In vitro evaluation of prebiotic properties derived from rice bran obtained by debranning technology

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An abbreviated running headline: prebiotic potential of rice fractions

Abstract

The prebiotic ability of several rice bran fractions obtained by debranning (RBD) using human microbiota was studied in anaerobic batch cultures with agitation and pH-controlled. Fraction C (3.8-5% w/w pearling) from RBD increased the number of bifidobacteria and lactobacteria compared with the positive control, raftilose P95. RBD fermentation induced changes in the short-chain fatty acid (SCFA) profile. In addition, Fraction C revealed the highest growth of positive lactobacteria than commercial control. The present work illustrates the prebiotic capacity of RBD to modulate human microbiota and highlights that fraction C could be an economical source for use in human food as well as an interesting alternative to valorize a by-product of cereal industry.

Keywords: rice bran; debranning; batch cultures; prebiotic; human microbiota.

Introduction

Rice bran derived from the outer layer of the rice grain is one of the most abundant, underutilised co-products (Brunschwiler et al., 2013; Sharp, 1991; Vergara, 1991). Rice bran is an important source of dietary fibres such as soluble fibre and insoluble fibre (arabinoxylans, hemicellulose, lignin, β -glucans). These polysaccharides can resist digestion and absorption in the upper human gut and enter the colon, where they undergo fermentation by the colonic microbiota. The consequences of fermentation by the gut microbiota include increased faecal bulk through bacterial proliferation and the formation of microbial metabolites, for example, short-chain fatty acids and gas (Álvarez & Sánchez, 2006; Beards et al., 2010; Cummings & Macfarlane, 1991). Dietary fibre has been shown to improve health and prevent diseases, with the more soluble, viscous, and fermentable fibre sources being proposed to reduce the glycemic index, insulin sensitivity (Hallfrisch & Behall, 2000) and cholesterol absorption (Kahlon et al., 1994).

There are many studies focusing on the prebiotic potential of cereal bran products (Berger et al., 2014; Kedia et al., 2009; Yang et al., 2014). A prebiotic is a “nondigestible food ingredient that affects the host by selectively targeting the growth and/or activity of one or a limited number of bacteria in the colon, and thus has the potential to improve host health”. This is currently accepted as including increases in the populations of *Bifidobacterium* spp. and *Lactobacillus* spp., as both genera are viewed as positive for host health and have a long history of use as probiotics (Hughes et al., 2007). A number of methods have been used to evaluate the prebiotic properties of a potential substrate

(Costabile et al., 2016; Costabile et al., 2015; Likotrafiti et al., 2014; Sarbini et al., 2014). For a rapid comparative evaluation, batch fermentation vessels inoculated with faecal slurries are useful as they represent the diverse gut microflora (Connolly et al., 2010; Gómez et al., 2010; Likotrafiti et al., 2014). In addition, several vessels can be set up simultaneously. They can be used on a small scale for screening potential substrates which are only available in small quantities and the process is completed within 24 h (Rycroft et al., 2001). The method of fluorescent *in situ* hybridisation (FISH) is used for the rapid and specific identification of individual microbial cells. FISH is the genetic technique whereby genus-specific fluorescently labelled oligonucleotide probes are hybridised to bacterial rRNA extracted from a faecal or a fermentation sample. The resultant fluorescently labelled cells are then enumerated by fluorescence microscopy (McCartney, 2002).

Many researches are attempting to focus on phytonutrients in rice bran (Ardiansyah et al., 2006; Cai et al., 2005; Godber & Wells, 1994; Jariwalla, 2001; Ogué-Bon et al., 2011). Some studies have developed strategies to improve the functional properties of rice bran (Lee et al., 2014; Saman et al., 2011). However, none reported on the effect of pearling on the prebiotic level in bran fractions. Identifying the layers of bran containing the majority of prebiotics would be helpful in maximising the economics of efficiently extracting these compounds. Therefore, this study provided information regarding prebiotic properties within the rice kernel based on bran collected from different degrees of debranning. A small-scale faecal batch culture representing the human gut was used in the experiment. The fermentability and bifidogenic activity of

different rice bran fractions was monitored by bacteria enumeration using FISH method. SCFA production was also determined by gas chromatography.

