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Selective Fermentation and Prebiotic Index of Sago (*Metroxylon sagu*) Resistant Starches Type III by Rat Fecal Cultures

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Abstract. One of the criteria for prebiotic substance is its selective fermentation by beneficial gut microbes. Previously, sago resistant starch type III (RS_3) produced from *Metroxylon sagu* starch had been proven to resist digestion by gastrointestinal acidity and enzymes. In this research, sago RS_3 was evaluated for its selective fermentation and prebiotic potential by fecal cultures. Sago RS_3 was incubated with fecal microbial cultures for 72 hours at 37°C and samples were measured for microbial growth, prebiotic index, organic acid concentration and β-glucuronidase activity during the fermentation. Commercial prebiotics FOS (fructooligosaccharides) and inulin were used as comparison. Sago RS_3 was selectively fermented by fecal beneficial lactobacilli and bifidobacteria while decreasing the growth of detrimental bacteroides, clostridia and enterobacteria. The prebiotic index of sago RS_3, FOS and inulin was +12.19, +9.45 and +6.82, respectively. The butyric acid molar concentration in media with sago RS_3 was comparable with FOS and inulin, and low activity of β- glucuronidase was detected in medium with sago RS_3. Sago RS_3 exhibited prebiotic characteristics comparable with commercial prebiotics and their potential prebiotic function is worth for further *in vivo* assessment.

1. Introduction.

Human large intestine is heavily populated by numerous and diverse species of microorganism, forming a complex microflora community. Colonic microflora plays a crucial role in maintaining the proper intestinal functions and this influences the host health by impacting the development of immune system, inhibiting the growth of pathogen and regulating metabolic pathways in the host [1]. Hence, colonic microflora must be maintained in a balanced state which predominantly constitutes of health promoting bacteria, for instance, lactobacilli and bifidobacteria. Imbalance in the composition of colonic microflora may be linked to numerous diseases such as colorectal cancer and inflammatory bowel disease [2].

A promising strategy, whereby involving the usage of prebiotic was introduced [3]. A prebiotic is a non-digestible carbohydrate that selectively stimulates the growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the [4]. A prebiotic ingredient should resist towards the digestions in the upper gastrointestinal tract and intestinal absorption, and be selectively fermented by intestinal microflora associated with beneficial effects [5]. The demand for prebiotic food is growing rapidly and is expected to reach USD$5.5 billion by the year 2020 [6]. Hence, many researches are in the progress of studying various sources of carbohydrate for their prebiotic potential. Oligosaccharides such as fructooligosaccharides,

galactooligosaccharides and trans-galactooligosaccharides are commonly available commercial prebiotics [7] whereas resistant starch as prebiotic is not commercially available. Emerging evidences had revealed that resistant starch could be a potential prebiotic [8]. Sago RS_3 is known for its resistance to digestion by gastric acidity and pancreatic enzymes, and resistant to absorption along the upper intestinal tract [9]. With the beneficial effects of resistant starch towards health [8], it is suggested to incorporate RS in the formulation of various food products as many of the commonly available foods contain low RS amount of less than 10 g/100 g [9] whilst recommended daily consumption of RS is 20 g [10]. No information on the advantages of sago RS3 compared to other prebiotics is available.

In this study, sago RS_3 was evaluated for its fermentation and prebiotic index by fecal cultures. The metabolic activities (organic acids production and β-glucuronidase activity) of the diverse species of gut microorganism will be monitored. The changes of these bacteria population allow us to determine the fermentation selectivity of sago RS_3. The fermentation of sago RS_3 by fecal cultures will be compared with commercial prebiotics FOS and inulin.

2. Materials and methods

2.1 Materials.
Native sago starch used to produce sago resistant starch type III (RS_3) was purchased from a local grocery in Kuching, Sarawak, Malaysia. Fructooligosaccharides (Orafti® P95) and inulin (Orafti® GR) were purchased from BENEO-Orafti, Tienen, Belgium. Pullulanase debranching enzyme (Promozyme®D2) was purchased from Novozymes (Bagsvaerd, Denmark) and used upon receipt. Unless otherwise specified, all other chemicals used were of analytical grade and were purchased from Sigma-Aldrich Inc., St. Louis, Missouri, USA.

