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Short-chain fatty acid profiles from flaxseed dietary fibres after *in vitro* fermentation of pig colonic digesta: Structure–function relationship

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ABSTRACT

Flaxseed (*Linum usitatissimum* L.) is rich in dietary fibres. There are four major soluble dietary fibre fractions, and their molecular structures have been characterised; however, the structure–function relationship on the short-chain fatty acid (SCFA) profiles is still unknown. SCFA profiles produced by *in vitro* fermentation of pig colonic digesta with soluble flaxseed dietary fibre fractions were determined by gas chromatography with psyllium arabinoxylans as the reference fibres. The results revealed that total SCFA production from all tested dietary fibres were significantly higher than negative control (NC) after 72 h fermentation. Acetic acid was the major SCFA produced, followed by propionic acid and butyric acid during 72 h fermentation. The acetic acid production from flaxseed kernel xyloglucans, and butyric acid production from flaxseed mucilage arabinoxylans were significantly higher than negative control (NC) after 48 h fermentation. Both psyllium and flaxseed mucilage arabinoxylans promoted butyric acid production more effectively than other tested dietary fibres during 48 h fermentation, while flaxseed kernel xyloglucans and flaxseed RG-I were more effective on promoting acetic acid production. The dietary fibre strucutre played major roles on promoting specific SCFAs, and the conformational characteristics might contribute to the difference of fermentability rates. Flaxseed dietary fibres were relatively slower fermentable dietary fibres as compared with psyllium fibres, and all flaxseed dietary fibres had higher level of total SCFA production than psyllium fibres after 72 h incubation. This research should further promotes the utilisation of flaxseed dietary fibres, and contributes to the optimisation of colonic health based on more balanced diets with both grain and oilseed.

1. Introduction

Flaxseed (*Linum usitatissimum* L.) is rich in dietary fibres (22–28%, w/w), most of which become by-products of the flaxseed oil industry. Rhamnogalacturonan-I (RG-I) and arabinoxylans are the major components in flaxseed mucilage (Cui, Mazza, Oomah, & Biliaderis, 1994; Qian, Cui, Nikiforuk & Goff, 2013), and xyloglucans and arabinan-rich RG-I are the major soluble dietary fibres in flaxseed kernel (Ding et al. 2015; Ding, Cui, Goff, Guo & Wang, submitted for publication). The health benefits of dietary fibres, as well as their overlapping definitions and legally permissible claims, have been extensively summarised and discussed by Phillips (2013). The adequate intakes of dietary fibres are recommended to be 25 and 38 g for adult women and men, respectively (Institute of Medicine, 2005).

In typical western diets, around 80% of dietary fibres are non-starch polysaccharide and resistant starch, and the others are non-digestible oligosaccharides and sugar alcohols, yielding 150–600 mmol short-chain fatty acids (SCFAs) (Wachtershauser & Stein, 2000). SCFAs could decrease colon pH and prevent pathogenic bacteria growing; moreover, they increase mucosal blood flow and colon mobility, and reduce secondary bile acid formation and osmotic pressure (Macdonald, Singh, Mahony, & Meier, 1978; Daly, Stewart, Flint, & Shirazi-Beechey, 2001; Cummings, Rombeau, & Sakata, 2004). It was also reported that fermentative production of butyrate from dietary fibres might decrease the inflammatory response in the colon (Rose, DeMeo, Keshavarzian & Hamaker, 2007).

SCFAs produced by large intestine microbes are mainly composed of acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, and other branched fatty acids (*e.g.* isobutyric acid and isovaleric acid). It is worth noting that lactic acid and formic acid

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serve as intermediates in the metabolism of SCFA and normally do not accumulate in the colon (Macfarlane & Gibson, 2004). The fermentation profiles of arabinoxylans from agricultural crops (*e.g.* maize, rice, wheat) and psyllium fibres have been widely studied (Rose, Patterson, & Hamaker, 2010; Elli, Cattivelli, Soldi, Bonatti, & Morelli, 2008; Pollet et al., 2012), and they are commonly accepted by the general public as prebiotics (Slavin, 2013; Kolida, Tuohy, & Gibson, 2002).

There are four major soluble dietary fibre fractions from flaxseed, flaxseed mucilage RG-I (FM-RGI) and arabinaxylans (FM-AX), and flaxseed kernel arabinan-rich RG-I (FK-RGI) and xyloglucans (FK-XG). Soluble flaxseed gum, which is mainly the mixture of FM-RGI and FM-AX, has been evaluated using *in vitro* fermentation model (Ying et al., 2013); however, the detailed structure–function relationship is still unknown. It was reported that changes in molecular weight, viscosity, and substituted groups of dietary fibres affected SCFA production (Nyman, 2003; Olano-Martin, Mountzouris, Gibson and Rastall, 2000; Al-Tamimi, Palframan, Cooper, Gibson, & Rastall, 2006; Rose et al., 2010). In order to better understand the structure–function relationship of flaxseed dietary fibres, SCFA profiles produced by *in vitro* fermentation of pig colonic digesta on four major soluble flaxseed dietary fibre fractions were studied with psyllium arabinoxylans as the reference fibres in the present study.

