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Characterisation of polysaccharides from gold kiwifruit (*Actinidia chinensis* Planch. 'Hort16A')

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ABSTRACT

The cell-wall polysaccharide components of gold kiwifruit (*Actinidia chinensis* Planch. 'Hort16A', ZESPRI® GOLD) have been investigated for the first time. Alcohol-insoluble residues (AIRs) were prepared from whole, unpeeled gold and green (*Actinidia deliciosa*) kiwifruit and the constituent sugar and glycosyl linkage compositions determined. AIRs from both kiwifruit contained a high proportion of cellulose; the gold kiwifruit contained a higher proportion of hemicellulosic polysaccharides and lower proportion of pectic polysaccharides compared with the green. The gold kiwifruit AIR was partitioned by sequential extraction with water, aqueous CDTA (0.05 M), aqueous Na_2CO_3 (0.05 M) and aqueous KOH (1 M then 4 M). The glycosyl linkage compositions of each fraction were determined and the types of cell-wall polysaccharides from the gold fruit were found to be similar to those previously reported for the green fruit.

1. Introduction

Kiwifruit (*Actinidia* sp.) are an important export crop in New Zealand, with *Actinidia deliciosa* [A. Chev.] C.F. Liang and A.R. Ferguson var. *deliciosa* 'Hayward' (green kiwifruit) and *A. chinensis* Planch. 'Hort16A' (marketed as ZESPRI® GOLD) the main cultivars grown commercially. A substantial amount of work has been published on the polysaccharide structure and composition of green kiwifruit cell walls. The major non-cellulosic polysaccharide constituents are a mixture of structurally diverse pectic polysaccharides. The fractions extracted from the cell walls with cyclohexane-*trans*-1,2-diamine tetraacetate (CDTA) and Na_2CO_3 contain homogalacturonans (HGs) and rhamnogalacturonans (RGs) substituted with galactan and arabinogalactan (AG) side-chains having varying degrees of branching, while the guanidinium thiocyanate (GTC)- and KOH-soluble fractions contain more highly branched RGs (Dawson & Melton, 1991; Redgwell, Melton, & Brasch, 1988; Redgwell, Melton, Brasch, & Coddington, 1992). There is also evidence that rhamnogalacturonan II (RG II) is a component of green kiwifruit cell walls (Fischer, Wegryzn, Hallett, & Redgwell, 1996; Redgwell et al., 1988). Hemicellulosic polysaccharides, also present in the GTC- and KOH-soluble fractions, comprise a smaller portion of the green kiwifruit cell walls and are predominantly xyloglucan (XG), with minor amounts of glucuronoxylan and galactoglucomannan also present (Redgwell et al., 1988; Schröder et al., 2001). The cellulosic component of the cell walls includes both the I_α and I_β crystalline forms of cellulose, though the relative proportions of each are unknown (Newman & Redgwell, 2002). During ripening in green kiwifruit, large amounts of pectic polysaccharides are solubilized in the cell walls but little structural modification of these cell-wall components occurs (Redgwell, Melton, & Brasch, 1990; Redgwell, Melton, & Brasch, 1991; Redgwell, Melton, & Brasch, 1992). The cell-wall related changes that occur during kiwifruit softening, which include pectin solubilization, galactose loss and XG depolymerization, have been recently reviewed (Schröder & Atkinson, 2006). The changes that occur in the cell-wall polysaccharide composition of green kiwifruit during fruit growth and development have also been investigated (Gallego & Zarra, 1997; Li, Nakagawa, Nevins, & Sakurai, 2006). Air drying kiwifruit at various temperatures, as a means of preserving the fruit postharvest, has been found to promote the degradation of cell-wall polysaccharides, particularly the pectic polysaccharides (Femenia et al., 2009).

In contrast, there has been little research published on the cell-wall polysaccharides of gold kiwifruit. Makabe, Yoshioka, Miki, and Fukumoto (1998) investigated the changes which occur in the constituent sugar composition of cell-wall polysaccharides from gold and green kiwifruit during on-vine softening of fruit. The galactose and galacturonic acid contents of the gold kiwifruit differed from those of the green kiwifruit throughout the study, and the content of these monosaccharides decreased during on-vine softening of the gold fruit. However, they used a different variety of gold kiwifruit, *Actinidia chinensis* Planch. cv. Yellow Koshin, from that analysed in the present study. In a more recent study, Yuliarti

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et al. (2008) used different techniques to extract water-soluble polysaccharides (WSP) from the commercially important *A. chinensis* Planch. 'Hort 16A' (ZESPRI® GOLD).

The NZ kiwifruit industry generates a large proportion of gold fruit that are not suitable for either export or domestic food markets (Forgie, Giltrap, & Andrew, 2008). These 'reject' fruit are a potential source of biologically active polysaccharides (Hunter et al., 2008) and we have recently investigated the bioactive properties of AIR prepared from whole, unpeeled gold kiwifruit (unpublished results). In this present study we report on differences in the carbohydrate composition of AIRs prepared from whole unpeeled gold and green kiwifruit and on the structure of cell-wall polysaccharides isolated by sequential solvent extraction of the gold kiwifruit AIR.