Methods

Rice bran fraction preparation

The paddy rice RD6 (glutinous rice cultivar from Sisaket, Thailand) was initially dehusked using a laboratory dehusker (THU35A, Satake, Japan). Samples (200 g, moisture content 12%) were pearled with a test mill (Model TM05, Satake Engineering Co, Japan) using an abrasive wheel (sieve size no. 40) rotating at 1450 rpm to obtain rice fractions. The weight percentage of rice layers removed by pearling was calculated from the weight of rice before and after pearling (Lamberts et al., 2007). The pearling fractions obtained after 5, 10, 20, 30, 40 s and the residual kernels were labelled as fractions A, B, C, D, E and F respectively.

Chemical analysis

Total fibre, soluble fibre and insoluble fibre were measured according to the method of Prosky et al. (1992). The concentration of soluble free amino nitrogen (FAN) during fermentation was assayed by the EBC-ninhydrine colorimetric method (European-Brewery-Convention, 1973). The concentration of total reducing sugars (TRS) was determined by following the method of Miller (1959), using 3,5-dinitrosalicylic acid solution.

Fermentation monitoring

Batch culture fermentations

The method for batch culture fermentation was modified from that of Mandalari et al. (2007). The 300 ml water-jacketed fermenters were filled with 180 ml presterilised basal growth medium at pH 7.0. The fermenters were inoculated with 20 ml of faecal slurry. This faecal slurry was prepared by homogenizing 10% (w/v) fresh faeces from a healthy donor, who had not taken antibiotics for 3 months beforehand, in 0.1 M phosphate buffered saline (Oxoid), pH 7.0. Each 2 g rice fraction was added to give a final concentration of 1% (w/v) and 2 g of raftilose P95 (95% fructo-oligosaccharides) was used as a positive control. Each vessel was magnetically stirred with temperature controlled at 37°C by circulating water bath (Type GD120, Grant Instruments, UK). Culture pH was controlled at pH 6.6-6.8 by a pH controller (model 260 Electrolab, UK). Anaerobic conditions were maintained by sparging the vessels with oxygen-free nitrogen gas at 15 ml/min. Samples (5 ml) were taken from the fermenters at the start and at intervals over a 24-h incubation period. The fermentation experiments were performed in triplicate for each substrate using faecal inoculum from three volunteers.

Enumeration of bacteria

Bacteria were counted using FISH as described by Daims et al. (2005) and Rycroft et al. (2001). Samples were fixed overnight at 4°C with 4% (w/v) filtered paraformaldehyde (pH 7.2) in a ratio of 1:3 (v/v). Samples were washed twice with filtered phosphate buffered saline (0.1 M, pH 7.0), and stored at -20°C in PBS/ethanol (1:1 v/v) until further processing. When required for counting, the

fixed cells were hybridised with the appropriate genus-specific probes for differential bacterial counts, or 4,6-diamidino-2-phenylindole (DAPI) for total cell counts. The probes used consisted of Bif 164 for *Bifidobacterium* spp. (Langendijk et al., 1995), His 150 specific for *Clostridium histolyticum* subgroup (Franks et al., 1998), Bac 303 specific for *Bacteroides* spp. (Manz et al., 1996), Erec 482 specific for *Clostridium coccooides-Eubacterium rectale* group (Franks et al., 1998), Ato 291 specific for *Atopobium* spp. and Lab 158 specific for *Lactobacillus* / *Enterococcus* spp. (Harmsen et al., 1999; Harmsen et al., 2002).

