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Research Article

Assessment of dietary fiber fermentation: Effect of *Lactobacillus reuteri* and reproducibility of short-chain fatty acid concentrations

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This investigation had two aims: (i) to determine the reproducibility of SCFA production of two fibers: wheat dextrin and inulin, in two separate *in vitro* batch fermentation systems, and (ii) to determine if the addition *Lactobacillus reuteri*, a probiotic bacterium, enhanced the fermentation of wheat dextrin, inulin, and psyllium using *in vitro* batch fermentation. Samples were removed at 0, 4, 8, 12, and 24 h. SCFAs were measured by GC. *L. reuteri* improved inulin's fermentation profile by reducing the total SCFA peak at 4 h and enhancing fermentation at 8 and 12 h. Wheat dextrin and psyllium were largely unaffected. Wheat dextrin's total SCFA and propionate production curves were steady and replicable, but concentration values varied between fermentations. Partially hydrolyzed guar gum (PHGG) and wheat dextrin had similar fermentation patterns from 0 – 8 h, but PHGG plateaued at 8 h for all measures. Psyllium produced peak SCFA concentrations at 8 h, similar to inulin. *L. reuteri* could be combined with inulin for enhancing fermentation, but it does not improve wheat dextrin or psyllium fermentation. Wheat dextrin will likely produce similar physiological within a group of individuals due to the reproducibility of fermentation.

Keywords: Dietary fiber / Fermentation / Gastrointestinal / Probiotic / SCFAs

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1 Introduction

Dietary fiber is a nutrient found in plant-based foods which escapes digestion in the upper gastrointestinal (GI) tract. The recommended dietary fiber intake in the United States is 25 g/day for women and 38 g/day for men; however, few Americans meet this recommendation [1]. Average fiber intake in the United States is only about 13 g/day for women and 18 g/day for men [2]. The average fiber intake varies among different European countries due to different diets and eating patterns of the European populations. Women in the United Kingdom consume the least amount dietary fiber (11 g/day), while Germans consume the most dietary fiber (26 g/day) [3].

Some physiological effects of dietary fiber depend on the degree to which it may be fermented by colonic microbiota. Nonfermentable or poorly fermentable fibers such as cellulose or wheat bran may improve gut health by increasing fecal bulk or speeding GI transit. Alternatively, fermentable fibers, such as oat β-glucan or inulin exhibit a wide range of physiological effects (such as prebiotic activity) [4]. Fermentation characteristics are influenced by the physicochemical properties of fiber, gut transit time, and gut microbiota composition.

The concept of *synbiotics*, the combination of prebiotic fibers with probiotic bacteria, is a topic of scientific interest [5]. Probiotics and prebiotics are both orally consumed food ingredients or supplements that alter gut microbiota in a beneficial manner [5, 6]. *Lactobacillus reuteri* is one such probiotic that is native to the human digestive tract and can also reach the lower GI tract intact and viable [7]. Many beneficial health effects of *L. reuteri* have been investigated: improved immunity [8], decreased cholesterol [8], and decreased diarrhea [9, 10].

Studying gut effects of fiber in humans is difficult due to the invasive and expensive nature of colonic observation, as well as the dynamic nature of the colon. Examination of excreted colon contents is currently the best way to assess fiber fermentability in a living system. However, this may

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Abbreviations: GI, gastrointestinal; **PHGG,** partially hydrolyzed guar gum

not represent colon contents in the proximal or even distal colon, due to continual fermentation of fiber and continual absorption of minerals and SCFA across the epithelium. *In vitro* fermentation with representative human colonic microbiota is a noninvasive, time-efficient means to estimate fiber fermentability. Batch fermentation has been shown to degrade nonstarch polysaccharides (NSP) to a similar extent as the human colon, based on residual NSP in fecal samples and fermentation flasks [11].

Physical and chemical structure of fibers is an important characteristic in determining fermentability. Inulin (fructan), partially hydrolyzed guar gum (PHGG) (galactomannan), wheat dextrin (RS3-type resistant starch), and psyllium (arabinose – xylose polymer) have distinctly different chemical and physical attributes, which support the hypothesis of unique fermentation characteristics [12]. Fermentability of PHGG and inulin has been documented by several sources [13 – 16]; however, the SCFA production of wheat dextrin has not been documented [17]. The physiological effects of wheat dextrin consumption in humans have been published and this data supports the fermentation of wheat dextrin in the human gut [17 – 19]. This study investigates the *in vitro* fermentability of wheat dextrin, inulin, PHGG, and psyllium and hypothesizes that each fiber will show unique fermentation patterns due to their varied chemical structure. SCFAs produced by bacteria *via* metabolism of different fibers were used to characterize fermentation. This study also aimed to identify changes in fiber fermentation associated with added *L. reuteri*.

2 Materials and methods

Two experiments were conducted to assess the fermentability of selected dietary fibers and to evaluate the effect of *L. reuteri*, a probiotic, on fiber fermentation.