2.2 Fecal samples.
The fecal samples were collected from eight healthy male Sprague Dawley rats, housed in the Animal Research and Service Centre, Universiti Sains Malaysia. The 3-month old rats were not on antibiotics or probiotics/prebiotics. The fresh feces were pooled and stored in sterile sealed bags and immediately transported to the laboratory and dispensed into sterile 0.17 M phosphate buffered saline [11] at a concentration of 10% (w/v) in an anaerobic condition. The feces were homogenized using a stomacher for 2 min and used as an inoculum for the fermentation experiment after filtering through two layers of muslin cloth.

2.3 Preparation of Sago RS_3.
Sago RS_3 was produced according to the method as described by Zi-Ni et al. [12].

2.4 Fermentation Media.
Basal fermentation medium [13] contained (per L): 2 g peptone, 2 g yeast extract, 2 g $NaHCO_3$, 0.5 g bile salts, 0.5 g L-cysteine.HCl, 0.1 g NaCl, 0.04 g K_2HPO_4, 0.04 g KH_2PO_4, 0.01 g $MgSO_4.7H_2O$, 0.01 g $CaCl_2.6H_2O$, 0.005 g Hemin, 2 mL Tween 80, 10 µL Vitamin K1 and 4 mL Resazurin solution (0.025%, w/w). The fermentation media was adjusted to pH 7.0 with 0.5 M of phosphoric acid and sterilized at 121°C for 15 min.

2.5 Fermentation.
Fermentations were carried out in 1 L capped bottles at 37°C for 72 h. Native sago starch, sago RS_3, glucose, inulin or FOS at 10% (w/v) was dispersed in 850 mL of the basal fermentation medium. Each bottle was inoculated with 10% (v/v) of fecal slurry consisted of 10^8-10^9 CFU/mL of total bacteria. Inoculated medium without a carbohydrate source served as a blank. Periodically, 20 mL of sample was withdrawn and immediately analyzed for bacterial viable count using agar plate count method. Sample was centrifuged at 2330 × *g*, 4°C for 15 min to obtain cell-free supernatant and the supernatant were analyzed for organic acids concentration and β-glucuronidase activity. The inoculation and plating were performed in an anaerobic chamber (Forma 1025 Anaerobic System, Thermo Scientific, Marietta, Ohio, USA) supplied with an atmospheric composition of 85% N_2, 10% CO_2 and 5% H_2.

2.6 Bacterial Viable Count.

One mL sample was serially diluted in 9 mL of half strength of Wilkins Chalgren Anaerobic Broth (HiMedia, Mumbai, India) in anaerobic condition. The mixture was pour plated on selective agars: nutrient agar (total aerobes); Wilkins Chalgren Anaerobic agar (total anaerobes); Bifidobacterium agar (bifidobacteria); Lactobacillus Selection agar (lactobacilli); Bacteroides Bile Esculin agar (bacteriodes); Clostridia agar (clostridia) and MacConkey agar with 0.15% Bile salts, crystal violet and NaCl (enterobacteria), incubated at 37°C for 48-72 h, and the viable count was reported as $\log_{10}$ CFU/mL. The agars were purchased either from Merck, Darmstadt, Germany, HiMedia, Mumbai, India or BD Diagnostic, Heidelberg, Germany.

2.7 Prebiotic Index

Prebiotic index of the substrates (native sago starch, sago RS, HCl-sago RS, inulin and FOS) were calculated using the equation as follows [14]:

$$\text{Prebiotic Index} = \left(\frac{\text{Bif}}{\text{Total}}\right) + \left(\frac{\text{Lac}}{\text{Total}}\right) - \left(\frac{\text{Bac}}{\text{Total}}\right) - \left(\frac{\text{Clos}}{\text{Total}}\right) \quad \text{Eq. (1)}$$

Whereby:

Bif was count of bifidobacteria at sampling time over count of bifidobacteria at 0 h of fermentation; Lac was count of lactobacilli at sampling time over count of lactobacilli at 0 h of fermentation; Bac was count of bacteroides at sampling time over count of bacteroides at 0 h of fermentation; Clos was count of clostridia at sampling time over count of clostridia at 0 h of fermentation; Total was total bacterial count (anaerobes and aerobes). Bacterial counts were expressed as colony forming unit (CFU) per mL.

2.8 pH and Organic Acids Concentration.

pH of the fermentation samples was measured by using a pH meter (Mettler Toledo, Shanghai, China). Organic acids concentrations were determined using a HPLC system [15]. Cell-free supernatant was filtered using 0.45 µm nylon membrane syringe filter (Titan 2, Sun Sri, Rockwood, Tennessee, USA) and injected onto a HPLC system (Shimadzu, Kyoto, Japan) equipped with a UV-VIS detector (Model SPD-20A, Shimadzu, Kyoto, Japan), fitted with an IC-PakTM ion-exclusion column (7.8 x 300 mm; Waters, Milford, Massachusetts, USA). The concentration of organic acids in the samples were quantified using the external standard calibration curves of lactic, acetic, succinic, propionic and butyric acids (Sigma-Aldrich, Steinheim, Germany).

