

Contents lists available at [SciVerse ScienceDirect](http://www.sciencedirect.com)

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchemEffects of simulated digestion *in vitro* on cell wall polysaccharides from kiwifruit (*Actinidia* spp.)Susan M. Carnachan^a, Tracey J. Bootten^a, Suman Mishra^b, John A. Monro^b, Ian M. Sims^{a,*}^a Industrial Research Limited, P.O. Box 31-310, Lower Hutt 5040, New Zealand^b The New Zealand Institute for Plant and Food Research Limited, Fitzherbert Science Centre, Private Bag 11600, Palmerston North 4442, New Zealand

ARTICLE INFO

Article history:

Received 8 December 2011

Received in revised form 19 December 2011

Accepted 21 December 2011

Available online xxxxx

Keywords:

Kiwifruit

*Actinidia chinensis**Actinidia deliciosa**in vitro* digestion

Polysaccharides

Structural analysis

ABSTRACT

Cell wall polysaccharides are resistant to digestion and absorption in the human small intestine and are considered to be delivered to the colon in a chemically unaltered state. In this paper, pulp from green and gold kiwifruit was subjected to *in vitro* upper-intestinal tract digestion and the chemical and physical changes to cell wall polysaccharides (dietary fibre) were investigated. Yields of insoluble fibre decreased slightly with simulated digestion while soluble fibre yields increased. Constituent sugar and glycosyl linkage analysis of the soluble and insoluble fibre fractions revealed that the chemical composition and structure of the non-starch polysaccharides remained largely unchanged. However, the degree of methylesterification of galacturonic acid residues present in the pectin-rich soluble fibre fractions of both fruit decreased with treatment; size-exclusion chromatography detected changes in the molecular weight profiles of these fractions. These changes may affect the physicochemical properties and fermentability of kiwifruit dietary fibre in the large intestine.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Kiwifruit contain about 2–3% non-starch polysaccharides (Ferguson & Ferguson, 2003) that make up the fruit cell walls and are considered a good source of both soluble and insoluble dietary fibre. Both green (*Actinidia deliciosa* [A. Chev.] C.F. Liang and A.R. Ferguson) and gold (*Actinidia chinensis* Planch.) kiwifruit contain pectic polysaccharides, hemicelluloses and cellulose in varying proportions (Dawson & Melton, 1991; Redgwell, Melton, & Brasch, 1988; Redgwell, Melton, & Brasch, 1992; Sauvageau, Hinkley, Carnachan, & Sims, 2010; Schröder, Nicolas, Vincent, Fischer, Reymond, & Redgwell, 2001). The major pectic polysaccharides include homogalacturonans and rhamnogalacturonans substituted with galactan and arabinogalactan side-chains, while the hemicellulosic polysaccharides include xyloglucan, glucuronoarabinoxylan and galacto-(gluco)-mannan.

The polysaccharides of plant cell walls are resistant to digestion by human enzymes in the small intestine and are delivered to the colon in what has been assumed to be a chemically unaltered state, where they are fermented by intestinal microbiota. However, there are very few data confirming the validity of this assumption. Whether or not chemical or structural changes occur when fruit cell walls are exposed to gastric acidity, followed by an influx of alkali during entry into the small intestine is uncertain, but important, be-

cause even minor chemical or structural changes in polysaccharides can substantially change the physicochemical properties that determine their impact on health. For instance, the viscosity of polysaccharides depends on the logarithm of chain length, so a single chain cleavage could greatly affect their digesta properties. Even under neutral conditions pectins can undergo depolymerisation by β -elimination (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995).

There are some reports detailing the amount of dietary fibre reaching the terminal ileum of human subjects. For example, Englyst and Cummings (1985 & 1987) found more than 90% of the non-starch polysaccharides of some cereals and potatoes were recovered in the ileostomy fluid, whereas most of the starch was digested. Similarly, Saito et al. (2005) showed that about 90% of the pectin fed to male volunteers reached the terminal ileum. However, the methods used for measuring dietary fibre and total pectin could not detect the subtle changes that may, nonetheless, alter polysaccharide structure and physical properties.

Methods for studying carbohydrate digestion *in vivo* are, generally, time-consuming and costly for routine analyses of digestion products. There are a large number of methods for simulating human carbohydrate digestion *in vitro* that vary in complexity (Woolnough, Monro, Brennan, & Bird, 2008). The TNO-Intestinal model of the stomach and small intestine is perhaps the most elaborate and enables manipulation of many parameters, including regulation of gastric and intestinal pH, flow of gastric and pancreatic juice including digestive enzymes, peristalsis for mixing, gastrointestinal transit times and continuous removal of digested

* Corresponding author. Tel.: +64 4 9313000; fax: +64 4 9313055.

E-mail address: i.sims@irl.cri.nz (I.M. Sims).

compounds (Minekus, Marteau, Havenaar, & Huis in't Veld, 1995). Monro, Mishra, and Venn (2010) have used a much simpler model that allows multiple treatments to be conducted in parallel, to monitor the digestibility of foods. This model is appropriate for an initial investigation of the effects of exposing fruit polysaccharides to conditions that would be typically encountered *in vivo*. In this study we have subjected green and gold kiwifruit to a similar *in vitro* digestion and report on the physical and chemical changes that occur in the cell wall polysaccharides.

2. Materials and methods

2.1. Materials

ZESPRI® GREEN (*Actinidia deliciosa* 'Hayward'; green kiwifruit) and ZESPRI® GOLD kiwifruit (*Actinidia chinensis* 'Hort16A'; gold kiwifruit) were harvested at maturity, in May 2010, from a commercial orchard in the Gisborne growing area of the East Coast, New Zealand, and ripened to a ready-to-eat state (0.5–0.8 kgf; Hopkirk, Maindonald, & White, 1996) under ambient conditions. The fruit were peeled, pulped by briefly blending in a laboratory blender (waring) on a low speed until all lumps of intact tissue had been dispersed but the seeds remained intact, and stored frozen until use.