2. Materials and methods

2.1. Materials

ZESPRI® GOLD kiwifruit (*A. chinensis* Planch. 'Hort16A'; gold kiwifruit) were harvested at maturity, in May 2007, from a commercial orchard in the Gisborne growing area of the East Coast, New Zealand, ripened to a ready-to-eat state (0.5–0.8 kgf; Hopkirk, Maindonald, & White, 1996) and stored frozen until analysis. Ripe green kiwifruit (*A. deliciosa*) were purchased from a local supermarket.

2.2. Preparation of an alcohol-insoluble residue (AIR) from gold and green kiwifruit

One quarter from each of four whole, unpeeled individual kiwifruit were combined and powdered in a mortar and pestle with liquid nitrogen. The ground material (30 g) was suspended in aqueous ethanol (100 mL, 70%, w/v) and stirred (4 h, 4 °C). The alcohol-insoluble residue (AIR) was collected on a sintered funnel and washed with ice cold ethanol (70%, w/v, 4× 100 mL), then acetone (4× 100 mL). The last ethanol and acetone washes each contained negligible amounts of carbohydrate as determined by colorimetric total carbohydrate assay (see Section 2.4).

2.3. Sequential extraction of polysaccharides from the AIR prepared from gold kiwifruit

A portion of AIR (0.85 g) from gold kiwifruit was sequentially extracted in duplicate (as a stirred suspension) with each of the following solvents (85 mL 2×): distilled water (1 h, 80 °C), CDTA (0.05 M, pH 6.5, 1 h, 40 °C), Na_2CO_3 (0.05 M containing 20 mM $NaBH_4$, 1 h, 40 °C, under $Ar_{(g)}$), KOH (1 M containing 20 mM $NaBH_4$, 1 h, 40 °C, under $Ar_{(g)}$), then KOH (4 M containing 20 mM $NaBH_4$, 1 h, 40 °C, under $Ar_{(g)}$). After each extraction, the solution was neutralised (acetic acid, 2 M), centrifuged (4000 rpm, 30 min, ambient temperature) and the supernatant was removed and filtered using a borosilicate microfiber filter (GD120, 70 mm, Advantec). Supernatants from duplicate extractions were combined, reduced in volume to ~25 mL by rotary evaporation (20 mbar, 40 °C) and dialysed exhaustively against distilled water (Medicell International Ltd, MWCO 12–14 kDa) with the exception of the CDTA extract that was first dialysed against NaCl (0.1 M) then against distilled water, and freeze-dried. The freeze-dried fractions were stored over silica gel, under vacuum, prior to weighing and analysis.

2.4. General analyses

Total carbohydrate was estimated colorimetrically by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using glucose (0–80 μg) as the standard. Total uronic acid was estimated colorimetrically by the *m*-hydroxydiphenyl method (Blumenkrantz & Asboe Hansen, 1973) using galacturonic acid (0–40 μg) as the standard. The amino acid composition was determined by the method of Boogers, Plugge, Stokkermans, and Duchateau (2008) using standard-H (Pears Biotech) and α-amino adipic acid as internal standard. Total protein was determined colorimetrically using the Bradford (1976) assay.

2.5. Constituent sugar analyses

The constituent neutral sugar compositions of AIRs from gold and green kiwifruit were determined by gas chromatography–mass spectrometry (GC–MS) after hydrolysis with TFA and derivatisation of the resulting neutral sugars to their corresponding alditol acetates (Blakeney, Harris, Henry, & Stone, 1983). Briefly, dried AIRs (~1 mg, with 100 μg *myo*-inositol as internal standard), were hydrolysed with aqueous TFA (2 M, 400 μL, 1 h, 120 °C), reduced to the alditols with $NaBH_4$ (2%, w/v, in water) and neutralised with NH_4OH (2 M, 0.4 mL). Acetylation gave the per-acetylated derivatives suitable for GC–MS analysis.

Portions of the AIRs from both fruit were hydrolysed with H_2SO_4 (Saeman, 1945). For this hydrolysis, samples (~10 mg, with 100 μg *myo*-inositol as internal standard) were dispersed in H_2SO_4 (72%, w/w), heated (2 h, 30 °C), diluted to one molar H_2SO_4 and the hydrolysis continued (3 h, 100 °C). The solutions were neutralised and derivatised following the same methodology as used for the TFA hydrolysis to generate alditol acetates.

Alditol acetates were separated by GC on a SGE BPX70 capillary column (25 m × 0.25 mm i.d., 0.25 μm film thickness) with the GC oven programmed from 70 °C (held for 1 min) to 140 °C at a rate of 25 °C min^{-1}, and then to 230 °C at a rate of 3 °C min^{-1} with analysis by MS using an Agilent 5973 Inert MSD. Identifications were based on peak retention times and on comparisons of electron impact spectra with the spectra obtained from reference compounds.