2.9 β-glucuronidase activity

The cell-free supernatants were assayed for β-glucuronidase activity according to [16]. One unit of enzyme activity was equivalent to 1 µmole of ρ-nitrophenol released from ρ-nitrophenyl-β-D-glucuronide per mL at 37°C after one hour of reaction. The specific activity was expressed as unit of β-glucuronidase activity (U) per mg of protein.

2.10 Statistical analysis

The triplicate data was subjected to repeated measures analysis of variance (ANOVA), and the significance of the differences between means was determined by the Duncan test, where $p<0.05$ was considered statistically significant. The Statistical Package for Social Sciences, Version 20 (SPSS Inc., USA) was used for the analysis.

3. Results and discussion

3.1 Bacterial growths

Table 1 shows the viable counts of fecal microorganisms; total anaerobes, total aerobes, bifidobacteria, lactobacilli, bacteroides, clostridia and enterobacteria during 72 h fermentation in blank medium (without carbohydrate source) and media containing glucose, native sago starch, sago RS_3, FOS and inulin. Initially, at 0 h the fecal cultures contained an average of 7.76 ± 0.03 $\log_{10}$ CFU/ml total aerobes, 6.04 ± 0.02 $\log_{10}$ CFU/ml total aerobes, 6.54 ± 0.02 $\log_{10}$ CFU/mL bifidobacteria, 5.75 ± 0.02 $\log_{10}$ CFU/mL lactobacilli, 4.83 ± 0.01 $\log_{10}$ CFU/mL bacteroides, 7.23 ± 0.02 $\log_{10}$ CFU/mL clostridia and 4.75 ± 0.01 $\log_{10}$ CFU/mL enterobacteria.

Table 1. Bacterial population (log_{10} CFU/mL) in media containing different substrates during 72 hours of fermentation by rat fecal culture

