Bioactive Carbohydrates and Dietary Fibre 6 (2015) 37–45

Contents lists available at ScienceDirect

Bioactive Carbohydrates and Dietary Fibre

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

Kiwifruit fermentation drives positive gut microbial and metabolic changes irrespective of initial microbiota composition

Paul Blatchford [a,b], Halina Stoklosinski [a], Gemma Walton [b], Jonathan Swann [b], Glenn Gibson [b], Richard Gearry [c], Juliet Ansell [d,*]

[a] The New Zealand Institute for Plant & Food Research Limited, Private Bag 11600, Palmerston North 4442, New Zealand
[b] Department of Food and Nutritional Sciences, The University of Reading, Reading RG6 6AP, United Kingdom
[c] Department of Medicine, University of Otago, Christchurch, New Zealand
[d] Zespri International Limited, 400 Maunganui Road, PO Box 4043, Mt Maunganui 3149, New Zealand

ARTICLE INFO

Article history:
Received 23 December 2014
Received in revised form
2 July 2015
Accepted 13 July 2015

Keywords:
16S rRNA gene sequencing
Bioinformatics
Gut models
Microbial ecology
Microbiota

ABSTRACT

It is well established that individuals vary greatly in the composition of their core microbiota. Despite differing ecology, we show here that metabolic capacity converges under the pressure of kiwifruit substrates in a model gut system. The impact of pre-digested green and gold kiwifruit on the human colonic microbiota and their metabolic products was assessed using *in vitro*, pH-controlled, anaerobic batch culture fermenters. Phylogenetic analyses revealed that bacterial composition changed over time, irrespective of whether a substrate was added or not, indicating a natural adjustment period to the gut model environment. Adding kiwifruit substrate caused additional changes in terms of growth of specific bacterial groups, bacterial diversity and metabolite profiles. Relative abundance of *Bacteroides* spp. increased with both green and gold kiwifruit substrate while *Bifidobacterium* spp. increased only with green kiwifruit. NMR spectroscopy and GC demonstrated an increase in organic acids (primarily acetate, butyrate, and propionate) and a concomitant decrease in several amino acids and oligosaccharides following addition of green and gold kiwifruit substrate. The experiments demonstrated that despite markedly different baseline profiles in individual donor inoculum, kiwifruit components can induce substantive change in microbial ecology and metabolism which could have consequences for human health.

1. Introduction

The gut microbiota is a highly diverse collection of trillions of microbes comprised of hundreds of species (Gill et al., 2006). In the densely colonized large intestine, the microbiota can reach numbers of up to 10^{12} cfu/g colon contents (Gueimonde & Collado, 2012). Such vast numbers of symbionts can have a considerable impact on the health of the host. The gut microbiota has evolved with humans to a complex inter-dependant state, where their genome in addition to our own generates a profound ability to metabolise the diverse array of substrates in the human diet (Xu et al., 2007). Predominant phyla in the human gut are Bacteroidetes and Firmicutes, making up over 90% of all resident colonic bacteria with the two other subdominant phyla being Actinobacteria and Proteobacteria (Eckburg et al., 2005; Ley, Turnbaugh, Klein, & Gordon, 2006). There have been numerous studies conducted recently on the use of purified and processed foods or food additives to modify bacterial composition. It is clear that diet has an effect on microbiota and this in turn affects health; as many as a third of all diseases, including cardiovascular disorders such as coronary heart disease and hypertension, type 2 diabetes, functional bowel problems and cancer, are lifestyle related and their risk may be mitigated through dietary means (Johnson, Chua, Hall, & Baxter, 2006; Shahidi, 2009; Tuomilehto et al., 2001). Prebiotic supplementation is commonly used to treat gastrointestinal dysfunction. These are a class of non-digestible food ingredients such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS) and xylo-oligosaccharides (XOS) (Gibson, 2004) that confer a health benefit to the host through selectively modulating bacterial composition (Gibson et al., 2010). Prebiotic molecules are often sourced from plants, where specific oligosaccharides are isolated and concentrated in order to be used as a supplement to a regular diet. An alternative to prebiotic supplementation is the use of whole fruits and vegetables as health promoting foods, which are easier to implement into a dietary routine (Lipsky, Cheon, Nansel, & Albert, 2012). In addition to the health benefits of whole foods,