2.6. Glycosyl linkage analysis

Prior to glycosyl linkage analysis, uronic acid and methyl-esterified uronic acid residues were reduced using the two-step carboxyl reduction method of Kim and Carpita (1992) as described by Sims and Bacic (1995). Carboxyl-reduced samples (~1 mg) were methylated as described by Ciucanu and Kerek (1984), except that samples were dispersed in DMSO (0.5 ml) and heated (50 °C) overnight under $Ar_{(g)}$. After extraction into chloroform, the methylated polysaccharides were hydrolysed with TFA and the products were reduced and acetylated before analysis by GC–MS, as described above. Additional analysis was completed with an Agilent HP5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) as some partially methylated alditol acetate sugar derivatives were observed to co-elute on the SGE BPX70 column. Identifications were based on peak retention times and on comparisons of electron impact spectra with the spectra obtained from reference compounds.

3. Results and discussion

3.1. Composition of the AIRs from gold and green kiwifruit

The powdered gold and green kiwifruit were extracted with 70% ethanol to remove low molecular weight compounds, including mono- and oligosaccharides (Fry, 1988; Nunan, Sims, Bacic, Robinson, & Fincher, 1997). The extraction was performed at low temperature (4 °C) to minimise the activity of cell-wall degrading enzymes and the resulting residue was washed with cold ethanol (Nunan et al., 1997). During the extraction of tissues with organic solvent, cytoplasmic components such as proteins, starch

Table 1
Amino acid composition of the AIRs from gold and green kiwifruit.

Amino acid	Relative amount (mol%)	
	Gold fruit	Green fruit
Hyp	1.3	1.5
His	2.5	2.5
Ser	6.7	7.4
Arg	5.1	5.2
Gly	13.0	11.8
Asx	10.0	10.8
Glx	9.2	9.7
Thr	6.7	6.0
Ala	6.9	6.8
Pro	4.4	5.3
Lys	5.0	5.9
Tyr	4.7	3.1
Val	6.5	6.4
Ile	5.9	5.6
Leu	7.3	7.5
Phe	5.1	4.4

and polyphenols can co-precipitate with the cell walls (Selvendran, 1975; Selvendran & Ryden, 1990) and are not always removed by washing the cell-wall residue (Nunan et al., 1997). The yields of AIR from the gold and green fruit were 36.7 and 32.1 mg g^{-1} fresh weight, respectively.

The AIRs isolated from gold and green kiwifruit were analysed for protein content. Both samples contained only small amounts of water-soluble proteins (1.1% and 1.5%, w/w, respectively), as determined both colorimetrically and by amino acid analysis. The amino acid compositions of the gold and green kiwifruit AIRs were very similar (Table 1). These amino acid compositions were also similar to that reported previously for cell-wall materials isolated from *A. deliciosa* fruit tissues (Redgwell et al., 1988). The most abundant amino acids were glycine, asparagine/aspartic acid and glutamine/glutamic acid, and only the amounts of tyrosine and glycine varied by more than 1 mol% between the gold and green fruit varieties.

Colorimetric analysis estimated that the total carbohydrate content of both the gold and green kiwifruit AIRs was at least 80% (w/w). Comparative sugar analysis of the AIRs from each kiwifruit variety showed some clear differences (Table 2). The low overall sugar content of the AIRs shown in Table 2 is partly explained by differences in the rates of hydrolysis of different glycosidic linkages and the acid stability of the released monosaccharides. The yield of neutral monosaccharides is reduced if they are glycosidically linked to acid-resistant uronic acid residues (Melton & Smith, 2001). The estimated uronic acid content of the gold kiwifruit AIR was about one-third lower than that of the green fruit, indicating that the gold fruit contained less pectic polysaccharides than the green. In the TFA hydrolysate from the gold fruit, xylose was the most abundant neutral monosaccharide, followed by galactose and glucose, with small amounts of arabinose, mannose and rhamnose. By comparison, the TFA hydrolysate from the green fruit contained less xylose and slightly more of galactose, arabinose and rhamnose, which together with the higher uronic acid content is consistent with the observation that the green fruit contain a greater proportion of pectic polysaccharides than gold fruit. Previous studies have similarly shown that galactose and uronic acids are the major constituents of green kiwifruit cell walls (Fischer et al., 1996; Gallego & Zarra, 1997; Redgwell et al., 1988). Together with rhamnose and arabinose, these monomers are components of pectic polysaccharides (Mohnen, 2008), the predominant non-cellulosic polysaccharides found in green kiwifruit cell walls (Redgwell et al., 1988; Redgwell, Melton, Brasch, et al., 1992; Redgwell, Melton, & Brasch, 1992).