2. Materials and methods

2.1. Materials

Flaxseed mucilage RG-I (FM-RGI) and arabinaxylans (FM-AX) were obtained through further fractionation of flaxseed mucilage gum through ion exchange column as described by Qian, Cui, Wu, and Goff (2012). Flaxseed kernel arabinan-rich RG-I (FK-RGI) and xyloglucans (FK-XG) were prepared using sequential extraction and fractionation as described in previous studies (Ding et al., 2014, 2015). Psyllium husk was purchased from the local market, and psyllium arabinoxylans were obtained after further water extraction at 70 °C, followed by protease hydrolysis and dialysis.

2.2. Chemical composition analysis

Total sugar, total uronic acid, protein content, molecular weight distribution, and monosaccharide composition were determined. Briefly, total sugar content was determined by phenol-H_2SO_4 method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956). Nitrogen content was determined by NA2100 Nitrogen and Protein Analyser (Thermo Quest, Milan, Italy), and then protein content was obtained by a conversion factor of 6.25. Total uronic acid content was determined by m-hydroxyphenyl colorimetric method (Blumenkrantz & Asboe-Hansen, 1973).

2.3. Molecular weight distribution

Molecular weight distribution was analysed on a high performance size-exclusion chromatography (HPSEC) system (Shimadzu Scientific Instruments Inc., Maryland, US) with multiple detectors:, a refractive index (RI) detector, a Photodiode Array (PDA) Detector (Viscotek 2600), a differential pressure viscometer, a right angle laser light scattering detector and a low angle laser light scattering detector (Viscotek TDA 305). The column systems combined two AquaGel PAA-M columns and a PolyAnalytik PAA-203 column (Polyanalytik Canada, London, Canada) in series. Detectors were calibrated by Pullulan standards (JM Science Inc., New York, US). The eluent was 0.1 M $NaNO_3$ with 0.05% (w/w) NaN_3 at a flow rate of 0.6 mL/min, and the columns and detectors were maintained at 40 °C. Data were analysed by OmniSEC 4.6.1 software.

2.4. Monosaccharide composition analysis

Monosaccharide composition was analysed on a high performance anion exchange chromatography (HPAEC) system equipped with a pulse amperometric detector (PAD) (Dionex-5500, Dionex Corporation, California, US). Polysaccharide samples (around 2 mg) were hydrolysed in 1 mL 1 M H_2SO_4 at 100 °C for 2 h, and then diluted 20 times with Milli-Q water. Each sample was injected through a CarboPac PA1 ($250 \times 4\ mm^2$ I.D.) column with 100–300 mM NaOH as gradient eluents at a flow rate of 1.0 mL/min. Post-column eluent was 600 mM NaOH solution at a flow rate of 0.5 mL/min. Six target monosaccharides (fucose, rhamnose, arabinose, galactose, glucose, and xylose) at various concentrations were chosen as standards. Each sample was measured in triplicate.

2.5. Digesta preparation

Digesta was collected from the central part of colons from three Yorkshire pigs with the market body weight of approximately 60 kg. The pigs were raised at the Arkell Swine Research Station of University of Guelph. The pigs were cared based on the guidelines from Canadian Council of Animal Care (Olfert, Cross, & Mcwilliam, 1993), and the use of pigs for this study was approved by the Animal Care Committee of the University of Guelph. Collected digesta of three pigs was mixed in an equal amount, and was stored at −80 °C until use.

2.6. Medium preparation

An anaerobic incubation medium (AIM) was prepared as per He, Young and Forsberg (1992). The ingredients and recipe are listed in Table 1, and the added dietary fibres were the sole carbon and energy source in the medium. Briefly, all ingredients except vitamins and cysteine-HCl were mixed and dissolved with pH adjusted to 6.8. The solution was heated to boil and flushed with mixed gas (10% H_2, 10% CO_2, and 80% N_2) in the anaerobic chamber (Coy Laboratory Products Inc., MI, US) prior to autoclaving for 20 min at 121 °C. Vitamin and cysteine-HCl solutions were sterilised using 0.22 μm filters, and then flushed with mixed gas before mixing with autoclaved medium in the anaerobic chamber.

2.7. Preparation of in vitro batch culture system

Dietary fibres were the sole carbon source with a concentration of 1% (w/v) in the system, and 10% (w/v) of digesta was selected for inoculation as per Ying et al. (2013). To specify, the digesta (0.6 g) were aliquoted into 15 mL Eppendorf tubes, and then dietary fibre samples (0.06 g) were added and mixed with 6 mL AIM, dissolving for 1 h in the anaerobic chamber. The culture tubes were placed in

Table 1
Composition of anaerobic incubation medium (AIM) in 1 L Milli-Q water.

Mineral (mg)		Vitamin (mg)		Other (mg)	
$CaCl_2$	50	Biotin	0.05	Cysteine-HCl	1000
$CoCl \cdot 6H_2O$	2	Calcium D-pantothenate	2	Resazurin	1
$FeSO_4 \cdot 7H_2O$	20	Cobalamine	0.005		
K_2HPO_4	900	Folic acid	0.05		
$MgSO_4$	50	Nicotinamide	2		
$MnSO_4 \cdot H_2O$	20	Para-aminobenzoic acid	0.1		
NaCl	900	Pyridoxine-HCl	2		
Na_2CO_3	4000	Riboflavin	2		
$(NH_4)_2SO_4$	900	Thiamine-HCl	2		
$ZnSO_4 \cdot 7H_2O$	20				