2.2. *In vitro* digestion of kiwifruit

Kiwifruit pulp samples were subjected to *in vitro* digestion based on that described by Monro et al. (2010), but scaled up to 200 g of kiwifruit pulp. Triplicate digestions were carried out in 500-mL beakers placed in a trough on a magnetic stirrer and maintained at 37 °C using a flow of warm water. Samples (200 g) of kiwifruit pulp were weighed accurately into the digestion beakers, and then subjected to one of three treatments (Fig. 1):

- (1) Water: As the non-starch polysaccharide concentration in the blended pulp is about 5%, the soluble fraction in the pulp was deemed to be the water-soluble fraction for analysis.
- (2) "Gastric": The kiwifruit pulp was adjusted to pH 2.5 by addition of 1 M HCl with stirring. Pepsin (Sigma–Aldrich, P7000) solution consisting of 1 g pepsin dissolved in 10 mL of 50 mM HCl was added, and the pulp digested for 30 min with constant stirring.
- (3) "Gastrointestinal (= gastric plus small-intestinal digestion)": A sample of kiwifruit pulp was subjected to the aforementioned gastric digestion and digestion continued into an ileal phase by adding 2 M NaOH with stirring until the pH had increased to 6.5. Pancreatin (Sigma–Aldrich P7545) solution (5% in maleate buffer pH 6.5, 20 mL) was added, followed by amyloglucosidase (Megazyme E-AMGDF, 0.5 mL) and the samples stirred for 120 min.

The water, "gastric" and "gastrointestinal" samples were transferred to 50-mL Falcon tubes and centrifuged (3000g, 15 min). Residues from the water and gastrointestinal treatments were washed with water (50 mL × 3), whereas residues from the gastric treatment were washed with water acidified to pH 2.5 to maintain the gastric pH. The supernatants and washes, within each treatment, were pooled, as were the residues within each treatment, providing a bulked residue and a bulked extract for each of the water, gastric and gastrointestinal treatments. To each of the pooled extracts, four volumes of ethanol were added; the extracts were mixed, covered and allowed to stand overnight at 20 °C. The samples were centrifuged to pellet the water-soluble but 80% ethanol-insoluble polymers and the supernatants removed and

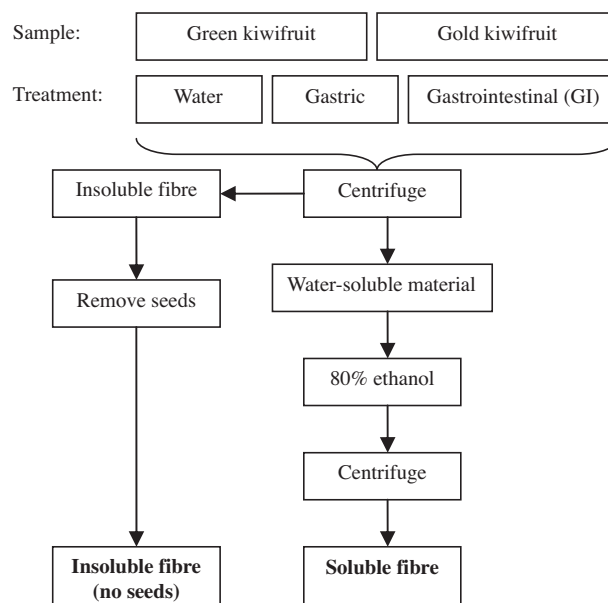


Fig. 1. *In vitro* digestion and extraction of kiwifruit.

retained. The pellets were redissolved in distilled water and freeze-dried to give friable soluble fibre fractions.

The bulked residues were frozen and freeze dried to give the water-insoluble fibre fractions. The seeds were removed from representative samples of these fractions, as they were considered to pass through the gastrointestinal tract intact, and found to constitute 40% and 18% w/w of the water-insoluble fractions from green and gold kiwifruit, respectively. The samples of insoluble fibre (no seeds) were ground to a fine powder in liquid nitrogen and used for all analyses discussed below.

2.3. Colorimetric analyses

Total carbohydrate was measured colorimetrically by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using glucose (0–80 µg) as the standard. Total uronic acid was measured colorimetrically by the *m*-hydroxydiphenyl method (Blumenkrantz & Asboe Hansen, 1973), after first hydrolysing the samples in sulphuric acid (Smith & Harris, 1995), using galacturonic acid (0–12.5 µg) as the standard.

2.4. Reduction of uronic acid residues

For constituent sugar and glycosyl linkage analyses of the soluble fibre fractions, 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). Briefly, samples (20 mg) were dissolved in 500 mM imidazole–HCl buffer (20 mL, pH 8) and esterified uronic acids reduced with NaBD₄. Excess NaBD₄ was destroyed with acetic acid and the samples dialysed (molecular weight cut-off 2 kDa) for 24 h against distilled H₂O and freeze dried. Samples were split into two aliquots, dissolved in distilled H₂O (1 mL) and 200 mM MES–KOH (200 µL, pH 4.75) and, following activation with 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide-metho-*p*-toluenesulfonate (carbodiimide reagent), free uronic acid residues were reduced with either NaBD₄ (for analysis of total uronic acids) or NaBH₄ (to yield the proportion of esterified uronic acids compared to the total uronic acids). Excess reductant was then destroyed

with acetic acid and the samples dialysed (molecular weight cut-off 2 kDa) extensively against distilled H₂O and freeze dried.