Time	Substrates					
(h)	Blank	Glucose	Native Starch	Sago RS	FOS	Inulin
	Total Anaerobes					
0	7.77±0.11abc	7.82±0.11cd	7.77±0.17d	7.73±0.10e	7.72±0.05d	7.77±0.13e
4	7.63±0.29bc	8.72±0.20a	7.85±0.13d	8.59±0.18b	8.61±0.07b	8.47±0.16bc
8	7.78±0.24abc	8.94±0.10a	8.01±0.12bcd	8.68±0.11b	8.83±0.08a	8.58±0.10ab
12	7.96±0.17ab	8.26±0.17b	8.12±0.11abc	8.95±0.24a	8.59±0.09b	8.82±0.24a
24	8.06±0.24a	8.12±0.21bc	8.21±0.17ab	8.61±0.12b	8.29±0.18c	8.26±0.19cd
48	7.57±0.17cd	7.59±0.17de	8.28±0.15a	8.32±0.12c	7.68±0.17d	8.05±0.22de
72	7.26±0.04d	7.28±0.04e	7.94±0.03cd	8.00±0.02d	7.59±0.10d	7.84±0.11e
	Total Aerobes					
0	6.02±0.07a	6.01±0.06e	6.04±0.07b	6.06±0.02b	6.05±0.08d	6.06±0.05c
4	6.17±0.26a	7.40±0.09a	6.43±0.12ab	6.99±0.16a	7.15±0.07a	7.24±0.19a
8	6.52±0.12a	7.14±0.14ab	6.76±0.13ab	7.10±0.18a	6.63±0.14bc	6.98±0.16ab
12	6.48±0.07a	6.83±0.19bc	6.91±0.22a	7.15±0.18a	6.87±0.17ab	6.84±0.18ab
24	6.38±0.21a	6.62±0.20cd	7.03±0.17a	7.02±0.17a	6.77±0.22abc	6.91±0.12ab
48	6.14±0.49a	6.32±0.35de	6.96±0.15a	6.99±0.15a	6.54±0.20bc	6.84±0.24ab
72	5.98±0.45a	6.18±0.26e	6.90±0.27a	6.95±0.14a	6.42±0.19cd	6.69±0.27b
	Bifidobacteria					
0	6.57±0.06a	6.51±0.10c	6.55±0.05c	6.53±0.11d	6.52±0.07d	6.56±0.08d
4	6.61±0.05a	6.82±0.12bc	6.79±0.03bc	6.80±0.09c	6.81±0.04c	6.79±0.09c
8	6.70±0.11a	7.33±0.06a	6.91±0.05b	7.19±0.06b	7.24±0.11b	7.13±0.09b
12	6.70±0.11a	7.46±0.17a	7.11±0.13ab	7.82±0.14a	7.88±0.13a	7.68±0.18a
24	6.62±0.23a	7.26±0.13a	7.12±0.22ab	7.81±0.14a	7.70±0.14a	7.57±0.15a
48	6.28±0.23a	6.91±0.10b	7.39±0.25a	7.80±0.15a	7.19±0.17b	7.46±0.15a
72	6.26±0.11a	6.65±0.14bc	7.09±0.30a b	7.69±0.16a	7.17±0.18b	7.11±0.11b
	Lactobacilli					
0	5.72±0.10abc	5.76±0.10bc	5.74±0.11b	5.76±0.10c	5.74±0.11b	5.76±0.11d
4	5.89±0.26ab	6.70±0.11a	6.02±0.20ab	6.61±0.13b	6.27±0.16a	6.00±0.20cd
8	5.98±0.20a	6.89±0.12a	6.32±0.11a	6.76±0.22ab	6.37±0.12a	6.13±0.11bc
12	5.96±0.08a	6.59±0.06a	6.39±0.11a	6.96±0.17a	6.51±0.11a	6.35±0.10ab
24	5.90±0.39ab	6.10±0.24b	6.41±0.18a	6.87±0.13ab	6.46±0.28a	6.59±0.16a
48	5.50±0.06bc	5.87±0.14bc	6.22±0.15a	6.77±0.11ab	6.36±0.05a	6.51±0.09a
72	5.37±0.18c	5.70±0.14c	6.01±0.11ab	6.76±0.08ab	6.20±0.13a	5.92±0.14cd
	Bacteroides					
0	4.84±0.10c	4.83±0.09c	4.82±0.11d	4.84±0.11b	4.83±0.09c	4.82±0.11
4	4.76±0.25c	5.16±0.15b	4.93±0.12d	5.14±0.15b	5.04±0.13b	5.00±0.15c
8	5.11±0.15bc	5.36±0.21a	7.02±0.10c	6.09±0.17a	5.99±0.15a	5.29±0.16b
12	5.27±0.25ab	0d	7.23±0.05b	6.22±0.14a	4.78±0.11c	6.32±0.16a
24	5.54±0.27a	0d	7.37±0.13ab	5.00±0.17b	0d	3.73±0.16d
48	5.07±0.15bc	0d	7.42±0.11a	3.66±0.30c	0d	0e
72	4.73±0.20c	0d	7.01±0.23c	2.39±0.14d	0d	0e
	Clostridia					
0	7.24±0.13a	7.24±0.19bc	7.24±0.12c	7.20±0.13c	7.24±0.13c	7.22±0.17b
4	7.10±0.14a	8.38±0.09a	7.47±0.08ab	8.07±0.10a	8.13±0.18a	8.04±0.13a
8	7.21±0.19a	7.99±0.11a	7.50±0.09ab	7.97±0.12a	7.86±0.10ab	8.00±0.09a
12	7.20±0.21a	7.55±0.14b	7.52±0.10ab	7.88±0.10a	7.70±0.22b	7.95±0.13a
24	7.30±0.17a	6.99±0.46cd	7.55±0.11ab	7.60±0.14b	7.12±0.26c	7.22±0.20b
48	7.02±0.24a	6.68±0.33d	7.61±0.11a	7.17±0.22c	6.71±0.27d	6.88±0.38b
72	6.18±0.06b	5.66±0.12e	7.39±0.11bc	6.65±0.09d	5.74±0.20e	5.78±0.18c

Table 1. Continued

Time (h)	Substrates					
	Blank	Glucose	Native Starch	Sago RS	FOS	Inulin
	Enterobacteria					
0	4.75±0.07c	4.75±0.10c	4.75±0.12c	4.76±0.10d	4.73±0.05c	4.75±0.08e
4	5.36±0.37bc	7.10±0.12a	5.36±0.38b	6.00±0.07b	6.80±0.10a	5.82±0.25bc
8	5.78±0.38ab	5.46±0.58b	6.11±0.23a	6.52±0.11a	5.91±0.29b	6.24±0.16a
12	6.05±0.18a	0d	6.28±0.19a	6.38±0.11ab	4.89±0.25c	6.07±0.17ab
24	5.80±0.14ab	0d	6.35±0.22a	5.99±0.11b	0d	5.60±0.24c
48	5.58±0.16ab	0d	6.43±0.11a	5.46±0.20c	0d	5.19±0.35d
72	5.33±0.15bc	0d	6.46±0.20a	5.19±0.21c	0d	5.05±0.31de