2.1 Study 1

SCFA production by three fibers: inulin, PHGG, and wheat dextrin was assessed using a batch *in vitro* fermentation system. The inulin (Cosucra, Warcoing, Belgium) tested in this study was a fructose polymer joined by β(1 – 2) bonds with chain length of 70% less than 10 U, 25% 10 – 20 U, 5% greater than 20 U. The PHGG (Taiyo Kagaku, Mies, Japan) tested in this study had a M_r of 20 000 – 30 000 Da. The wheat dextrin (Nutriose FB, Roquette Frères, France) tested in this study was a glucose polymer linked by indigestible (1 – 6), (1 – 2), and (1 – 3) bonds. Glucose was used as a positive control to ensure the bacteria were metabolically active. No fiber was added as a negative control. Chemical reagents were obtained from Fisher Scientific (Pittsburgh, PA, USA), Sigma – Aldrich (St. Louis, MO, USA), and VWR Scientific (West Chester, PA, USA).

Fecal inoculum was prepared as described by McBurney and Thompson [20]. Briefly, fecal samples from three human subjects consuming a nonspecified Western diet were pooled (125 g total) and diluted with 400 mL distilled water. The solution was homogenized in a blender. Reducing solution (950 mL distilled water, 6.25 g cysteine hydrochloride, 40 mL 1 N NaOH, 6.25 g sodium sulfide nonahydrate) was added to the fecal inoculum to obtain a ratio of 15 parts fecal inoculum to 2 parts reducing solution [21]. Five 100 mL serum bottles were prepared for each fiber sample (0.5 g), one for each of the five time points: 0, 4, 8, 12, and 24 h. Control bottles containing either 0.5 g glucose or no added carbohydrate were prepared in the same manner. Glucose was chosen as a positive control because the monomers are completely available to the bacteria, *i. e.*, no glycosidic bonds must be broken for metabolism of the carbohydrate. No carbohydrate was added as a negative control to quantify the SCFAs produced by substrate originating from the fecal sample. Forty milliliter of sterile trypticase peptone fermentation media (2.49 g trypticase peptone, 1.0 g ammonium bicarbonate, 8.75 g sodium bicarbonate, 1.43 g anhydrous sodium phosphate, 1.55 g anhydrous potassium phosphate monobasic, 0.6 g magnesium sulfate, 0.12 mg resazurin, 1.12 mmol calcium chloride, 0.63 mmol manganous chloride, 0.15 mmol cobalt chloride, 0.04 mmol ferric chloride, diluted to 1 L with distilled water) plus reducing solution (2 mL) were added to each bottle [20]. The bottles were sealed with a rubber stopper and crimped metal seal for storage at 4°C for 12 h to hydrate samples. Two hours prior to inoculation with fecal solution, sample bottles were warmed to 37°C.

Ten milliliter of fecal inoculum was added into each serum bottle along with 0.8 mL Oxyrase® oxygen reducing enzyme (Oxyrase, Mansfield, OH, USA). The bottles were immediately flushed with carbon dioxide gas to eliminate oxygen and generate anaerobic conditions. The bottles were gently shaken in a 37°C water bath. One sample bottle for each fiber was removed at 0, 4, 8, 12, and 24 h. Immediately upon removal, 1 mL of copper sulfate (200 g/L) was added to each bottle to kill the bacteria and cease fermentation. Two 2 mL aliquots were removed for SCFA analysis.

2.2 Study 2

SCFA production by three fibers: inulin, psyllium, and wheat dextrin was assessed using a batch *in vitro* fermentation system. Wheat dextrin and inulin samples are described in study 1. The psyllium used in this study was commercially available dietary fiber supplement (Proctor and Gamble, Cincinnati, OH, USA). Glucose was used as a positive control to ensure the bacteria were metabolically active. No fiber was added as a negative control. *L. reuteri* (Biogaia, Stockholm, Sweden) was added to selected fiber samples to simulate the effects of a probiotic bacterium.

Chemical reagents were obtained from Fisher Scientific, Sigma–Aldrich, and VWR Scientific.

Fermentation bottles were prepared as described in study 1 with the following changes: 30 mL trypticase peptone media was used to hydrate the fibers overnight. Fecal inoculum was prepared as described in study 1, but the three donors in study 2 were different than the donors in study 1. After the bottles were warmed to 37°C, 10 mL of fecal inoculum was added into each serum bottle along with 0.8 mL Oxyrase oxygen reducing enzyme (Oxyrase). After the fecal inoculum was added, an additional 10 mL trypticase peptone media containing *L. reuteri* (0.05 g/mL to yield ~10^{12} cfu/10 mL) or 10 mL stock trypticase peptone media was added to each fermentation bottle. Fermentation conditions were the same as study 1.