* Corresponding author. Fax: +64 7 574 8031.
E-mail address: juliet.ansell@zespri.com (J. Ansell).

http://dx.doi.org/10.1016/j.bcdf.2015.07.001
2212-6198/

they make for a more marketable product. An absence of processing maintains the natural structure of nutrients which are potentially more bioavailable in whole foods (Chandrasekara, Naczk, & Shahidi, 2012; van der Sluis, Dekker, Skrede, & Jongen, 2002).

The most commonly sold kiwifruit are from the species *Actinidia deliciosa* (typically green fleshed e.g. 'Hayward') and *Actinidia chinensis* (typically yellow fleshed e.g. 'Gold3'). Kiwifruit are rich in vitamin C, potassium, folate, and phytochemicals (Ferguson & Ferguson, 2003). The principal carbohydrate found in kiwifruit is starch, with non-starch polysaccharides (NSP) such as pectic polysaccharides, hemicelluloses and celluloses amounting to 2–3% of total kiwifruit constituents (Carnachan, Bootten, Mishra, Monro, & Sims, 2012; Dawson & Melton, 1991; Ferguson & Ferguson, 2003; Seager & Haslemore, 1993). NSP are essentially resistant to digestion by enzymes encountered in the human stomach and small intestine. Therefore, they reach the colon largely intact where pectic polysaccharides and, to a lesser extent, hemicelluloses and celluloses are fermented by the gut microbiota (Cummings & Englyst, 1987). Prebiotic effects, namely beneficial changes to the composition of the existing microbiota and colonic metabolites, may subsequently be observed. Several studies have examined kiwifruit fibre digestion *in vitro,* finding a chemically unaltered structure with only minor modifications to galacturonic acid residues and molecular weight profiles in the soluble fibre fraction (Carnachan et al., 2012; Dawson & Melton, 1991). In a recent study, upper gastrointestinal tract digestion had little effect on either green or gold kiwifruit in an *in vivo* porcine model, with the dietary fibre fraction being completely undigested at the terminal ileum (Henare et al., 2012).

Changes in bacterial composition can lead to a modified metabolite profile which can have direct consequences for host health. Recent research has shown that the observed metabolic profile can be altered by changing the substrates available for fermentation. Substrates that can induce changes in metabolic profiles include: carbohydrates such as resistant starch, unabsorbed sugars, non-starch polysaccharides, gums and cellulose; and proteins from the diet and endogenous sources such as mucin (Cummings & Englyst, 1987; Cummings & Macfarlane, 1991; Louis, Scott, Duncan, & Flint, 2007). Some of the main end products of fermentation are short chain fatty acids (SCFA), branched chain fatty acids (BCFA) and gases like hydrogen, carbon dioxide and methane (Blaut, 2002; Rosendale, Cookson, Roy, & Vetharaniam, 2011). This study determined the effect of whole kiwifruit components that escape gastric and small intestinal digestion on the colonic microbiota and metabolites in an *in vitro* batch culture gut model.