The hydrolysates from both kiwifruit varieties contained similar amounts of glucose, as determined by both TFA and sulfuric acid hydrolysis. In both sulfuric acid hydrolysates, glucose was the most abundant neutral monosaccharide, comprising 60–70% of the neutral sugars; in the TFA hydrolysates, glucose accounted for less than 20% of the neutral sugars, as cellulose is not hydrolysed under these conditions. A portion of the glucose detected in the TFA hydrolysates is derived from XGs, the major hemicellulosic polysaccharide components of kiwifruit cell walls (Redgwell et al., 1988), although some may also be derived from starch. Kiwifruit can contain large quantities of starch, particularly the unripe fruit (Schröder et al., 2001). The amount of glucose detected in the TFA hydrolysates indicates gold and green fruit used in the present study contained relatively small amounts of starch. No attempt was made to remove starch from the AIRs because of the possibility of removing other cell-wall polysaccharides, particularly xylans, when extracting with DMSO (Bian, Peng, Xu, Sun, & Kennedy, 2010; Teleman, Tenkanen, Jacobs, & Dahlman, 2002) or water-soluble cell-wall derived polysaccharides during enzymic digestion of starch in aqueous solutions.

Linkage analysis showed that the glycosidic linkages between the monosaccharide residues in the polysaccharides of the gold and green kiwifruit AIRs were similar, suggesting that the same types of polysaccharides were present in both varieties (Table 3). The most abundant glycosyl linkage in the AIR from both varieties was 4-linked glucopyranosyl (4-Glc*p*), with 4-linked galacturonic acid (4-GalA*p*) and 4-linked xylopyranosyl (4-Xyl*p*) the next most abundant linkages. The gold fruit contained a higher proportion of 4-Xyl*p* residues than green fruit, while the green fruit contained more 4-GalA*p* residues than gold. The differences in the glycosyl linkage compositions of the AIRs further highlighted the differences observed in the sugar compositions between the two kiwifruit varieties and indicated that the gold fruit contained a higher proportion of hemicellulosic polysaccharides and lower proportion of pectic polysaccharides compared with the green.

3.2. Sequential fractionation and polysaccharide composition of gold kiwifruit AIR

In order to characterise the structure of the polysaccharides present in gold kiwifruit the AIR was fractionated by sequential chemical extraction. The green fruit AIR was not fractionated, due to the extensive existing literature on green kiwifruit cell-wall polysaccharides. The total recovery of material from the sequential fractionation process was 86% (w/w). The water (14%), CDTA

Table 2
Monosaccharide composition of the AIRs from gold and green kiwifruit.

Sample	Monosaccharide composition (wt%)[a]								
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	Total
Gold	0.2 (tr)	–	1.1 (0.4)	8.6 (5.6)	0.2 (0.7)	3.5 (2.8)	2.8 (21.6)	21.0	37.4 (52.1)
Green	0.7 (0.3)	tr (tr)	1.7 (1.1)	3.6 (3.8)	0.5 (1.5)	5.4 (4.9)	2.7 (20.9)	29.0	43.6 (61.5)

–: Not detected; tr: trace.

[a] Monosaccharides recovered from TFA hydrolysis (values in parentheses are monosaccharides recovered from Saeman hydrolysis). Uronic acids determined colorimetrically. Values are averages of duplicate determinations.

Table 3
Glycosyl linkage composition of AIRs from gold and green kiwifruit and fractions obtained from the gold kiwifruit.

Sugar	Deduced linkage[a]	Relative amount (mol%)[b]							
		Green fruit	Gold fruit	Water	CDTA	Na_2CO_3	1 M KOH	4 M KOH	Residue
Rha	t*p*	–	–	0.4	0.3	0.3	–	–	–
	2*p*	0.4	1.4	2.2	2.9	6.4	–	–	–
	2,4*p*	–	1.0	1.9	0.9	4.7	–	–	–
Fuc	t*p*	–	–	0.4	0.6	0.2	0.6	0.5	–
Ara	t*f*	2.4	3.3	8.2	3.6	4.4	0.2	–	0.5
	2*f*	–	0.1	0.2	0.8	0.3	–	–	–
	5*f*	–	1.2	3.0	0.9	4.4	–	–	–
	2,5*f*	2.5	–	–	–	–	–	–	–
	2,3,5*f*	–	–	–	3.5	0.8	–	–	–
Xyl	t*p*	1.2	2.2	1.6	1.4	1.3	4.3	6.0	–
	2*p*	0.8	1.8	0.9	0.4	0.4	7.2	9.0	–
	4*p*	5.7	7.9	2.5	2.3	1.8	54.5	34.1	2.4
	2,4*p*	–	0.7	0.2	–	–	3.3	1.2	–
	2,3,4*p*	0.4	–	–	–	–	–	–	14.2
Man	t*p*	–	0.9	0.2	0.2	1.1	–	–	–
	4*p*	1.6	5.6	0.7	3.8	2.9	1.3	3.6	1.0
	4,6*p*	–	0.6	0.2	–	–	0.3	2.5	–
Glc	t*p*	1.8	–	1.1	1.2	3.7	0.7	1.4	0.5
	3*p*	–	–	0.2	–	–	–	–	–
	4*p*	56.0	51.5	9.2	9.4	6.6	15.3	17.8	75.7
	2,4*p*	–	–	–	–	–	–	–	0.4
	3,4*p*	0.2	–	0.1	–	–	–	–	0.6
	4,6*p*	3.2	6.7	2.8	0.7	0.5	7.1	17.2	1.1
	3,4,6*p*	0.1	–	–	–	–	–	–	0.4
	2,3,4,6*p*	0.2	0.8	0.1	–	0.1	–	–	3.1
Gal	t*p*	1.5	2.4	3.3	1.4	3.1	1.1	2.9	–
	2*p*	–	0.6	0.5	–	–	0.8	1.9	–
	3*p*	–	–	1.5	–	2.4	–	–	–
	4*p*	6.7	0.5	0.5	1.2	12.9	0.4	0.3	0.3
	6*p*	1.6	–	2.0	0.4	0.3	–	–	–
	2,3*p*	–	–	0.3	–	–	–	–	–
	2,4*p*	–	–	0.2	0.4	0.7	–	–	0.4
	3,4*p*	0.2	–	1.6	1.4	4.1	–	–	–
	3,6*p*	1.0	2.0	7.3	1.2	1.1	–	–	–
	4,6*p*	–	–	0.1	0.1	1.5	0.2	–	–
	2,3,4,6*p*	1.5	0.5	1.7	1.5	3.1	–	–	0.1
GlcA	tA*p*	–	–	–	–	–	2.6	1.5	–
GalA	4A*p*	10.9(53.7)	6.8(40.7)	44.4(45.9)	62.5(66.3)	28.4(2.2)	–	–	–
	2,4A*p*	–	–	0.3	–	0.6(4.9)	–	–	–
	4,6A*p*	–	–	0.8(51.0)	1.1(80.0)	1.7(6.1)	–	–	–