2.5. Constituent sugar analyses

The neutral constituent sugar compositions of the insoluble fibre fractions were determined by gas chromatography-mass spectrometry (GC–MS) of alditol acetate derivatives after hydrolysis of the polysaccharides present to their component monosaccharides, using a two-step hydrolysis procedure. In the first step, non-cellulosic polysaccharides were hydrolysed with 2 M TFA, and in the second step, a two-stage sulphuric acid hydrolysis was used to hydrolyse remaining polysaccharides, mostly cellulose (Melton & Smith, 2001).

Samples (5 mg, in duplicate) were first hydrolysed with TFA (2 M, 500 μ L, containing 0.4 mg/mL *myo*-inositol, 1 h, 120 °C). After cooling, 2 mL methanol were added to each sample, the tubes were centrifuged (1120g, 5 min) and the soluble TFA hydrolysates removed to a clean tube. This procedure was repeated and the two supernatants from each sample were combined and evaporated to dryness in a stream of air. Toluene (500 μ L) was added to the dried TFA hydrolysates and the resolubilised samples were dried in a stream of air. Ammonium hydroxide (2 M, 200 μ L) was added to all tubes to ensure that the solutions were alkaline during the reduction (Albersheim, Nevins, English, & Karr, 1967).

The TFA-insoluble material was dried under a stream of air to remove residual methanol, *myo*-inositol (25 μ L, 20 mg mL^{−1}) added as an internal standard and the samples were freeze dried. Samples were dissolved in sulphuric acid (125 μ L, 72% w/w) and incubated (3 h, 30 °C); then the acid was diluted to 1 M by addition of water (1.37 mL) and the polysaccharides hydrolysed by incubating at 100 °C for a further 3 h. Following hydrolysis, the solutions were neutralised by adding ammonia (15 M, 350 μ L), filtered (0.22 μ m) to remove insoluble material, and an aliquot (200 μ L) of the filtered hydrolysate was reduced and acetylated.

The neutralised TFA and sulphuric acid hydrolysates were reduced with 2 M NaBH₄ in 2 M NH₄OH (200 μ L) for 60 min at 60 °C. The reaction was stopped by adding glacial acetic acid (3 $\times$ 50 μ L). Borate was removed as volatile trimethylborate by adding 5% v/v acetic acid in MeOH (3 $\times$ 0.5 mL), and the sample concentrated under an air stream at 40 °C, followed by addition of MeOH (3 $\times$ 0.5 mL) and evaporating to dryness under an air stream at 40 °C. The resulting alditols were acetylated in acetic anhydride (600 μ L), ethyl acetate (200 μ L), acetic acid (40 μ L) and perchloric acid (60%, 23 μ L) for 15 min at room temperature. Water (2 mL) and 1-methylimidazole (40 μ L) were added to the acetylated sugars to decompose the acetic anhydride. Dichloromethane (DCM) (2 mL) was added to extract the alditol acetates from the aqueous phase. The aqueous phase was removed and the DCM extracts were washed, successively, with 0.5 M sodium carbonate (2.5 mL) and then 2x water (2.5 mL). The washed solvent phase containing the alditol acetates was evaporated to dryness in a stream of air. The alditol acetates were redissolved in acetonitrile (0.5 mL) and evaporated to dryness to remove any residual water, and resuspended in an appropriate volume of acetone.

The neutral constituent sugar compositions of the unreduced and carboxyl-reduced soluble fibre fractions were determined by GC–MS of alditol acetate derivatives after hydrolysis of the polysaccharides present to their component monosaccharides. Unreduced soluble fibre fractions (4–5 mg, in duplicate) were hydrolysed with TFA (2 M, 500 μ L, containing 0.816 mg/mL *myo*-inositol, 1 h, 120 °C). After cooling, samples were filtered (0.2 μ m) to remove insoluble material, and the filtered hydrolysates dried in a stream of air and neutralised with ammonium hydroxide (2 M, 200 μ L). The neutralised hydrolysates were reduced as described above and the resulting alditols acetylated as described by Stevenson

and Furneaux (1991), for the reductive hydrolysis method, except that 200 μ L each of acetic anhydride and TFA were used and the acetylation was performed at 50 °C for 30 min. The carboxyl-reduced soluble fibre fractions (1–2 mg, in duplicate) were hydrolysed with TFA (2 M, 500 μ L, containing 0.4 mg/mL *myo*-inositol, 1 h, 120 °C) as described above for the unreduced fractions. The neutralised hydrolysates were reduced and acetylated as described previously for the insoluble fibre fractions.

The alditol acetate derivatives produced were separated by GC on an Agilent HP-5MS fused silica capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness; Agilent, Santa Clara, CA) with the GC oven programmed from 50 °C (held for 1 min) to 130 °C at a rate of 25 °C min^{−1}, then to 230 °C at a rate of 3 °C min^{−1} and detected by MS using a Hewlett Packard 5973 MSD. Identifications were based on peak retention times and by comparison of electron impact mass spectra with standard spectra. Weight calibration constants were determined from a seven sugar standard mix (derivatised at the same time as the samples) following the TAPPI standard method T 249 cm-85 (1985). *Myo*-inositol was used as an internal standard. Monosaccharide yields are means of duplicate determinations and are expressed as weight per anhydro-sugar because this is the form of sugar present in a polysaccharide.

2.6. Glycosyl linkage analysis

Samples (~0.5 mg) were methylated as described by Ciucanu and Kerek (1984), except that they were dispersed in DMSO (0.5 mL) and heated (50 °C) overnight under Ar. 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 SGE BPX90 fused silica capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness; SGE, Ringwood, Australia) as some partially methylated alditol acetate sugar derivatives were observed to co-elute on the Agilent HP5MS capillary column. Identifications were based on peak retention times and on comparisons of electron impact spectra with the spectra obtained from reference compounds. The data are means of duplicate analyses. The proportions of the various polysaccharides present in the samples were deduced from the linkage compositions by totalling the proportions of individual glycosyl linkage residues that are characteristic of well-defined cell-wall polysaccharides already described in the literature and based on the structures of kiwifruit polysaccharides described previously (Sauvageau et al., 2010).