Short-chain fatty acid (SCFA) analysis

The method was carried out as described by Zhao et al. (2006). The 300 µl of sample solution was acidified by adding 100 µl of 6 M HCl and left at room temperature for 10 min. The suspension was centrifuged at 13,793 g (Centrifuge model 5402, Eppendorf, West Germany) for 10 min, giving a clear supernatant and filtrated through 0.22 µm PVDF Whatman filter. Then, 100 µl of the internal standard, 10 mM ethylbutyric acid solution, were added into 400 µl of the filtered supernatant. The supernatant was finally injected in the GC (model 5890 Series II, Hewlett Packard, USA) under specific conditions. The column was an FFAP column (30 m × 0.53 mm diameter 0.50 µm, J&W Scientific, USA). Helium was used as the carrier gas with a flow rate of 14 ml/min. The head pressure was set at 10 psi and the split ratio is 10:1. The total flow is 140 ml/min. The initial oven temperature was 100°C, maintained for 0.5 min, raised to 150°C at 8°C/min, then increased to 250°C at 50°C/min, and finally held at 250°C for 2 min. The temperatures of the injector and the detector were set at 280°C and 300°C respectively. The injected volume was 1 µl and

the runtime was 10.75 min. Concentrations of SCFA were determined (in mM) and then the areas under the kinetic curves (AUC, in mM h) were calculated as (Amado et al., 2016):

$$AUC = (y_0 + 2y_1 + 2y_2 + \dots + 2y_{n-2} + 2y_{n-1} + y_n) \frac{\Delta t}{2} \quad (1)$$

where y_0 to y_n are the $n+1$ y -values defining the curve, and Δt is the sampling interval (h).

Statistical evaluations

Statistical analysis was performed using Windows Excel (Microsoft Corporation). Differences between bacteria numbers and SCFA concentrations at 0, 5, 10 and 24 hours of fermentation from each batch culture were checked for significance by paired t test assuming variances and considering both sides of distribution. Univariate analysis of variance (ANOVA) two factors with replication was used to compare the difference between each substrate and positive control in terms of their effects on bacteria population and SCFA production. Differences were significant at $P \leq 0.05$.

Results

The chemical composition of the different rice fractions obtained by debranning is shown in Table 1. The highest levels of fiber were observed in A, B and C fractions, and the higher soluble fiber was presented in B and C fractions. The lower fiber presence was detected in E and F (endosperm and embryo) that are richest in starch. Highest concentrations of FAN were obtained in B, C and D

fractions. Seed coat including spermoderm and nucellar layer are the main tissue present in the samples C and D.

In vitro batch culture fermentations were used to investigate the prebiotic activities of rice bran fractions. Samples were taken at intervals in order to monitor the levels of different bacterial groups (Figure 1) and the SCFA production. Results shown in Table 2 indicate that a significant increase in the level of total bacteria was obtained in all batch cultures from 5 h until 24 h. The number of *Bifidobacterium* spp. obviously increased, especially in the cultures of rice bran fraction B, C, and D compared with the positive control, raffinose P95 oligofructose. This result can be due to the higher content of soluble fiber and protein present in those fractions. Increments of *Lactobacillus* spp. and *Enterococcus* spp. were slightly and observed in each culture. In addition, a significant increase in the number of eubacteria and bacteroides was obtained in batch cultures of rice fraction E and F, the latter showing a greater increase. The population of *Clostridium histolyticum* and *Atopobium* spp. notably decreased in each culture after faecal inoculation while significant decrease of *C. histolyticum* in the positive control was observed after 5 h.

When all substrates were compared with positive control in terms of their effects on bacteria population, the results showed that a significant difference in bifidobacterial population was obtained in the culture of rice fraction F while the level of *E. rectale*/*C. coccoides* was significantly different in cultures of rice fraction A, B and C. The population of *C. histolyticum* was significantly different

in cultures of rice fraction D and F. However, the total bacterial population of all cultures was not significantly different.

Significant increase in total SCFA occurred in all substrates, especially in raftilose P95 and rice fraction F. The level of acetic acid increased significantly in all cultures by 24 h, while a significant increase in propionic acid was observed in rice fraction B at 10 h (data not shown). The concentration of n-butyric acid was significantly increased in both cultures of rice fraction A and F. The level of i-butyric acid, i-valeric acid, n-valeric and n-capronic acid increased slightly in all cultures (data not shown). The global productions of SCFA measured in the fermentations and calculated as AUC (in mM h) are presented in Table 3.

