Food Chemistry 133 (2012) 132–139

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects 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 13 January 2012

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.

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, because 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).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.12.084

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

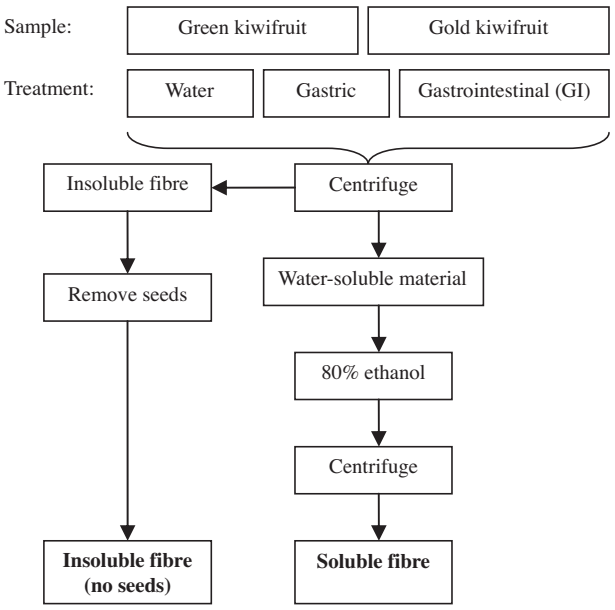

Fig. 1. *In vitro* digestion and extraction of kiwifruit.

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 $NaBD_4$. Excess $NaBD_4$ was destroyed with acetic acid and the samples dialysed (molecular weight cut-off 2 kDa) for 24 h against distilled H_2O and freeze dried. Samples were split into two aliquots, dissolved in distilled H_2O (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 $NaBD_4$ (for analysis of total uronic acids) or $NaBH_4$ (to yield the proportion of esterified uronic acids compared to the total uronic acids). Excess reductant was then destroyed