2.7. Size-exclusion chromatography (SEC)

The SEC system consisted of a Waters 2690 Alliance separations module, a Waters 490E variable wavelength detector set at 280 nm, a DAWN-EOS multi-angle laser light scattering detector with a laser at 690 nm (Wyatt Technology Corp., Santa Barbara, CA) and a Waters 2410 refractive index monitor.

Samples of the insoluble fibre fractions dissolved in 8% w/v LiCl/dimethylacetamide (LiCl/DMAC; 5 mg mL^{−1}) were centrifuged (14,000g, 10 min) before injection (20 μ L) and eluted with 0.5% w/v LiCl/DMAC (0.25 mL min^{−1}, 60 °C) from two columns (TSK-Gel Super HM-H and Super HM-L, 150 $\times$ 4.6 mm, Tosoh Corp., Tokyo, Japan) connected in series. Molecular weights were estimated by comparison of elution volumes with those of polystyrene molecular weight standards (0.6–1110 kDa).

Samples of the soluble fibre fractions dissolved in 0.1 M LiNO₃ (2 mg mL^{−1}) were centrifuged (14,000 g, 10 min) before injection (100 μ L) and eluted with 0.1 M LiNO₃ (0.7 mL min^{−1}, 60 °C) from two columns (TSK-Gel G4000_{PWXL} and G3000_{PWXL}, 300 $\times$ 7.8 mm, Tosoh Corp) connected in series. Molecular weights were estimated

by comparison of elution volumes with those of pullulan molecular weight standards (12–830 kDa).

3. Results

3.1. *In vitro* digestion of kiwifruit

Changes in the proportions of the soluble and insoluble dietary fibre fractions with *in vitro* digestion are shown in Fig. 2. The presence of a substantial proportion of seed in the insoluble material would have had a significant influence on the results of the carbohydrate analysis, had the seeds been included in the analysis, because the seed coat consists of carbohydrates resistant to digestion and also encapsulates storage polysaccharides, protecting them against digestion. As the seed coat is rigid and insoluble it does not have digesta properties typical of dietary fibre, which arise from the relatively disperse and highly hydrated polysaccharides of plant tissue cell walls (Guillon & Champ, 2000). Exclusion of seeds from the present analyses was justified, since it was the nutritional effects of kiwifruit that were of interest.

Analysis of the dietary fibre fractions revealed that the proportion of soluble material obtained from green kiwifruit increased following both the gastric and gastrointestinal digestions, with a corresponding decrease in the proportion of the insoluble material, compared with the water control (Fig. 2). The proportion of soluble material obtained from gold kiwifruit was not greatly affected by the gastric digestion but increased following the gastrointestinal digestion, compared with the water control.

3.2. Changes in constituent sugar compositions

Table 1 shows the constituent sugar composition of the dietary fibre fractions obtained from green and gold kiwifruit following simulated gastric and gastrointestinal digestion, in comparison to water-treated controls. In general, green kiwifruit had a higher overall sugar content than gold fruit. The neutral sugar composition of the non-cellulosic components of the insoluble fibre fractions from both green and gold kiwifruit, obtained by TFA hydrolysis, following gastric digestion, was similar to that of the water controls, but that of the gastrointestinal digested samples contained more neutral sugars, particularly glucose, than the water controls. The cellulose content of the insoluble fibre fractions, as shown by glucose in the H_2SO_4 hydrolysates, was lower in the gastric digests but higher in the gastrointestinal digests compared to the water controls. The uronic acid content of the samples decreased with gastric and gastrointestinal digestion compared with the water

controls. This decrease in uronic acid content of the insoluble fibre fractions of the gastric digests was reflected in an increase in uronic acid content of the soluble fibre fractions. However, the uronic acid content of the soluble fibre fractions of the gastrointestinal digests was substantially lower than that of both the gastric digests and water controls. The only other major change to the sugar composition of the soluble fibre fractions is an increase in glucose content with gastrointestinal digestion.

3.3. Glycosyl linkage analyses

Glycosyl linkage analysis showed that the insoluble fibre fractions of both green and gold kiwifruit contained mostly cellulose (59.8–66.6 relative mol %), xyloglucan (7.8–13.2 mol %), arabinoxylan (7.8–11.3 mol %) and galactomannan (6.0–9.1 mol %), but there were no observable changes in either composition or structure with *in vitro* digestion (Table 2). Glycosyl linkage analysis showed that the soluble fibre fractions of both green and gold kiwifruit contained mostly (rhamno)-galacturonan (85.5–87.7 relative mol %), with low proportions of type I and type II arabinogalactans and arabinan (Table 3). As observed for the insoluble fibre fractions, the types of sugars and their linkages did not change with *in vitro* digestion. However, the 4-linked GalAp content of the gastrointestinal digests of both fruit showed a much lower degree of methylesterification; 51% for green kiwifruit, compared with 65% for the water control and 43% for gold fruit, compared with 59% for the water control.

3.4. Size-exclusion chromatography (SEC)

SEC of the insoluble fibre fractions from both green and gold kiwifruit showed two distinct peaks, eluting at 3.33 and 4.07 mL (Fig. 3). Comparison with polystyrene molecular weight standards indicated that the earlier eluting peak had a molecular weight in excess of 800 kDa, while the later eluting peak had an estimated molecular weight of 45 kDa. Also, this second peak co-chromatographed with microcrystalline celluloses (Sigmacell® cellulose, Type 101, Sigma, St. Louis, MO; Avicel® cellulose, Art. 2330, Merck, Darmstadt, Germany), which had molecular weights of 30 kDa, determined by multi-angle laser light scattering (MALLS) using a dn/dc of 0.104 mL g^{-1} (Hinkley & Sims, unpublished observations). Simulated *in vitro* digestion of both green and gold kiwifruit resulted in changes in the relative proportions of the two peaks detected by SEC, but did not show any observable changes in molecular weight. For green kiwifruit, the peak eluting at 4.07 mL decreased with gastric and gastrointestinal digestion compared with the water control, but there was relatively little change in the size of the peak eluting at 3.33 mL. There was also a decrease in size of the later eluting peak with the two *in vitro* digests of gold kiwifruit, together with an increase in the high molecular weight peak.