2.3 SCFA analysis

All samples were prepared for GC as described by Pylkas *et al*. [15]. Prior to GC analysis, samples were stored at –20°C. Acetate, propionate, and butyrate were determined by GC using a Hewlett Packard model 6890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) with a 4% Carbowax® 20M/80/120 Carbopack B-DA column, 2 m length × 2 mm id, TightSpec (Supelco, Bellefonte, PA, USA) a temperature of 175°C [22]. Flow rates for nitrogen, hydrogen, and air were 24, 40, and 450 mL/min, respectively. Nitrogen flow on the column was 25.5 mL/min. Inlet and detector temperatures were 200 and 230°C, respectively.

2.4 Statistical design and analysis

The experiment was a randomized complete block. In study 1, one block consisting of pooled fecal samples from three humans was used. In study 2, two blocks, one with *L. reuteri* and one without *L. reuteri* were used. The treatments were arranged in a 4 × 5 factorial pattern with four fibers measured at five time points. Each fiber *x* time point combination was measured in duplicate. Statistical analyses were completed with SAS statistical software package, version 8.0 (SAS Institute, Cary, NC, USA). Analysis of variance with Tukey pairwise comparison was conducted to compare the mean SCFA concentrations with and without *L. reuteri* and to compare the mean SCFA concentrations in study 1 and study 2. Statistical significance was achieved at *p*-values less than 0.05.

3 Results

3.1 Comparison of two fermentations – study 1 and study 2

Wheat dextrin, inulin, psyllium, and PHGG exhibited unique fermentation patterns (Table 1). All SCFAs were produced by bacteria that fermented the added fibers, as well as components in the fermentation media and fecal inoculum. Wheat dextrin resulted in a steady rate of SCFA concentrations over the 24 h fermentation in both study 1 and study 2; however, in study 2 lower total SCFA concentrations were produced compared to study 1 at 12 and 24 h. Total SCFA production by inulin peaked early in both study 1 (4 h) and study 2 (8 h), but concentrations decreased over the remainder of the fermentation in study 2 while concentrations rebounded at 24 h in study 1. Psyllium produced SCFAs similar to inulin while PHGG produced SCFAs similar to wheat dextrin until 8 h when SCFA production plateaued. Wheat dextrin was verified to be a fermentable fiber and produced total SCFA concentrations similar to inulin, but was significantly greater than PHGG and psyllium at 24 h. Glucose produced TSCFA concentrations similar to the other fibers in study 1 (data not shown) and negative control TSCFA concentrations were low (Table 1). However, control TSCFA concentrations in study 1 were greater than study 2.

Acetate was the most abundant SCFA produced, comprising 50–90% of total SCFAs. As seen with TSCFA concentrations, study 1 produced greater acetate concentrations in presence of inulin at all time points and wheat dextrin at 12 and 24 h (Table 1). Wheat dextrin produced greater acetate concentrations than PHGG at 12 and 24 h. Acetate concentrations in the presence of psyllium plateaued from 4 to 24 h. Control concentrations of acetate were greater in study 1 than study 2.

Study 2 produced lower propionate concentrations than study 1 at all time points for wheat dextrin, control, and at all time points except 12 h for inulin (Table 1). Wheat dextrin produced propionate at a steady rate in study 1 and study 2. Inulin produced propionate in a similar manner from 0 to 8 h for both studies, but peaked at 12 h in study 2 and slightly decreased from 8 to 12 h and increased from 12 to 24 h in study 1. Psyllium and PHGG produced steady concentrations of propionate but were lower than wheat dextrin. Glucose produced propionate concentrations similar to the other fibers in study 1 (data not shown). Negative control produced high propionate concentrations in study 1 (Table 1).

Wheat dextrin produced greater butyrate concentrations in study 1 at 4 h, but fermentation patterns were similar from 0 to 12 h (Table 1). At 24 h in study 1, wheat dextrin produced twice the concentration of butyrate in study 2. Inulin's butyrate production pattern was different in study 1 (peak at 8 h) compared to study 2 (peak at 12 h). Butyrate production by PHGG was similar to wheat dextrin. Psyllium produced peak butyrate concentrations at 8 h. Glucose produced butyrate concentrations similar to other fibers (data not shown) and negative control produced low butyrate concentrations in study 1 and study 2 (Table 1).

Lactate concentrations were detected at 4 and 8 h for most samples, but inulin was the only fiber to produce

Table 1. SCFA concentrations produced from inulin, wheat dextrin, psyllium, and PHGG by bacteria in model colonic fermentation