–: Not detected.
[a] Terminal Rha*p* deduced from 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tetra-*O*-methylrhamnitol, etc.
[b] Values are averages of duplicate determinations (values in parentheses are degree of methylesterification).

(12%) and Na_2CO_3 (9%) fractions accounted for about one-third of the extracted material, whereas the 1 M (7%) and 4 M KOH (4%) fractions accounted for only 11% of the extracted material. Approximately half of the AIR remained as an insoluble residue after the sequential extraction procedure. Colorimetric analysis estimated the uronic acid content of the water and CDTA fractions to be more than 45% (w/w), whereas that of the Na_2CO_3 fraction was 18%, that was consistent with the presence of pectic polysaccharides. The high uronic acid content was similar to that shown previously for water extracts of gold kiwifruit (Yuliarti et al., 2008). Colorimetric analysis estimated the uronic acid content of the KOH fractions and the insoluble residue at only 3%.

Glycosyl linkage analysis showed that the most abundant glycosyl linkage in the water, CDTA and Na_2CO_3 fractions was 4-GalA*p* (Table 3). Together with the smaller proportions of 2-Rha*p* and 2,4-Rha*p* also found in these fractions, these results are consistent with the presence of HG and pectic polysaccharides with a RG backbone (Mohnen, 2008). The Na_2CO_3 fraction contained higher proportions of 2- and 2,4-Rha*p* and a lower proportion of 4-GalA*p* than the water or CDTA fractions, indicating the RG extracted under these conditions was more heterogeneous and highly branched. The Na_2CO_3 fraction also contained a high proportion of 4-Gal*p* that, together with the presence of 3,4- and 4,6-Gal*p*, is consistent with the presence of Type I AGs which are neutral side-chains on the RG backbone of pectic polysaccharides (Mohnen, 2008). The 4-GalA*p* linkages in the Na_2CO_3 fraction had a lower degree of methyl esterification than the GalA*p* linkages in the water and CDTA fractions (Table 3), which may be partially due to saponification that can occur under basic conditions (Renard & Thibault, 1996). The Na_2CO_3 fraction also contained a small proportion of 5-Ara*f* which is consistent with the presence of neutral arabinan side-chains on the pectic polysaccharides. In addition to the deduced HG and RG linkages, the water fraction also contained relatively high proportions of 3,6-Gal*p* and terminal Ara*f* that, together with the occurrence of 3- and 6-Gal*p*, are typical of Type II AGs, which are often associated with protein and present as arabinogalactan-proteins (AGPs). The presence of small proportions of 2,4-Gal*p* in the water, CDTA and Na_2CO_3 fractions indicates that these fractions may contain some RG II (Bacic, Harris, & Stone, 1988). The predom-

Table 4
Estimated polysaccharide compositions of the fractions obtained from gold kiwifruit AIR.