SEC of the soluble fibre fractions showed molecular weights ranging from less than 200 kDa to more than 800 kDa, compared with pullulan standards (Fig. 4). The light-scattering response at 90° (data not shown) gave large peaks at the exclusion limit of the columns and considerably lower responses for peaks in the refractive index traces, similar to that observed previously for gold kiwifruit pectin (Yuliarti, Matia-Merino, Goh, Mawson, & Brennan, 2011). Thus, the MALLS data were not used to compare the molecular weight of these fractions. The water controls and gastric digests of both the green and gold kiwifruit showed a broad peak eluting between about 10 and 16 mL, with an apparent weight-average molecular weight of around 500 kDa. Following gastrointestinal digestion, there was a decrease in the apparent molecular weight of the soluble fibre fractions, with a reduction in higher molecular weight material and an increase in lower molecular

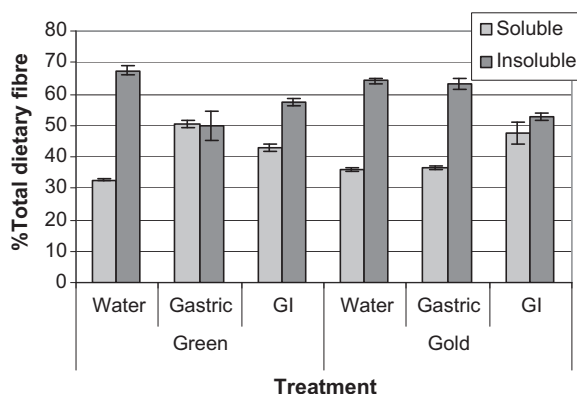


Fig. 2. Proportions of soluble and insoluble fibre fractions of *in vitro* digested green and gold kiwifruit. GI: gastrointestinal. The error bars are standard deviation of the mean ($n = 3$).

Table 1Constituent sugar compositions of the soluble and insoluble fibre fractions of *in vitro* digested kiwifruit.

Fruit	Extraction	Fraction	Hydrolysis agent	Sugar (mg per 100 gram pulp)								Total	UA
				Rha	Fuc	Ara	Xyl	Man	Gal	Glc			
Green	Water	Insol.	TFA	4	4	14	117	15	61	56	271	242	
			H ₂ SO ₄	–	–	–	17	51	–	1029	1097		
		Sol.	TFA	7	1	10	2	–	13	10	43	423	
	Gastric	Insol.	TFA	4	6	15	116	17	60	57	275	206	
			H ₂ SO ₄	–	–	–	19	43	3	848	913		
		Sol.	TFA	4	1	7	1	–	9	5	27	450	
	GI	Insol.	TFA	4	5	14	127	23	66	75	314	187	
			H ₂ SO ₄	–	–	–	17	56	–	1175	1248		
		Sol.	TFA	2	–	4	1	2	11	31	51	304	
Gold	Water	Insol.	TFA	3	–	7	70	7	23	21	132	177	
			H ₂ SO ₄	–	–	–	16	34	–	574	624		
		Sol.	TFA	5	–	9	2	–	7	5	28	267	
	Gastric	Insol.	TFA	1	2	7	57	7	29	19	122	134	
			H ₂ SO ₄	–	–	–	9	24	–	562	595		
		Sol.	TFA	3	–	7	2	–	6	3	21	360	
	GI	Insol.	TFA	3	4	10	78	12	39	31	177	108	
			H ₂ SO ₄	–	–	–	13	33	–	705	751		
		Sol.	TFA	3	–	5	–	3	15	48	74	87	

GI: gastrointestinal.

Table 2Polysaccharide compositions of the insoluble fibre fractions of *in vitro* digested kiwifruit. Bold values indicate the total mol% for each type of polysaccharide.

Polysaccharide	Deduced linkage ^a	Relative amount mol % ^b					
		Green			Gold		
		Water	Gastric	GI	Water	Gastric	GI
Xyloglucan	T-Fucp	0.8	1.0	0.7	0.6	0.7	0.6
	T-Xylp	2.5	0.7	2.4	2.7	2.8	2.8
	2-Xylp	1.6	1.9	1.7	1.4	1.6	1.5
	T-Galp	0.5	0.3	1.0	0.8	0.9	0.9
	2-Galp	1.5	1.3	1.7	1.2	1.7	1.5
	4-Glcp	1.0	0.5	1.0	1.0	1.1	1.1
	4,6-Glcp	3.8	2.1	4.1	4.1	4.4	4.3
		11.7	7.8	12.6	11.8	13.2	12.7
Galactomannan	T-Galp	1.4	1.1	1.4	0.7	1.0	0.9
	4-Manp	6.3	5.7	6.2	4.6	5.9	5.2
	4,6-Manp	1.4	1.1	1.4	0.7	1.0	0.9
		9.1	7.9	9.0	6.0	7.9	7.0
Arabinoxylan	T-Araf	1.2	1.4	1.0	1.3	1.5	1.0
	4-Xylp	7.0	8.7	6.9	5.1	5.6	4.9
	2,4-Xylp	1.3	1.0	1.3	1.3	1.6	1.2
	2,3,4-Xylp	0.2	0.2	0.3	2.2	0.9	0.7
		9.7	11.3	9.5	9.9	9.6	7.8
Cellulose	4-Glcp	60.0	61.7	62.3	64.1	59.8	66.6
Others		9.5	11.3	6.6	8.2	10.5	5.9

GI: gastrointestinal.