		Inulin			Wheat dextrin			Psyllium		PHGG	Control		
		Study 1	Study 2 (−LR)	Study 2 (+LR)	Study 1	Study 2 (−LR)	Study 2 (+LR)	Study 2 (−LR)	Study 2 (+LR)	Study 1	Study 1	Study 2 (−LR)	Study 2 (+LR)
TSCFA	4 h	67.6 ± 2.2[a)]	45.2 ± 0.7	50.4 ± 0.8[b)]	37.3 ± 0.4	27.3 ± 2.6	31.2 ± 1.7	47.6 ± 1.5	50.1 ± 1.2	35.9 ± 3.7	22.8 ± 1.9[a)]	10.4 ± 0.4	12.7 ± 0.5[b)]
	8 h	62.6 ± 0.8[a)]	73.2 ± 2.3	58.5 ± 2.6[b)]	48.7 ± 1.3	41.2 ± 0.7	48.0 ± 0.6[b)]	65.9 ± 2.7	70.6 ± 1.7	54.2 ± 0.8	37.5 ± 7.4	23.6 ± 0.2	26.2 ± 0.8[b)]
	12 h	53.8 ± 0.4[a)]	70.7 ± 0.6	62.0 ± 0.9[b)]	76.4 ± 2.7[a)]	52.2 ± 1.1	53.8 ± 1.0	54.8 ± 0.7	51.1 ± 0.1[b)]	51.0 ± 3.7	50.0 ± 0.3[a)]	25.1 ± 2.0	27.0 ± 1.5
	24 h	103.5 ± 1.4[a)]	59.0 ± 2.5	70.2 ± 3.2	106.5 ± 2.4[a)]	72.3 ± 1.8	67.5 ± 1.4	57.9 ± 1.4	60.6 ± 0.3	62.8 ± 3.7	64.7 ± 5.2[a)]	29.1 ± 0.8	33.9 ± 2.0
Acetate	4 h	49.2 ± 1.1[a)]	32.3 ± 0.1	28.2 ± 0.2[b)]	23.5 ± 0.3	15.3 ± 0.2	18.3 ± 0.5[b)]	31.9 ± 1.3	28.9 ± 0.9	21.7 ± 1.5	11.0 ± 1.6[a)]	4.0 ± 0.1	5.9 ± 0.2[b)]
	8 h	39.6 ± 0.7[a)]	34.7 ± 0.2	46.1 ± 1.1[b)]	27.5 ± 0.8	27.1 ± 1.6	34.5 ± 0.7[b)]	37.7 ± 0.3	33.7 ± 0.7[b)]	31.6 ± 0.8	14.9 ± 3.0	8.9 ± 0.2	13.8 ± 0.4[b)]
	12 h	36.9 ± 0.05[a)]	44.4 ± 0.6	45.7 ± 1.2	45.6 ± 1.9[a)]	36.3 ± 0.8	36.2 ± 0.9	35.5 ± 0.5	31.1 ± 0.4[b)]	29.7 ± 2.0	20.1 ± 0.3[a)]	14.1 ± 1.0	14.4 ± 1.1
	24 h	69.9 ± 0.2[a)]	40.8 ± 2.1	36.0 ± 0.4	50.8 ± 1.4[a)]	39.8 ± 1.0	38.8 ± 0.9	32.9 ± 0.6	33.2 ± 0.1	30.0 ± 2.1	28.7 ± 4.1[a)]	13.1 ± 0.7	16.4 ± 1.1
Propionate	4 h	5.2 ± 0.1[a)]	2.0 ± 0.01	2.2 ± 0.4[b)]	5.8 ± 0.03[a)]	2.4 ± 0.02	2.8 ± 0.05[b)]	2.6 ± 0.1	2.5 ± 0.1	5.4 ± 0.2	5.8 ± 0.5[a)]	1.5 ± 0.1	1.5 ± 0.1
	8 h	10.6 ± 0.3[a)]	4.6 ± 0.1	4.0 ± 0.8[b)]	14.7 ± 0.3[a)]	6.2 ± 0.5	7.0 ± 0.1	5.4 ± 0.1	5.5 ± 0.1	15.0 ± 0.1	18.6 ± 4.7[a)]	3.8 ± 0.02	5.1 ± 0.1[b)]
	12 h	8.4 ± 0.3	10.1 ± 0.3	5.8 ± 0.9[b)]	23.3 ± 0.6[a)]	9.7 ± 0.2	9.9 ± 0.07	8.3 ± 0.1	5.6 ± 0.01[b)]	15.4 ± 1.2	23.3 ± 0.1[a)]	8.1 ± 0.3	8.3 ± 0.2
	24 h	15.9 ± 0.3[a)]	7.5 ± 0.6	10.8 ± 0.4[b)]	39.8 ± 2.2[a)]	25.2 ± 0.6	21.7 ± 0.3[b)]	15.2 ± 0.6	14.7 ± 0.01	22.8 ± 1.2	26.3 ± 0.5[a)]	12.3 ± 0.1	12.5 ± 0.6
Butyrate	4 h	6.1 ± 0.1[a)]	1.5 ± 0.01	1.7 ± 0.04[b)]	3.4 ± 0.01[a)]	1.3 ± 0.02	1.6 ± 0.004[b)]	2.6 ± 0.05	2.3 ± 0.05[b)]	3.3 ± 0.1	2.4 ± 0.8	3.3 ± 2.4	4.4 ± 0.1
	8 h	10.4 ± 0.2[a)]	7.5 ± 0.2	5.5 ± 0.1[b)]	5.1 ± 0.2	4.5 ± 0.5	5.6 ± 0.1	11.6 ± 0.05	12.6 ± 0.4	6.6 ± 0.001	2.4 ± 0.5	1.6 ± 0.01	3.2 ± 0.1[b)]
	12 h	7.3 ± 0.2[a)]	12.8 ± 0.4	11.6 ± 0.2[b)]	5.3 ± 0.2	4.8 ± 0.02	5.6 ± 0.02[b)]	10.5 ± 0.06	13.5 ± 0.1[b)]	4.6 ± 0.4	3.3 ± 0.03	2.7 ± 0.3	2 .7 ± 0.4
	24 h	14.7 ± 1.8	10.9 ± 0.1	16.9 ± 0.5[b)]	12.4 ± 1.4[a)]	5.7 ± 0.07	5.1 ± 0.1[b)]	8.0 ± 0.2	9.8 ± 0.2[b)]	7.4 ± 0.2	4.9 ± 0.6[a)]	2.1 ± 0.1	3.1 ± 0.2[b)]
Lactate	4 h	5.3 ± 0.8[a)]	13.4 ± 0.6	14.0 ± 0.6	3.2 ± 0.02	8.3 ± 2.3	8.4 ± 1.5	10.4 ± 0.3	16.3 ± 0.5[b)]	5.0 ± 0.5	2.3 ± 0.8	3.9 ± 0.2	4.4 ± 0.1
	8 h	0.8 ± 0.1[a)]	14.9 ± 1.0	14.2 ± 2.9	0	2.3 ± 2.3	0.01 ± 0.01	10.7 ± 2.5	18.3 ± 2.3	0	0[a)]	9.1 ± 0.2	3.6 ± 0.3
	12 h	0	1.5 ± 1.2	0	0	0.01 ± 0.01	0	0	0	0	0	0	0
	24 h	0	4.0 ± 2.1	0.2 ± 0.1	0	0	0	0	0	0	0	0	0