Fraction F and control showed the higher productions of acetic but the difference were not significant between them and in comparison with the other fractions ($p>0.05$). Similar descriptions can be defined for the production of propionic acid. Moreover, no significant differences between fractions effects were observed for the fermentative production of n-butyric, n-valeric and n-capronic ($p>0.05$). Fractions A and D were statistically the best options for the production of i-valeric and i-butyric, respectively.

The concentration of total SCFA was significantly higher in fraction F followed by raftilose P95 oligofructose and both significantly superiors to the rest of cultures ($p\leq 0.05$). These findings were confirmed by the ratio acetate:propionate:butyrate at 24 h displayed in Figure 2.

Discussion

This study has shown the prebiotic potential of rice bran fractions with different degrees of debranning. Rice bran generated from a rice milling process contains various nutritional substrates. Doesthae et al. (1979) reported that rice bran constituted approximately 10% by weight of the whole kernel which consisted of the pericarp, seed coat (with spermoderm and nucellar layers), endosperm including aleurone layer and the embryo or germ.

During initial pearling, the outer layers of the rice kernel, comprised of pericarp, seed coat, and nucellus, are removed. Subsequent pearling removes the more inner layers such as aleurone cells, and the embryo (Juliano and Bechtel, 1985), leaving polished rice, composed mainly of starchy endosperm. The concentrations of chemical components that are concentrated with abundance in the bran layers and the germ of rice have been related to the degree of pearling (Ha et al., 2006; Mohapatra & Bal, 2006). Among six different rice fractions, fraction A contained mainly the outer layer of rice kernel following by fractions B, C, D and E. Fraction F was the remains of the kernel after 40-s pearling consisting mostly endosperm.

To evaluate the prebiotic activity, it is necessary to analyse the evolution of the bacterial populations in the presence of the substrate being tested (Costabile et al., 2016). The behaviour of different bacteria in the fermentation could be monitored in mixed cultures due to synergistic, antagonistic and/or competitive effects (Mandalari et al., 2007). The fermentation process in the gut is a complex process whereby many metabolic pathways are carried out by different

groups of bacteria (Gibson & Roberfroid, 1995; Likotrafiti et al., 2014). Therefore, in this study the rice bran fractions were tested to evaluate the potential prebiotic using mixed faecal cultures. The fermentation conditions were maintained anaerobically at 37°C and pH 6.6-6.8 (Hughes et al., 2007; Rycroft et al., 2001). Raftilose P95 as a source of fructo-oligosaccharides was used as a positive control (Tuohy et al., 2005; Beards et al., 2010).

According to the prebiotic concept, changes of bacterial population could be monitored in both numbers of beneficial bacteria and disadvantageous bacteria (Hughes et al., 2007). The genera *Bifidobacterium* and *Lactobacillus* do not contain any known pathogens, and they are primarily carbohydrate-fermenting bacteria in comparison to clostridia. The products of carbohydrate fermentation, principally SCFA are beneficial to host health, while those of protein breakdown and of amino acid fermentation, which include ammonia, phenols, indoles, thiols, amines and sulphides are not healthy (Macfarlane et al., 2006). Eubacteria are involved in the production of butyric acid which is the main fuel for colonic epithelial cells (Pryde et al., 2002), while *Atopobium* spp. for instance may be considered in specific cases, certain vaginal disorders, as a potential pathogen (De Backer et al., 2006). Thus, an increase in the populations of bifidobacteria, lactobacilli and eubacteria could be used as the indicators.

In the fermentations, a significant increase of total bacteria was found in all cultures. However, the majority of the bifidobacteria and lactobacilli/enterococci grew better on the rice fraction C compared to the other selected gut bacteria. On the other hand, all of the *Bacteroides* spp. and *C. histolyticum* showed very

low growth on this fraction. This clearly indicates that the rice fraction C may allow a prebiotic effect by preferentially enhancing the growth of bifidobacteria and reducing the growth of bacteroides and clostridia. This fraction C is composed by a medium percentage of total fiber among the fractions tested but the highest concentration, together with B, of soluble fiber, FAN and TRS. This fraction B was in fact the substrate in which the second best results were observed. Similar prebiotic capacity was defined by oat bran rich in soluble fiber and FAN (Kedia et al., 2008 and 2009). In this context, rice bran with high contents of dietary fiber showed functional effect as prebiotics in human and canine microbiota (Inness et al., 2011; Kanauchi et al., 2014).