^a Terminal Fucp deduced from 1,5-di-O-acetyl-6-deoxy-2,3,4-tetra-O-methylfucitol, etc.^b Values are averages of duplicate determinations.

weight material. A peak at 18.5 mL (about 30 kDa) increased greatly following gastrointestinal digestion and may represent low molecular weight proteins or oligosaccharides resulting from this treatment (Yuliarti et al., 2011).

4. Discussion

The aim of this work was to measure the effects of exposing kiwifruit cell wall polysaccharides (dietary fibre) to simulated upper-intestinal tract digestion, in order to provide insights into

the composition and structure of the polysaccharides that would be available for bacterial fermentation in the large intestine. Our data indicate that, although there was a small decrease in the yields of insoluble fibre with simulated gastric and gastrointestinal digestion, the types and chemical structure of the polysaccharides in these fractions from both green and gold kiwifruit were largely unaltered. The relative proportions of cellulose and hemicellulosic polysaccharides remained constant with treatment. These findings are consistent with the study of ileostomy patients in which non-starch polysaccharides, including insoluble cellulose and non-cellulosic polysaccharides, are almost completely recovered from the terminal ileum (Englyst & Cummings, 1985 & 1987). However, the changes observed in the molecular weight profiles of the insoluble fibre fractions suggested that some physical changes may have occurred.

The changes in the yields of the soluble fibre fractions obtained following simulated gastric digestion, compared to the water controls, showed that the low pH (2.5) and enzyme conditions of this treatment increased the yields of the pectin-rich material from green, but not gold kiwifruit. Yuliarti et al. (2008) showed that the yield of soluble material obtained from gold kiwifruit by extraction with 1% (w/v) citric acid (pH 2.2), compared to water alone, was higher at 25 °C, but lower at 50 °C. In our experiments, the simulated gastric digestion was carried out at physiological temperature (37 °C), intermediate to that used by Yuliarti et al. (2008) and showed no change in the yield of soluble material from gold kiwifruit.

Chemical analysis of the pectin-rich soluble fibre fractions suggested that, similar to the insoluble fibre fractions, there was little change in the overall chemical composition of the polysaccharides. However, there was a distinct reduction in the degree of methylesterification of the galacturonic acid residues and an overall reduction in the molecular weight of these fractions with simulated gastrointestinal digestion. These changes may alter the physical properties of these polysaccharides; high-methoxy pectins (>50% degree of esterification, DE) form gels with sugar and acid, while low-methoxy pectins (<50% DE) gel in the presence of divalent cations (Voragen et al., 1995). Under the pH conditions of the gastrointestinal tract (2 h at pH 1–2, followed by 2 h each at pH 6.0, 7.0 and 8.0) and in the ileal contents of rats, Dongowski and Anger (1996) showed that there was little depolymerisation of pectin. Also, in contrast to the large decrease in methylesterification we

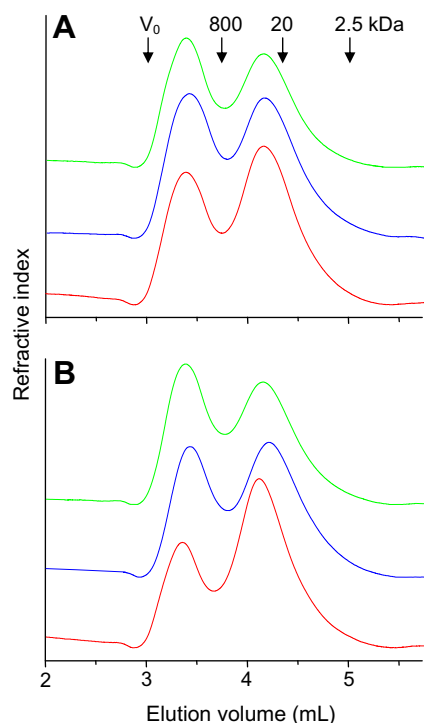


Fig. 3. Molecular weight profiles of insoluble fibre fractions of *in vitro* digested, (A) green and (B) gold kiwifruit preparations analysed by size-exclusion chromatography and monitored by refractive index. Profiles for the different treatments are shown in red (water), blue (gastric) and green (gastrointestinal). The molar mass of polystyrene standards is shown and V_0 was the void volume. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

observed with gastrointestinal digestion, they found only a slight decrease in the degree of esterification of pectins in the ileal contents of both conventional and germ-free rats. We speculated as to whether the reduction in esterification we observed was due to the increase in pH of the solution in the simulated gastrointestinal digestion, or the result of esterase activity of the added pancreatin. Miller, Buchanan, Eastwood, and Fry (1995) showed that artificial pancreatic juice (pH 8.8, 37 °C) was able to hydrolyse methyl ester groups of spinach pectins, but pancreatin did not de-esterify the pectin. In our experiments small-intestinal digestion was done at pH 6.5, similar to previous *in vitro* intestinal digestions that have typically been done at pH 6–6.9 (Woolnough et al., 2008). At this pH some de-esterification might be expected to occur, but at a lower rate than at higher pH (BeMiller, 1986). Miller et al. (1995) also showed that cell wall polysaccharides were not hydrolysed to low-molecular-weight products by either pancreatin or pepsin or by the pH conditions of artificial gastric (pH 1.85) and pancreatic (pH 8.8) juices. Depolymerisation of pectins through β -elimination can occur above pH 6, with the rate of reaction increasing with increasing pH (BeMiller, 1986). Therefore, it is more likely that the observed reduction in methylesterification of galacturonic acid residues and the reduction in molecular weight of the kiwifruit soluble fibre were due to chemical modifications during simulated gastrointestinal digestion.