Polysaccharides	Composition (mol%)[a]					
	Water	CDTA	Na_2CO_3	KOH (1 M)	KOH (4 M)	Residue
(Rhamno)-galacturonan	49	66	40	–	–	–
Type I (arabino)galactan	4	4	24	1	tr	tr
Type II arabinogalactan	18	3	5	–	–	–
Arabinan	3	1	4	–	–	–
Xyloglucan	9	3	3	23	41	2
Xylan	3	2	2	61	37	2
Mannan	1	4	3	2	9	1
Cellulose/starch	8	9	6	13	12	75
Other[b]	5	8	13	0	1	20

–: Not detected; tr: trace.
[a] mol% of total polysaccharide present in the fractions, calculated from the mol% of methylated alditol acetates characteristic of polysaccharides.
[b] Glycosyl linkages that cannot be assigned to well-defined cell-wall polysaccharides.

inance of pectic polysaccharides in the water, CDTA and Na_2CO_3 fractions of the gold kiwifruit AIR is consistent with the literature; CDTA and Na_2CO_3 are routinely used to extract non-covalently and covalently bound pectic polysaccharides, respectively, from plant cell walls (Nunan, Sims, Bacic, Robinson, & Fincher, 1998; Redgwell & Selvendran, 1986; Redgwell et al., 1988). The increased water solubility of pectic polysaccharides during kiwifruit development is also well documented (Gallego & Zarra, 1997; Redgwell et al., 1990; Redgwell, Melton, & Brasch, 1992). The other linkage present in the water, CDTA and Na_2CO_3 fractions in proportions >5 mol%, was 4-Glc*p*, derived from starch present in the original AIR, or from cellulose.

The most abundant glycosyl linkage in both the 1 and 4 M KOH fractions was 4-Xyl*p* (Table 3). Together with the occurrence of smaller proportions of 2,4-Xyl*p* and terminal GlcA*p* and Ara*f*, this is consistent with the presence of acidic xylans. These two fractions also contained 4,6-Glc*p*, terminal and 2-Xyl*p*, 2-Gal*p* and terminal Fuc*p* that are typical of XGs. In addition, the 4 M KOH fraction contained small proportions of galactomannan type linkages, 4-Man*p*, 4,6-Man*p* and t-Gal*p*. Considerable proportions of 4-Glc*p* were also found in both the 1 and 4 M KOH fractions. While a some of this 4-Glc*p* is derived from XGs, it is unclear whether the remainder is derived from cellulose or starch. The insoluble residue contained mostly 4-Glc*p* and was thus deduced to be predominantly composed of cellulose (Table 3). The other most abundant linkage in the residue was 2,3,4-Xyl*p* (14 mol%), which was probably derived from undermethylation of xylan.

The proportions of various polysaccharides present in the fractions prepared from the gold kiwifruit AIR (Table 4) were deduced from the linkage compositions (Table 3) by totalling the proportions of individual glycosyl linkage residues that are characteristic of well-defined cell-wall polysaccharides already described in the literature (Bacic et al., 1988; Gorshkova et al., 1996; Nunan et al., 1997, 1998). This requires making a number of assumptions about the structures of polysaccharides but these calculations are widely used to estimate the proportions of different types of polysaccharides present in cell-wall samples (Nunan et al., 1998; Shea, Gibeaut, & Carpita, 1989). The values in Table 4 are expressed as mol% of total polysaccharide present in the fractions; other cell-wall and cytoplasmic components have not been included. The HG + RG content was estimated by adding the following glycosyl linkage residues: 4-GalA*p*, 2-Rha*p*, 2,4-Rha*p* and 2,4-GalA*p*. The Type I AG content was estimated by adding: 4-Gal*p*, 3,4-Gal*p*, 4,6-Gal*p*, and/or terminal Gal*p*/terminal Ara*f* (equal to 3,4-Gal*p* + 4,6-Gal*p*). The Type II AG content was estimated by adding: 3,6-Gal*p*, 3-Gal*p*, 6-Gal*p*, and terminal Ara*f* (equal to 3,6-Gal*p*). The arabinan content was estimated from the amount of 5-Ara*f*. The XG content was estimated by adding: 4,6-Glc*p*, 4-Glc*p* (equal to one-third of the 4,6-Glc*p*), terminal Xyl*p*, 2-Xyl*p*, terminal Gal*p*, 2-Gal*p*, and terminal Fuc*p*. It was assumed that most of the remaining 4-Glc*p* was derived from cellulose, with a portion probably derived from starch. The xylan content was estimated by adding 4-Xyl*p*, 2,4-Xyl*p*, and/or terminal Ara*f*/terminal GlcA*p* (equal to 2,4-Xyl*p*). The mannan content was estimated by adding: 4-Man*p*, 4,6-Man*p*, and terminal Gal*p* (equal to 4,6-Man*p*).

The differences between the extracted fractions of the gold fruit are logically related to their dissolution properties. Pectic polysaccharides were the predominant components (73–74 mol%) in the water, CDTA and Na_2CO_3 fractions (Table 4). While the CDTA fraction contained mostly HG and RG with some neutral pectic side-chains (8 mol% of type I AG, type II AG and arabinan), the water and Na_2CO_3 fractions contained less HG and RG and higher proportions of neutral side-chains (25 and 33 mol%, respectively). The water fraction contained type II AGs (18 mol%), present either as neutral pectic side-chains or as AGP, whereas the predominant neutral pectic side-chains in the Na_2CO_3 fraction were type I AGs (24 mol%) (Mohnen, 2008). Small amounts (1–9 mol%) of XG, xylan and mannan were also detected in the water, CDTA and Na_2CO_3 fractions (Table 4). The 1 M KOH and 4 M KOH fractions comprised 84 and 78 mol% XGs and xylans, respectively. The 1 M KOH fraction was comprised predominantly of xylan (61 mol%) while the 4 M KOH fraction contained approximately equal amounts of xylan (37 mol%) and XG (41 mol%). Some of the cellulose or starch identified in these fractions, may represent XG with longer unsubstituted regions. The residue, as expected, contained mostly cellulose. Small amounts ($\leq$2 mol%) of XG, xylan and mannan were also detected in the residue (Table 4). Of the glycosyl linkages from the insoluble residue, 20 mol% could not be assigned to well-defined cell-wall polysaccharides, but was probably derived from xylan, strongly bound to cellulose.