A bifidogenic effect was observed in all cultures of rice fractions except the culture of rice fraction F. In this fraction the levels of FAN, TRS and soluble fiber were certainly poor and it would explain the low prebiotic yield. A small increase in the numbers of lactobacilli/enterococci was also obtained in all cultures. Furthermore, an increase in eubacteria population was obtained in all cultures with the highest cell population in the cultures of rice fraction F. Pryde et al. (2002) reported that the proliferation of eubacteria possibly relates to an increment of butyric acid production. Bacteroides are a metabolically versatile group with respect to their ability to utilise many types of plant polysaccharides as substrates (Hopkins et al., 2003). This correlates with the significant increases in bacteroides observed in response to all rice fractions. However, the increases were modest when compared to those in bifidobacteria.

Gibson (1998) reported that the main saccharolytic species in the colonic microflora belonged to the genera *Bacteroides*, *Bifidobacterium*, *Ruminococcus*, *Eubacterium*, *Lactobacillus* and *Clostridium*. Carbohydrates that resist digestion and escape absorption in the small intestine are available for colonic bacterial fermentation in the colon resulting in the production of SCFA and a number of other metabolites such as lactate, pyruvate, ethanol, succinate as well as the gases H₂, CO₂, CH₄ and H₂S (Topping & Clifton, 2001; Likotrafiti et al., 2014). Butyric acid has been hypothesized to reduce the risk of colon cancer and to benefit inflammatory bowel disease (Floch & Hong-Curtiss, 2002). Propionate is metabolised in the liver, acting as a precursor in gluconeogenesis and lipogenesis (Velazquez et al., 1996). Acetate is the main SCFA produced and utilised by peripheral tissues (Hijova & Chmelarova, 2007).

During batch fermentation, various metabolic processes in the anaerobic bacteria took place. The major products of fermentation were SCFA mainly acetate, propionate and butyrate, but n-valerate, n-caproate, i-butyrate and i-valerate were also present. Results in Table 3 and Figure 2 show that the concentrations of SCFA produced from different rice fractions were variable. The highest product of SCFA was acetic acid followed by propionic acid and butyric acid respectively. The concentrations of branched SCFA (i-butyric, i-valeric, n-valeric and n-capronic) were trace. Hijova & Chmelarova (2007) reported that in human colon, carbohydrates are fermented by saccharolytic bacteria resulting in the production of linear SCFA (acetic acid, propionic acid and butyric acid) while fermentation of proteins and amino acids by proteolytic bacteria yield branched SCFA. However, in the present case the production of

SCFA was not concordant with the bifidogenic effect of fractions B and C and the microbial growths associated to these fractions. The fermentations in both fractions led to the lowest concentrations of SCFA in the postincubates and the highest quantity of lactic acid bacteria. In the cultures formulated with raffilose P95 and fraction F the growth of lactic acid bacteria was lower but the counts of total bacteria and the corresponding SCFA productions were greater.

Conclusions

Fraction C (3.8 to 5% pearling) has the highest prebiotic potential among all the fractions tested. The degree of pearling clearly influences the prebiotic content of bran in cereals. The prebiotic-rich layer tends to be removed earlier in the milling process since it is primarily located in the bran obtained during the first 30 seconds of pearling (higher content in proteins and soluble fiber). This suggests that a selected debranning strategy could produce a fraction with a high oligosaccharide content that could be later used to isolate the prebiotic ingredients. Results reveal that this fraction would have a prebiotic effect higher than the one found in commercial prebiotic formulations.

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Disclosure statement

The authors declare that there is not conflict of interest

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TABLE CAPTIONS

Table 1. Chemical composition of rice fractions (% in g/100 g of dry fractions).

Table 2. Bacterial populations in small-scale batch cultures using rafterlose P95 oligofructose and difference from 10 h ($p\leq0.05$).

Table 3. AUC of SCFA kinetics in faecal batch cultures using rafterlose P95 oligofructose and six different rice fractions as substrates. Results are means of three different volunteers $\pm$ SD.