Values presented are mean ± SEM.

a) Indicates SCFA concentrations produced in study 1 were significantly different than SCFA concentrations produced in study 2 within a specific time point and fiber ($p < 0.05$).

b) Indicates SCFA concentrations produced with *L. reuteri* (+LR) were significantly different than those without *L. reuteri* (−LR) within a specific time point and fiber ($p < 0.05$).

detectable lactate concentrations during the entire fermentation (Table 1). Lactate concentrations between study 1 and study 2 only differed for inulin.

3.2 Effect of added *L. reuteri* on fermentation of fibers

The presence of *L. reuteri* affected SCFA production differently for each fiber: inulin, psyllium, and wheat dextrin (Table 1). Fermentation of wheat dextrin and psyllium were largely unaffected by the addition of *L. reuteri*, with wheat dextrin exhibiting increased SCFA concentrations at 8 h and psyllium exhibiting decreased SCFA concentrations at 12 h. Inulin showed the most pronounced effect of *L. reuteri*; although, this is not reflected as a consistent change in total SCFA concentrations over 24 h since total SCFA produced from inulin in the presence of *L. reuteri* increased at 4 h and decreased at 8 and 12 h. The end result is a smoother SCFA production curve; the peak at 8 h was blunted by *L. reuteri*. Glucose produced SCFA concentrations similar to psyllium with and without *L. reuteri* (data not shown). Negative control SCFA concentrations were low with and without *L. reuteri* (Table 1).

Acetate concentrations produced by inulin in the presence of *L. reuteri* decreased at 4 h and increased at 8 h, which is opposite to what was observed for total SCFA concentrations (Table 1). *L. reuteri* decreased the concentrations of acetate produced in the presence of psyllium at 8 and 12 h. Acetate concentrations were low with and without *L. reuteri* in the control flasks.

The presence of *L. reuteri* increased propionate production by inulin at 4 and 24 h and decreased propionate production at 8 and 12 h, resulting in a steady propionate production curve (Table 1). Propionate production by wheat dextrin and psyllium were minimally affected by *L. reuteri*. Wheat dextrin produced steady, high propionate concentrations while psyllium produced steady, low propionate concentrations regardless of the presence of *L. reuteri*. Propionate production by glucose was low, but measurable, without *L. reuteri* and similar to inulin in the presence of *L. reuteri* (data not shown). Negative control fermentation flasks had low propionate concentrations at 4 and 8 h (with and without *L. reuteri*), but were similar to inulin's propionate values at 12 and 24 h (Table 1).

L. reuteri affected butyrate production differently for each fiber (Table 1). Inulin was affected inconsistently by *L. reuteri* with increased production at 4 and 24 h and decreased production at 8 and 12 h in the presence of *L. reuteri*. Butyrate production by wheat dextrin was minimally affected by *L. reuteri*. *L. reuteri* increased butyrate production at 12 and 24 h for psyllium fermentation. Glucose control butyrate concentrations were similar to fibers throughout the fermentation with and without *L. reuteri* (data not shown). Negative control butyrate concentrations were low with and without *L. reuteri*.