4. Conclusions

The polysaccharide composition of gold kiwifruit has been reported for the first time and comparison with green kiwifruit has shown that gold fruit contain proportionally more hemicelluloses and less pectic polysaccharides than green fruit. Sequential fractionation of the gold fruit indicated that the types of polysaccharides from this variety was similar to those of green fruit. The water, CDTA and Na_2CO_3 fractions contained mostly pectic polysaccharides, consisting of HG + RGs and variable amounts of type I AG. The Na_2CO_3 fraction contained a much higher amount of type I AG than either the water or the CDTA fractions. In addition to soluble HG and RG, the water fraction also contained a considerable amount of type II AG and some water-soluble XG. The 1 and 4 M KOH fractions contained mostly acidic xylan and XG, with the proportion of xylan much higher for the 1 M KOH fraction than the 4 M KOH fraction. Conversely, the proportion of XG was

almost two times in the 4 M KOH fraction than in the 1 M KOH fraction.

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References

Bacic, A., Harris, P. J., & Stone, B. A. (1988). Structure and function of plant cell walls. In J. Preiss (Ed.), *The biochemistry of plants*. New York: Academic Press, pp. 297–371.

Bian, J., Peng, F., Xu, F., Sun, R.-C., & Kennedy, J. F. (2010). Fractional isolation and structural characterization of hemicelluloses from *Caragana korshinskii*. *Carbohydrate Polymers*, *80*, 753–760.

Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research*, *113*, 291–299.

Blumenkrantz, N., & Asboe Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.

Boogers, I., Plugge, W., Stokkermans, Y. Q., & Duchateau, A. L. L. (2008). Ultra-performance liquid chromatographic analysis of amino acids in protein hydrolysates using an automated pre-column derivatisation method. *Journal of Chromatography A*, *1189*, 406–409.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*, *72*, 248–254.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, *131*, 209–217.

Dawson, D. M., & Melton, L. D. (1991). Two pectic polysaccharides from kiwifruit cell walls. *Carbohydrate Polymers*, *15*, 1–11.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Femenia, A., Sastre-Serrano, G., Simal, S., Garau, M. C., Eim, V. S., & Rossello, C. (2009). Effects of air-drying temperature on the cell walls of kiwifruit processed at different stages of ripening. *LWT-Food Science and Technology*, *42*, 106–112.

Fischer, M., Wegryzn, T. F., Hallett, I. C., & Redgwell, R. R. (1996). Chemical and structural features of kiwifruit cell walls: Comparison of fruit and suspension-cultured cells. *Carbohydrate Research*, *295*, 195–208.

Forgie, V., Giltrap, D., & Andrew, R. (2008). *Life cycle assessment of producing biogas from waste kiwifruit*. Palmerston North, NZ: New Zealand Centre for Ecological Economics and Landcare Research., 32 pp.

Fry, S. C. (1988). *The growing plant cell wall: Chemical and metabolic analysis*. New Jersey: The Blackburn Press.

Gallego, P. P., & Zarra, I. (1997). Changes in cell wall composition and water-soluble polysaccharides during kiwifruit development. *Annals of Botany*, *79*, 695–701.

Gorshkova, T. A., Wyatt, S. E., Salnikov, V. V., Gibeaut, D. M., Ibragimov, M. R., Lozovaya, V. V., et al. (1996). Cell-wall polysaccharides of developing flax plants. *Plant Physiology*, *110*, 721–729.

Hopkirk, G., Maindonald, J. H., & White, A. (1996). Comparison of four new devices for measuring kiwifruit firmness. *New Zealand Journal of Crop and Horticultural Science*, *24*, 273–286.

Hunter, D. C., Denis, M., Parlane, N. A., Buddle, B. M., Stevenson, L. M., & Skinner, M. A. (2008). Feeding ZESPRI™ GOLD kiwifruit puree to mice enhances serum immunoglobulins specific for ovalbumin and stimulates ovalbumin-specific mesenteric lymph node cell proliferation in response to orally administered ovalbumin. *Nutrition Research*, *28*, 251–257.

Kim, J. B., & Carpita, N. C. (1992). Changes in esterification of the uronic acid groups of cell wall polysaccharides during elongation of maize coleoptiles. *Plant Physiology*, *98*, 646–653.