FIGURE CAPTIONS

Figure 1. The fluorescent cells: *Lactobacillus* spp. (a), *Bifidobacterium* spp. (b), *Bacteroides* spp. (c), *Clostridium* spp. (d), *Eubacterium* spp. (e) and *Atopobium* spp. (f).

Figure 2. Production of the three most important SCFA (acetate, propionate and butyrate) at 24 h of faecal batch cultures using rice fractions, obtained by debranning, and rafterlose P95 oligofructose as control.

Figure 1

Figure 2

Table 1

		Chemical Composition				
		Total Fiber (%)	Soluble Fiber (%)	Insoluble Fiber (%)	TRS (g l ⁻¹)	FAN (mg l ⁻¹)
A: 0-2.3% Fraction	Pearling	34.5	1.8	32.7	1.2	8.9
B: 2.3-3.8% Fraction	Pearling	28.9	13.3	15.6	1.3	9.0
C: 3.8-5% Fraction	Pearling	19.2	9.2	10.0	1.3	9.1
D: 5-6.3% Fraction	Pearling	10.5	4.0	6.5	1.3	9.1
E: 6.3-7.3% Fraction	Pearling	8.2	3.1	5.1	1.1	7.4
F: 7.3-100% Fraction	Pearling	4.6	0.9	3.7	0.7	2.9

Table 2

Treatment	Time (h)	Bacteria population (log ₁₀ cells/ml)						
		<i>Bifidobacterium</i>	<i>Lactobacillus /Enterococcus</i>	<i>E. rectale/ C. coccoides</i>	<i>Bacteroides</i>	<i>C. histolyticum</i>	<i>Atopobium</i>	Total cell counts
Raftilose P95	0	7.5±0.1	6.7±0.1	8.0±0.2	7.7±0.1	5.8±0.2	7.9±0.3	8.7±0.1
	5	8.4±0.1 ^a	6.8±0.2	8.4±0.1	8.1±0.3	6.0±0.2	7.9±0.3	9.1±0.1 ^a
	10	8.6±0.1 ^a	7.0±0.2	8.7±0.1 ^{ab}	8.5±0.2 ^a	5.7±0.1 ^b	7.7±0.2	9.3±0.1 ^a
	24	8.8±0.1 ^{abc}	7.2±0.4	8.9±0.1 ^{abc}	8.7±0.1 ^a	5.4±0.0 ^{bc}	7.6±0.2	9.5±0.1 ^{abc}
Fraction A	0	7.6±0.1	6.7±0.2	8.0±0.2	7.7±0.0	5.9±0.1	7.9±0.2	8.7±0.2
	5	8.1±0.4	6.9±0.4	8.2±0.1	8.0±0.2	5.9±0.2	7.8±0.2	9.0±0.1
	10	8.4±0.3 ^a	7.1±0.5	8.2±0.0	8.1±0.2	5.7±0.2	7.6±0.1	9.1±0.2
	24	8.8±0.2 ^a	7.3±0.6	8.3±0.0	8.2±0.4	5.6±0.0	7.4±0.2 ^a	9.2±0.1 ^a
Fraction B	0	7.5±0.1	6.7±0.2	7.9±0.1	7.7±0.1	5.9±0.1	7.9±0.2	8.7±0.2
	5	8.4±0.3	6.8±0.2	8.1±0.1	8.2±0.0 ^a	5.8±0.1	7.8±0.2	9.0±0.1 ^a
	10	8.6±0.3 ^a	7.2±0.4	8.1±0.1	8.3±0.1 ^{ab}	5.6±0.1	7.7±0.2	9.2±0.1 ^a
	24	8.8±0.2 ^a	7.3±0.6	8.2±0.1	8.6±0.1 ^{abc}	5.6±0.1 ^a	7.6±0.1 ^a	9.2±0.1 ^a
Fraction C	0	7.6±0.1	6.7±0.2	7.9±0.1	7.7±0.0	5.9±0.1	7.9±0.2	8.7±0.2
	5	8.5±0.2 ^a	7.1±0.5	8.1±0.1	8.0±0.1 ^a	5.8±0.1	7.9±0.2	8.8±0.1
	10	8.8±0.2 ^a	7.2±0.6	8.2±0.0 ^a	8.2±0.1 ^a	5.6±0.0	7.7±0.2	9.1±0.1 ^{ab}
	24	9.0±0.2 ^a	7.3±0.6	8.2±0.1 ^a	8.4±0.2 ^a	5.6±0.1 ^a	7.5±0.1	9.2±0.1 ^{ab}
Fraction D	0	7.6±0.2	6.7±0.1	7.9±0.1	7.7±0.0	5.9±0.2	8.0±0.2	8.7±0.2
	5	8.3±0.1 ^a	6.7±0.0	8.1±0.1	8.0±0.1 ^a	5.8±0.2	7.9±0.2	9.0±0.2
	10	8.6±0.2 ^a	6.9±0.1 ^b	8.4±0.1 ^a	8.3±0.1 ^{ab}	5.8±0.4 ^b	7.8±0.2	9.2±0.2 ^a
	24	8.8±0.2 ^{ab}	6.9±0.1 ^b	8.5±0.2 ^{ab}	8.5±0.0 ^{abc}	5.7±0.3 ^b	7.8±0.2	9.3±0.1 ^a
Fraction E	0	7.5±0.1	6.7±0.2	7.9±0.1	7.7±0.0	5.9±0.2	7.9±0.2	8.7±0.2
	5	8.1±0.2 ^a	6.8±0.4	8.3±0.1 ^a	8.3±0.3	5.9±0.2	8.0±0.2	8.9±0.2
	10	8.3±0.1 ^a	7.0±0.5	8.6±0.0 ^{ab}	8.6±0.2 ^a	5.7±0.2 ^a	8.0±0.1	9.1±0.1
	24	8.6±0.2 ^{ab}	7.1±0.7	8.9±0.0 ^{abc}	8.8±0.1 ^a	5.7±0.2 ^a	8.0±0.1	9.2±0.1 ^a
Fraction F	0	7.5±0.2	6.6±0.2	8.0±0.2	7.7±0.1	5.9±0.1	7.9±0.2	8.7±0.2
	5	7.8±0.2	6.7±0.2	8.3±0.1	8.3±0.2 ^a	5.9±0.1	8.0±0.2	9.0±0.1
	10	8.2±0.1 ^a	6.7±0.2	8.6±0.1 ^{ab}	8.7±0.2 ^a	5.8±0.1 ^a	8.1±0.0	9.2±0.2 ^a
	24	8.3±0.2 ^a	6.9±0.3	8.9±0.0 ^{abc}	8.9±0.1 ^{ab}	5.7±0.1 ^{ab}	8.2±0.0	9.4±0.1 ^{ab}