Lactate concentrations were not affected by the presence of *L. reuteri* (Table 1). Most fibers had detectable lactate concentrations at 4 and 8 h, but not at 12 and 24 h. Psyllium was the only fiber to have a significant difference in lactate production (4 h).

4 Discussion

This study is the first to compare *in vitro* fermentability of wheat dextrin with that of inulin, PHGG, and psyllium. Chemical structure of the four fibers varies significantly and is a contributor to variations in fermentability. Wheat dextrin is a branched glucose polymer composed of indigestible (1–2), (1–3), and (1–6) bonds, and as a result is primarily indigestible, with only 10–15% being degraded in the small intestine by host enzymes, 76% being fermented in the colon, and the remainder being excreted in the feces [23]. Inulin is a fructose polymer often with a terminal glucose, joined by β(1–2) linkages [24]. Guar gum is composed of galactose and mannose residues in a ratio of 1:1.5–1.8 [25]. Mannose units form the structural backbone, linked by β(1–4) bonds. Galactose units branch from the mannose O-6 position, linked by α(1–6) bonds. PHGG, a lower M_r form of native guar gum, is produced by enzymatic hydrolysis with endo-β-D-mannanase [26]. Approximately 5% of the 1,4-β-D-mannose–pyranose bonds are susceptible to enzymatic hydrolysis, resulting in an average M_r of 20 k Da. Psyllium, derived from ispaghula husk, is a polymer containing arabinose and xylose.

Variations seen in SCFA profiles are likely due to the chemical differences between the fibers. 3-D structure, chain length, monosaccharide type, and bond type all dictate which bacterial metabolic pathways will be utilized for fiber metabolism. Inulin may be rapidly fermentable due to the presence of short fructose chains (2–5 U). Availability of bacterial enzymes to cleave specific bonds, as well as the availability of enzymes to convert nonglucose monomers into fermentation pathway intermediates dictates the fermentation properties of wheat dextrin and PHGG. Wheat dextrin was likely more fermentable than PHGG due to the monomer composition of each fiber. Once cleaved from the dextrin chain, glucose can enter bacterial fermentation pathways immediately, while mannose and galactose must be metabolized to fructose 1,6-bisphosphate and glucose 6-phosphate, respectively before entering bacterial fermentation pathways [27, 28].

High propionate concentrations were reported for the control fermentation flasks, indicating that components of the media or remaining substrate in the fecal inoculum were contributing to fermentation. Although the fecal inoculum was homogenized with a blender, it is possible that the composition of bacteria and residual substrate was not exactly uniform for each fermentation flask. The control fermenta-

tion flasks were expected to produce lower concentrations of SCFA than flasks containing fiber.

Lactate concentrations were undetectable at 12 and 24 h for most fibers, regardless of the presence of *L. reuteri*. This may indicate the use of lactate in other SCFA production pathways. Lactate can be converted to pyruvate, which may be used to produce other SCFAs [28]. Inulin is known for its prebiotic effects and is a preferred substrate for *Lactobacillus* and bifidobacteria. This preference may explain why lactate concentrations were detected during the entire fermentation.

L. reuteri likely exerted its effect on fermentation *via* enzyme contributions toward glycosidic bond cleavage and monosaccharide degradation [29]. This study showed that *L. reuteri* improved fermentation patterns for inulin by reducing the total SCFAs produced at 4 h compared to inulin fermentation without *L. reuteri* at 4 h. The rapid fermentation of inulin is thought to contribute to high gas production *in vivo*. The addition of *L. reuteri* produced a more favorable, steady fermentation curve which may lead to fewer GI side effects *in vivo*; however, this idea should be investigated in future research. *L. reuteri* did not enhance the fermentation of wheat dextrin or psyllium. These characteristics should be considered when recommending the use of a fiber supplement in conjunction with a probiotic.

Variations in SCFA concentrations between study 1 and study 2 are likely due to differences in microbiota. Reproducibility of fermentation with varying fecal donors indicates the fiber might confer similar physiological effects across the human population. Further research should be conducted in this area to identify if specific gut microbe communities contribute to fiber fermentation and to identify an optimal gut microbiota composition. Gut microbiota has been implicated with obesity; therefore, the role of dietary fiber fermentation in the development of obesity should be further examined [30].

Batch *in vitro* fermentation of PHGG and inulin has provided consistent fermentation data across studies and supports the data from this study. Absolute SCFA concentrations are difficult to compare between studies due to differences in representative microbiota and methods, but trends in SCFA production are comparable. The relative propionate concentrations produced by PHGG and inulin in this study were similar to those previously reported [14, 16]. Although wheat dextrin has not been subjected to *in vitro* fermentation previously, SCFA production by other RS3-type resistant starches has been published. Steady SCFA production by tapioca-based resistant maltodextrin (RS3) was seen during the first 12 h of fermentation, with a plateau at 24 h [31, 32]. Butyrate production followed the same pattern as total SCFA production. In contrast, the present study reported low, but steady butyrate production during the first 12 h with a sharp increase from 12 to 24 h.