2. Materials and methods

2.1. Simulated gastrointestinal digestion (SGD)

Two kiwifruit substrates were used in the batch culture models: Green kiwifruit (*A. deliciosa*) 'Hayward' and Gold kiwifruit (*A. chinensis*) 'Hort16A'. The compositional data of these two kiwifruit are outlined in Table S1. As a control, no exogenous substrate was added. Green and gold kiwifruit were peeled, chopped and mashed finely. The samples were subjected to the simulated gastric digestion procedure as detailed by Mills et al. with minor modifications (Mills et al., 2008). Briefly, 60 g of sample was weighed and added to 150 mL of autoclaved distilled water in a stomacher bag where it was homogenised (Stomacher 400) for 5 min at normal speed (460 paddle beats/min). After addition of 0.001 mol/L salivary α-amylase the solution was incubated for 30 min on a shaker at 37 °C. The pH was adjusted to 2.0 using 6 M HCl. Pepsin solution was added to the mixture which was incubated at 37 °C gently shaking for 2 h. The pH was adjusted to 7.0 following addition of a pancreatin/bile mixture (P8096/B8631 Sigma) and the solution was incubated at 37 °C for 3 h. Samples were then transferred to a 500 Da dialysis membrane (Spectra/Por, Spectrum Laboratories Inc.) to remove most di- and mono-saccharides. This was dialysed for 15 h against a 10 mM NaCl solution at 4 °C. The dialysis fluid was replenished and the samples dialysed for a further 2 h. Samples were then frozen at −80 °C and freeze-dried.

2.2. pH controlled anaerobic faecal batch cultures

Batch culture systems allow the study of microbial fermentation in a simulated colonic environment. The apparatus was set up the day before the experiment and sterilised by autoclaving. The basal culture medium used for the batch cultures contained (per L): 2 g peptone, 2 g yeast extract, 0.1 g NaCl, 0.04 g K_2HPO_4, 0.04 g KH_2PO_4, 0.01 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2 \cdot 6H_2O$, 2 g $NaHCO_3$, 2 mL Tween 80, 0.05 g haemin (dissolved in a few drops of NaOH), 10 μl vitamin K, 0.5 g cysteine HCl, 4 mL resazurin solution (0.025 g/100 mL) and 0.5 g No.3 bile salts. The solution was made up to 1 L with distilled water and sterilised by autoclaving. All chemicals were obtained from Sigma. One hundred and thirty-five millilitres of freshly autoclaved medium was aseptically poured into 280 mL capacity water-jacketed batch culture vessels. The medium was continually mixed using a magnetic stirrer and maintained at 37 °C with a circulating waterbath. Oxygen free N_2 gas was bubbled through the media overnight to establish an anoxic environment. Excess gas was vented outside through a 0.22 μm filter.

On the morning of the experiment, calibrated pH electrodes were inserted into each vessel. A freshly voided stool sample was obtained from a healthy volunteer who had not taken any supplemental probiotics, prebiotics or antibiotics for 6 months prior. The stool was diluted 1:10 in sterile PBS, stomached for 2 min and 15 mL was added to the vessels, yielding a total volume in each vessel of 150 mL. Then 1.5 g (1% w/v) of each kiwifruit substrate was added to the vessels (excluding the control). Approximately 5.5 mL of sample was taken from each vessel immediately upon addition of substrate representing the 0 h time point. Samples were then taken at 5, 10, 24 and 48 h time points. Each sample was placed on ice, dispensed into aliquots and stored at −80 °C for metabolomics and −20 °C for all other samples. The batch culture systems were monitored throughout the 48 h run, with any adjustments of stirrer speed, N_2 flow rates or temperature carried out as required. This initial batch culture experiment was repeated twice with different faecal donors giving a total of three biological replicates.

2.3. Nuclear magnetic resonance (NMR) spectroscopy

One millilitre of fermenta was taken and centrifuged at 16,200*g* for 10 min; then the supernatant was decanted and frozen at −80 °C until analysis. Samples were then defrosted, vortexed and 400 μL transferred into a sterile eppendorf. Two hundred microlitres of phosphate buffer (containing 1 mM of the internal standard TSP (3-(trimethylsilyl)-[2,2,3,3-d4]-propionic acid sodium salt), the bacteriostatic sodium azide in 100% D_2O) was added to the samples which were then vortexed and centrifuged at 10,000*g* for 10 min. The supernatant (550 μL) was then transferred to a 5 mm glass NMR tube. All samples (and a batch culture medium only control) were run on a Bruker Avance III 700 MHz NMR spectrometer. Initial spectral processing was conducted using Bruker's Topspin software. Spectra were baseline corrected to remove systemic offsets, phased to yield accurate peak integration and peak shape and the TSP (internal chemical shift standard) adjusted to 0 ppm. Further data processing was carried out using