Table 3

Treatment	SCFA (mM h)							Total SCFA (mM h)
	acetic	propionic	i-butyric	n-butyric	i-valeric	n-valeric	n-capronic	
Raftilose P 95	173.2±20.5	161.6±73.2	0.56±0.49	51.8±53.0	0.77±0.49	1.82±1.48	0.35±0.36	390.5±25.8
Fraction A	151.6±32.7	95.2±38.8	2.07±0.62	31.5±8.9	4.14±1.00	5.69±6.34	0.22±0.21	289.9±8.4
Fraction B	133.2±42.9	87.4±29.1	1.67±1.45	32.2±29.4	3.52±3.27	6.01±6.28	0.07±0.06	264.1±58.1
Fraction C	139.2±33.8	80.7±36.0	1.77±1.06	38.4±16.6	3.78±1.77	6.75±6.19	0.02±0.02	277.8±31.1
Fraction D	158.1±35.0	106.7±46.3	3.20±0.13	42.8±22.3	3.05±0.33	4.25±3.52	0.33±0.32	318.4±31.2
Fraction E	151.0±27.6	102.2±56.7	1.90±0.50	49.5±26.3	3.13±0.52	7.56±4.79	0.07±0.14	315.5±36.3
Fraction F	237.7±86.7	157.1±82.5	1.57±0.18	35.9±2.4	2.28±0.43	5.49±4.93	0.00±0.00	440.2±20.5