides) (Pellerin et al., 1996). More recently, an acid-extracted (0.05 N HCl, 85 °C) pectin fraction of dried industrial citrus peels was found to contain RG II (Yapo et al., 2007). The present study demonstrates the presence of low levels (approx. 0.5%) of RG II in orange fruit.

In conclusion, the walls of orange albedo, an important alternative source of commercially valuable citrus pectin, are similar in composition to other dicot primary walls. Cellulose, the crystalline component of the wall, is associated with alkali extractable non-cellulosic polysaccharides, predominantly xylogucan but also small amounts of xylan and mannan. The cell wall pectin matrix is a major component of the wall and is composed of HG, RG I and small amounts of RG II. It is this readily extractable pectin component that is important in pectin manufacture. The data presented contributes to a better understanding of the wall polymers and the basis of their functionality in the commercially important citrus (orange) fruits and their extracts.

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