The comparison of RS3 data across studies should be done with caution. Many RS3 type resistant starches differ in starch source and production methods, which may alter physiological effects and fermentability [33]. Starch source and processing influenced SCFA production when fermentation of three different RS2-types of resistant starches were tested in rats [34]. A comparison of RS3 with varying starch sources and production methods is necessary to determine if RS3-type resistant starches are fermented similarly.

The relationship between dietary fiber, SCFAs, and colon health is currently under debate [35, 36]. As more information regarding the long-term health effects of dietary fiber becomes available, the fermentability of fibers will likely become a key characteristic to consider when incorporating dietary fiber into one's diet. The interaction between probiotics and dietary fiber should be carefully considered and researched further. As this study has illustrated that the choice of probiotic and dietary fiber may influence the physiological effects seen in humans.

In conclusion, the effect of *L. reuteri* on SCFA formation is dependent on the fiber, with inulin fermentation responding favorably to the presence of *L. reuteri* and wheat dextrin and psyllium being unaffected. Wheat dextrin is a fermentable fiber with favorable total SCFA and remarkable propionate production. Wheat dextrin produced SCFAs gradually and completely over the 24 h period, in contrast to the rapid peaks and decline seen with inulin; this suggests less likely production of gas and consequently better tolerability with regards to flatulence. Wheat dextrin should produce similar physiological effects across individuals due to the reproducibility of fermentation. Combined administration of inulin with *L. reuteri* may reduce gas production *in vivo*, resulting in a more pleasant dietary intervention.

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5 References

[1] Institute of Medicine, *Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, National Academies Press, Washington DC 2005.

[2] Variyam, J. N., *Factors affecting the macronutrient intake of US adults*, Rep. No. Technical Bulletin 1901, United States Department of Agriculture, Washington, DC 2003.

[3] Elmadafa, I., Weichselbaum, E., *Energy and nutrient intake in the European Union*, Rep. No. 58 Forum Nutr., Basel, Karger, 2005.

[4] Wong, J. M., de Souza, R., Kendall, C. W., Emam, A., Jenkins, D. J., Colonic health: Fermentation and short chain fatty acids. *J. Clin. Gastroenterol.* 2006, *40*, 235 – 243.

[5] Schrezenmeir, J., de Vrese, M., Probiotics, prebiotics, and synbiotics – approaching a definition. *Am. J. Clin. Nutr.* 2001, *73*, 361S – 364S.

[6] Gibson, G. R., Dietary modulation of the human gut microflora using prebiotics. *Br. J. Nutr.* 1998, *80*, S209 – S212.

[7] Casas, I. A., Dobrogosz, W. J., Validation of the probiotic concept: Lactobacillus reuteri confers broad-spectrum protection against disease in humans and animals. *Microb. Ecol. Health Dis.* 2000, *12*, 247 – 285.

[8] Valeur, N., Engel, P., Carbajal, N., Connolly, E., Ladefoged, K., Colonization and immunomodulation by Lactobacillus reuteri ATCC 55730 in the human gastrointestinal tract. *Appl. Environ. Microbiol.* 2004, *70*, 1176 – 1181.

[9] Rosenfeldt, V., Michaelsen, K. F., Jakobsen, M., Larsen, C. N., *et al*., Effect of probiotic Lactobacillus strains on acute diarrhea in a cohort of nonhospitalized children attending day-care centers. *Pediatr. Infect. Dis. J.* 2002, *21*, 417 – 419.

[10] Rosenfeldt, V., Michaelsen, K. F., Jakobsen, M., Larsen, C. N., *et al*., Effect of probiotic Lactobacillus strains in young children hospitalized with acute diarrhea. *Pediatr. Infect. Dis. J.* 2002, *21*, 411 – 416.

[11] Wisker, E., Daniel, M., Rave, G., Feldheim, W., Fermentation of nonstarch polysaccharides in mixed diets and single fibre sources: Comparative studies in human subjects and in vitro. *Br. J. Nutr.* 1998, *80*, 253 – 261.

[12] Robertson, J. A., Ryden, P., Botham, R. L., Gibson, G., Ring, S. G., Structural properties of diet-derived polysaccharides adn their influence on butyrate production during fermentation. *Lebensm. Wiss. Technol.* 2001, *34*, 567 – 573.

[13] Rycroft, C. E., Jones, M. R., Gibson, G. R., Rastall, R. A., A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. *J. Appl. Microbiol.* 2001, *91*, 878 – 887.

[14] Velazquez, M., Davies, C., Marett, R., Slavin, J. L., Feirtag, J. M., Effect of oligosaccharides and fibre substitutes on short-chain fatty acid production by human faecal microflora. *Anaerobe* 2000, *6*, 87 – 92.

[15] Pylkas, A. M., Juneja, L. R., Slavin, J. L., Comparison of different fibers for invitro production of short-chain fatty acids by intestinal microflora. *J. Med. Foods* 2005, *8*, 113 – 116.