Li, X., Nakagawa, N., Nevins, D. J., & Sakurai, N. (2006). Changes in the cell-wall polysaccharides of outer pericarp tissues of kiwifruit during development. *Plant Physiology and Biochemistry*, *44*, 115–124.

Makabe, T., Yoshioka, H., Miki, A., & Fukumoto, M. (1998). Changes in cell wall polysaccharides in kiwifruit (*Actinidia chinensis* Planch. cv. Yellow Koshin) during softening on the vine. *Journal of the Japanese Society for Horticultural Science*, *67*, 59–65.

Melton, L. D., & Smith, B. G. (2001). Factors to consider when estimating water vapor pressure. *Current Protocols in Food Analytical Chemistry*, E3.2.1–E3.2.13.

Mohnen, D. (2008). Pectin structure and biosynthesis. *Current Opinion in Plant Biology*, *11*, 266–277.

Newman, R. H., & Redgwell, R. J. (2002). Cell wall changes in ripening kiwifruit: ^{13}C solid state NMR characterisation of relatively rigid cell wall polymers. *Carbohydrate Polymers*, *49*, 121–129.

Nunan, K. J., Sims, I. M., Bacic, A., Robinson, S. P., & Fincher, G. B. (1997). Isolation and characterization of cell walls from the mesocarp of mature grape berries (*Vitis vinifera*). *Planta*, *203*, 93–100.

Nunan, K. J., Sims, I. M., Bacic, A., Robinson, S. P., & Fincher, G. B. (1998). Changes in cell wall composition during ripening of grape berries. *Plant Physiology*, *118*, 783–792.

Redgwell, R. J., & Selvendran, R. R. (1986). Structural features of cell-wall polysaccharides of onion *Allium cepa*. *Carbohydrate Research*, *157*, 183–199.

Redgwell, R. J., Melton, L. D., & Brasch, D. J. (1988). Cell-wall polysaccharides of kiwifruit (*Actinidia deliciosa*): Chemical features in different tissue zones of the fruit at harvest. *Carbohydrate Research*, *182*, 241–258.

Redgwell, R. J., Melton, L. D., & Brasch, D. J. (1990). Cell wall changes in kiwifruit following post harvest ethylene treatment. *Phytochemistry*, *29*, 399–407.

Redgwell, R. J., Melton, L. D., & Brasch, D. J. (1991). Cell-wall polysaccharides of kiwifruit (*Actinidia deliciosa*): Effect of ripening on the structural features of cell-wall materials. *Carbohydrate Research*, *209*, 191–202.

Redgwell, R. J., Melton, L. D., Brasch, D. J., & Coddington, J. M. (1992). Structures of the pectic polysaccharides from the cell walls of kiwifruit. *Carbohydrate Research*, *226*, 287–302.

Redgwell, R. J., Melton, L. D., & Brasch, D. J. (1992). Cell wall dissolution in ripening kiwifruit (*Actinidia deliciosa*): Solubilization of the pectic polymers. *Plant Physiology*, *98*, 71–81.

Renard, C. M. G. C., & Thibault, J. F. (1996). Degradation of pectins in alkaline conditions: Kinetics of demethylation. *Carbohydrate Research*, *286*, 139–150.

Saeman, J. F. (1945). Kinetics of wood saccharification. *Industrial and Engineering Chemistry*, *37*, 43–52.

Schröder, R., & Atkinson, R. G. (2006). Kiwifruit cell walls: Towards an understanding of softening. *New Zealand Journal of Forestry Science*, *36*, 112–129.

Schröder, R., Nicolas, P., Vincent, S. J. F., Fischer, M., Reymond, S., & Redgwell, R. J. (2001). Purification and characterisation of a galactoglucomannan from kiwifruit (*Actinidia deliciosa*). *Carbohydrate Research*, *331*, 291–306.

Shea, E. M., Gibeaut, D. M., & Carpita, N. C. (1989). Structural analysis of the cell walls regenerated by carrot protoplasts. *Planta*, *179*, 293–308.

Sims, I. M., & Bacic, A. (1995). Extracellular polysaccharides from suspension cultures of *Nicotiana plumbaginifolia*. *Phytochemistry*, *38*, 1397–1405.

Selvendran, R. R. (1975). Analysis of cell wall material from plant tissues: Extraction and purification. *Phytochemistry*, *14*, 1011–1017.

Selvendran, R. R., & Ryden, P. (1990). Isolation and analysis of plant cell walls. In P. M. Dey (Ed.), *Methods in plant biochemistry*. London: Academic Press, pp. 549–579.

Teleman, A., Tenkanen, M., Jacobs, A., & Dahlman, O. (2002). Characterization of *O*-acetyl-(4-*O*-methylglucurono)xylan isolated from birch and beech. *Carbohydrate Research*, *337*, 373–377.

Yuliarti, O., Goh, K., Matia-Merino, L., Mawson, J., Drummond, L., & Brennan, C. S. (2008). Effect of extraction techniques and conditions on the physicochemical properties of the water soluble polysaccharides from gold kiwifruit (*Actinidia chinensis*). *International Journal of Food Science and Technology*, *43*, 2268–2277.