The influence of the degree of methylesterification of pectins on colonic fermentation has been studied, with contrasting results. Both Dongowski and Lorenz (1998) and Olano-Martin, Gibson, and Rastall (2002) found that low-methoxyl pectins were fermented faster by human faecal microorganisms than high-methoxyl pectins. In contrast, more recent studies reported only small differences in the fermentability of pectins with differing degrees

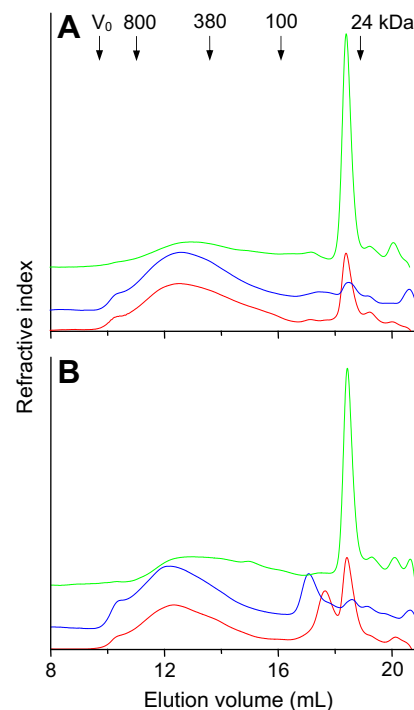


Fig. 4. Molecular weight profiles of soluble fibre fractions of *in vitro* digested, (A) green and (B) gold kiwifruit preparations analysed by size-exclusion chromatography and monitored by refractive index. Profiles for the different treatments are shown in red (water), blue (gastric) and green (gastrointestinal). The molar mass of pullulan standards is shown and V_0 was the void volume. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

of methylesterification (Gulfi, Arrigoni, & Amadò, 2005 & 2006). Thus, the significance for subsequent colonic fermentation of the reduction in degree of methylesterification of the pectic polysaccharides in soluble fibre fractions observed in this present study is unclear. Gulfi et al. (2005) also found that partially hydrolysed pectins behaved similarly to their corresponding starting materials during *in vitro* fermentation, suggesting that molecular weight had little influence on the fermentability of these substrates.

Overall, the results from the current study were largely consistent with the generally accepted definition of dietary fibre as polysaccharides and associated substances that are resistant to digestion and absorption in the monogastric foregut. However, reduction in the molecular weight and degree of methyl-esterification of the pectic polysaccharides were observed following simulated small-intestinal digestion and these changes could have a substantial effect on the physicochemical characteristics of the polysaccharides and may influence their functionality in the gut. These changes would not be detected using standard dietary fibre analytical techniques and, therefore, the results of the current study and the analytical techniques used have implications for future studies of the effect of foregut transit on the composition of dietary fibre. Others have detected changes in dietary fibre during foregut transit, but attributed them to the microbial population that is resident in the small intestine, although at much lower levels than in the colon (Robertson, Murison, & Chesson, 1992). As there were no bacteria within the *in vitro* system studied here, all changes arose from enzymatic and/or chemical means. This implies that, apart from changes that may occur *in vivo* as a result of bacterial fermentation, the chemical environment in the foregut makes some significant contributions to altered chemical structure of fruit dietary fibre between consumption and its passage to the colon.

Table 3Polysaccharide compositions of the soluble fibre fractions of *in vitro* digested kiwifruit. Bold values indicate the total mol% for each type of polysaccharide.

Polysaccharide	Deduced linkage ^a	Relative amount mol% ^b					
		Green			Gold		
		Water	Gastric	GI	Water	Gastric	GI
(Rhamno)-galacturonan	2-Rhap	1.0	0.9	1.4	1.0	1.4	1.0
	2,4-Rhap	0.8	0.6	0.7	0.7	0.7	0.7
	4-GalAp	79.6 (65)	79.5 (62)	80.8 (51)	80.4 (59)	81.1 (62)	84.6 (43)
	2,4-GalAp	1.0	1.1	0.9	0.8	0.6	0.5
	3,4-GalAp	2.4	2.8	3.0	3.0	1.2	0.7
	4,6-GalAp	0.7	0.9	0.4	0.4	0.5	0.2
		85.5	85.8	87.2	86.3	85.5	87.7
Type I AG	T-Araf	0.9	0.6	0.4	0.4	0.2	0.2
	4-Galp	1.8	1.8	1.8	1.8	1.8	1.9
	3,4-Galp	0.9	0.6	0.4	0.4	0.2	0.2
		3.6	3.0	2.6	2.6	2.2	2.3
Type II AG	T-Araf	0.9	0.8	0.8	0.8	1.0	0.8
	3-Galp	0.3	0.3	0.1	0.1	1.0	0.7
	3,6-Galp	0.9	0.8	0.8	0.8	1.0	0.8
		2.1	1.9	1.7	1.7	3.0	2.3
Arabinan	5-Araf	0.6	0.5	0.4	0.4	0.6	0.4
Others		8.2	8.8	7.2	9.0	8.7	7.3

GI; gastrointestinal.

^a 2-Rhap deduced from 1,2,5-di-O-acetyl-6-deoxy-3,4-tetra-O-methylrhamnitol, etc.^b Values are averages of duplicate determinations (values in parentheses are degree of methylesterification).

5. Funding Sources

This work was funded by Zespri International Limited (Contract No. 25534).

Acknowledgements

We gratefully acknowledge the expert advice of Lynley Drummond (Zespri International Limited, New Zealand) and Juliet Ansell (Plant and Food Research Ltd., Palmerston North New Zealand).