[16] Khan, K. M., Edwards, C. A., In vitro fermentation characteristics of a mixture of Raftilose and guar gum by human faecal bacteria. *Eur. J. Nutr.* 2005, *44*, 371 – 376.

[17] van den Heuvel, E. G., Wils, D., Pasman, W. J., Saniez, M. H., Kardinaal, A. F., Dietary supplementation of different doses of NUTRIOSE FB, a fermentable dextrin, alters the activity of faecal enzymes in healthy men. *Eur. J. Nutr.* 2005, *44*, 445 – 451.

[18] van den Heuvel, E. G., Wils, D., Pasman, W. J., Bakker, M., *et al*., Short-term digestive tolerance of different doses of NUTRIOSE FB, a food dextrin, in adult men. *Eur. J. Clin. Nutr.* 2004, *58*, 1046 – 1055.

[19] Pasman, W., Wils, D., Saniez, M. H., Kardinaal, A., Long-term gastrointestinal tolerance of NUTRIOSE FB in healthy men. *Eur. J. Clin. Nutr.* 2006, *60*, 1024 – 1034.

[20] McBurney, M. I., Thompson, L. U., Effect of human faecal inoculum on in vitro fermentation variables. *Br. J. Nutr.* 1987, *58*, 233 – 243.

[21] Goering, H. K., Van Soest, P. J., Forage fiber analysis: Apparatus, reagents, procedures, and some applications, in: US Department of Agriculture (Ed.), *Agriculture Handbook No. 379*, US Government Printing Office, Washington, DC 1970, pp. 1 – 20.

[22] Raeth-Knight, M. L., Linn, J. G., Jung, H. G., Effect of direct-fed microbials on performance, diet digestibility, and rumen characteristics of holstein dairy cows. *J. Dairy Sci.* 2007, *90*, 1802 – 1809.

[23] Vermorel, M., Coudray, C., Wils, D., Sinaud, S., *et al*., Energy value of a low-digestible carbohydrate, NUTRIOSE FB, and its impact on magnesium, calcium and zinc apparent absorption and retention in healthy young men. *Eur. J. Nutr.* 2004, *43*, 344 – 352.

[24] Niness, K. R., Inulin and oligofructose: What are they? *J. Nutr.* 1999, *129*, 1402S – 1406S.

[25] Ellis, P. R., Wang, Q., Rayment, P., Ren, Y., Ross-Murphy, S. B., Guar gum: Agricultural and botanical aspects, physicochemical and nutritional properties, and its use in the development of functional foods, in: Cho, S. S., Dreher, M. L. (Eds.), *Handbook of Dietary Fiber*, Marcel Dekker, New York 2001, pp. 613 – 657.

[26] Greenberg, N. A., Sellman, D., Partially hydrolyzed guar gum as a source of fiber. *Cereal Foods World* 1998, *43*, 703 – 707.

[27] Fraenkel, D. G., Vinopal, R. T., Carbohydrate metabolism in bacteria. *Annu. Rev. Microbiol.* 1973, *27*, 69 – 100.

[28] Singleton, P., Sainsbury, D., *Dictionary of Microbiology and Molecular Biology*, John Wiley and Sons, New York 1987.

[29] Arskold, E., Lohmeier-Vogel, E., Cao, R., Roos, S., *et al*., Phosphoketolase pathway dominates in lactobacillus reuteri ATCC 55730 containing dual pathways for glycolysis. *J. Bacteriol.* 2008, *190*, 206 – 212.

[30] Backhed, F., Ding, H., Wang, T., Hooper, L. V., *et al*., The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. USA* 2004, *101*, 15718 – 15723.

[31] Brouns, F., Arrigoni, E., Langkilde, A. M., Verkooijen, I., *et al*., Physiological and metabolic properties of a digestion-resistant maltodextrin, classified as type 3 retrograded resistant starch. *J. Agric. Food Chem.* 2007, *55*, 1574 – 1581.

[32] Fassler, C., Arrigoni, E., Venema, K., Brouns, F., Amado, R., In vitro fermentability of differently digested resistant starch preparations. *Mol. Nutr. Food Res.* 2006, *50*, 1220 – 1228.

[33] Dongowski, G., Jacobasch, G., Schmiedl, D., Structural stability and prebiotic properties of resistant starch type 3 increase bile acid turnover and lower secondary bile acid formation. *J. Agric. Food Chem.* 2005, *53*, 9257 – 9267.

[34] Ferguson, L. R., Tasman-Jones, C., Englyst, H., Harris, P. J., Comparative effects of three resistant starch preparations on transit time and short-chain fatty acid production in rats. *Nutr. Cancer* 2000, *36*, 230 – 237.

[35] Park, Y., Hunter, D. J., Spiegelman, D., Bergkvist, L., *et al*., Dietary fiber intake and risk of colorectal cancer: A pooled analysis of prospective cohort studies. *JAMA* 2005, *294*, 2849 – 2857.

[36] Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., *et al*., Review article: The role of butyrate on colonic function. *Aliment. Pharmacol. Ther.* 2008, *27*, 104 – 119.