References

- Albersheim, P., Nevins, D. J., English, P. D., & Karr, A. (1967). A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. *Carbohydrate Research*, 5, 340–345.
- BeMiller, J. N. (1986). An Introduction to Pectins: Structure and Properties. In M. L. Fishman & J. J. Jen (Eds.), *Chemistry and Function of Pectins* (pp. 2–12). ACS Symposium Series. Washington: American Chemical Society.
- Blumenkrantz, N., & Asboe Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, 54, 484–489.
- 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.
- Dongowski, G., & Anger, H. (1996). Metabolism of pectin in the gastrointestinal tract. *Progress in Biotechnology*, 14, 659–666.
- Dongowski, G., & Lorenz, A. (1998). Unsaturated oligogalacturonic acids are generated by *in vitro* treatment of pectin with human faecal flora. *Carbohydrate Research*, 314, 237–244.
- 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.
- Englyst, H. N., & Cummings, J. H. (1985). Digestion of the polysaccharides of some cereal foods in the human small intestine. *American Journal of Clinical Nutrition*, 42, 778–787.
- Englyst, H. N., & Cummings, J. H. (1987). Digestion of polysaccharides of potato in the small intestine of man. *American Journal of Clinical Nutrition*, 45, 423–431.
- Ferguson, A. R., & Ferguson, L. R. (2003). Are kiwifruit really good for you? *Acta Horticulturae*, 610, 131–137.
- Guillon, F., & Champ, M. (2000). Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Research International*, 33, 233–245.
- Gulfi, M., Arrigoni, E., & Amadò, R. (2005). Influence of structure on *in vitro* fermentability of commercial pectins and partially hydrolysed pectin preparations. *Carbohydrate Polymers*, 59, 247–255.
- Gulfi, M., Arrigoni, E., & Amadò, R. (2006). The chemical characteristics of apple pectin influence its fermentability *in vitro*. *LWT—Food Science and Technology*, 39, 1001–1004.
- 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.
- 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.
- Melton, L. D., & Smith, B. G. (2001). Determination of neutral sugars by gas chromatography of their alditol acetates. *Current Protocols in Food, Analytical Chemistry*, E3.2.1–E3.2.13.
- Miller, J. G., Buchanan, C. J., Eastwood, M. A., & Fry, S. C. (1995). The solubilisation and hydrolysis of spinach cell wall polysaccharides in gastric and pancreatic fluids. *Journal of the Science of Food and Agriculture*, 68, 389–394.
- Minekus, M., Marteau, P., Havenaar, R., & Huis in't Veld, J. H. J. (1995). A multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. *ATLA*, 23, 197–209.
- Monro, J. A., Mishra, S., & Venn, B. (2010). Baselines representing blood glucose clearance improve *in vitro* prediction of the glycemic impact of customarily consumed food quantities. *British Journal of Nutrition*, 103, 295–305.
- Olano-Martin, E., Gibson, G. R., & Rastall, R. A. (2002). Comparison of the *in vitro* bifidogenic properties of pectins and pectic-oligosaccharides. *Journal of Applied Microbiology*, 93, 505–511.
- 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., & Coddington, J. M. (1992). Structures of the pectic polysaccharides from the cell walls of kiwifruit. *Carbohydrate Research*, 226, 287–302.
- Robertson, J. A., Murison, S. D., & Chesson, A. (1992). Particle size distribution and solubility of dietary fibre in swede (*Brassica napus*) based and wheat bran-based diets during gastrointestinal transit in the pig. *Journal of the Science of Food and Agriculture*, 58, 197–205.
- Saito, D., Nakaji, S., Fukuda, S., Shimoyama, T., Sakamoto, J., & Sugawara, K. (2005). Comparison of the amount of pectin in the human terminal ileum with the amount of orally administered pectin. *Nutrition*, 21, 914–919.
- Sauvageau, J., Hinkley, S. F., Carnachan, S. M., & Sims, I. M. (2010). Characterisation of polysaccharides from gold kiwifruit (*Actinidia chinensis* Planch. 'Hort16A'). *Carbohydrate Polymers*, 82, 1110–1115.
- Schröder, R., Nicolas, P., Vincent, S. J. F., Fischer, M., Reymond, S., & Redgwell, R. J. (2001). Purification and characterisation of a galactoglucan from kiwifruit (*Actinidia deliciosa*). *Carbohydrate Research*, 331, 291–306.
- Sims, I. M., & Bacic, A. (1995). Extracellular polysaccharides from suspension cultures of *Nicotiana glauca* L. *Phytochemistry*, 38, 1397–1405.
- Smith, B. G., & Harris, P. J. (1995). Polysaccharide composition of unignified cell walls of pineapple [*Ananas comosus* (L.) Merr.] fruit. *Plant Physiology*, 107(1399–1), 409.

- Stevenson, T. T., & Furneaux, R. H. (1991). Chemical methods for the analysis of sulfated galactans from red algae. *Carbohydrate Research*, 210, 277–298.
- TAPPI standard method T 249 cm-85 (1985). Carbohydrate composition of extractive-free wood and wood pulp by gas-liquid chromatography, TAPPI Press, Atlanta.
- Voragen, A. G. J., Pilnik, W., Thibault, J. –F., Axelos, M. A. V., & Renard, C. M. G. C. (1995). Pectins. In A. M. Stephen (Ed.), *Food polysaccharides and their applications* (pp. 287–339). New York: Marcel Dekker, Inc.
- Woolnough, J. W., Monro, J. A., Brennan, C. S., & Bird, A. (2008). Simulating human carbohydrate digestion *in vitro*: A review of methods and the need for standardisation. *International Journal of Food Science and Technology*, 43, 2245–2256.
- 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.
- Yuliarti, O., Matia-Merino, L., Goh, K., Mawson, J. A., & Brennan, C. S. (2011). Effect of Celluclast 1.5L on the physicochemical characterization of gold kiwifruit pectin. *International Journal of Molecular Sciences*, 12, 6407–6417.