Results are expressed as means with standard deviation (N=3). Mean values in the same column followed by different lower case alphabets[abcde] within a particular substrate are significantly different (p<0.05) throughout the fermentation. Statistical Significance of Effect (within each group of bacteria): Effect of time, t: p<0.05; Effect of substrate, S: p<0.05; Interaction, t x S: p<0.05.

3.2 Selective bacterial growth as affected by different carbohydrate sources

It was observed that the growth of bifidobacteria and lactobacilli were sustained until 48 h of fermentation whereas bacteroides, clostridia and enterobacteria had started to show a significant decrease (p<0.05) at 24 h, 12 h and 24 h of fermentation, respectively. Sago RS_3 media exhibited higher growths of the beneficial bacteria than FOS and inulin suggesting that sago RS_3 is a potential prebiotic. Media containing sago RS_3 could sustain the growth of beneficial bacteria for 48 h of fermentation with a concomitant impediment in the counts of detrimental bacteria. In a previous *in vivo* research, an increase in the growth of bifidobacteria and lactobacilli was observed in the rat caecal content with a concomitant decrease in the counts of enterobacteria after 4 weeks of consuming retrograded high amylose corn starch [17]. However, neither *in vitro* nor *in vivo* studies had investigated the selective fermentation of sago RS_3.

3.3 Prebiotic index (PI)

PI is a quantitative approach used to magnitude the prebiotic effect of a substrate. Positive PI value indicates that the substrate exerts an increase in the counts of beneficial bacteria (bifidobacteria and/or lactobacilli) whereas negative value of PI indicates an increase in the counts of detrimental bacteria, which were bacteroides and/or clostridia [14]. Generally, all the substrates exhibited prebiotic characteristics with positive PIs were achieved by the substrates after 12-48 h of fermentation (Figure 1). The highest PI (p<0.05) was displayed by sago RS_3 (+12.19), followed by FOS (+9.45) and inulin (+6.82). PI of sago RS_3 at 48 h (+7.00) was not significantly different from that of inulin. Thus, this suggested that sago RS_3 could pronounce prebiotic effect similar to the commercial prebiotics.

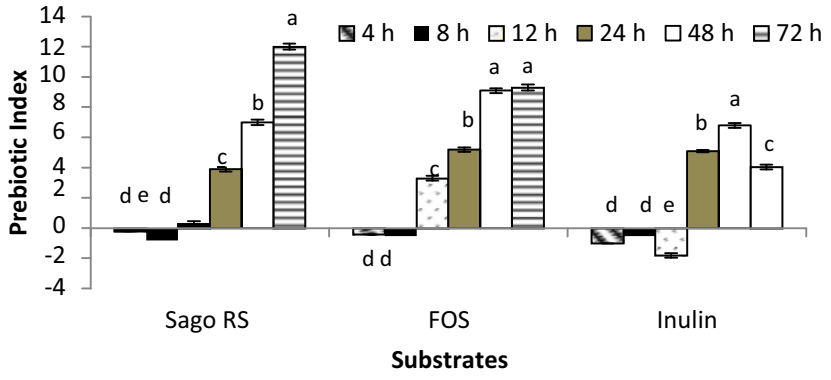

Figure 1. Prebiotic index of sago RS, HCl-sago RS, FOS and inulin. Error bars represent standard deviations of N=3. Bars bearing different alphabets within a particular substrate are significantly different (p<0.05). Value with * is significantly the highest (p<0.05) index. Statistical Significance of Effect: Effect of time, t: p<0.05; Effect of substrates, S: p<0.05; Interaction, t x S: p<0.05.

3.4 pH and organic acids production

Generally, all media with the addition of carbohydrate sources could show a significant decrease (p<0.05) in the pH value from the initial pH of ~6.52 to pH 3.38 (Data not shown). Reducing the fermentation pH is regarded as the mechanism of action of dietary carbohydrate attributed to the production of organic acids [18].

Overall, all the media display significant (p<0.05) increase in the production of lactic and short chain fatty acids (acetic, propionic and butyric acids) (Table 2).

Table 2. Molar concentration of organic acids (mM) in media containing different substrates during 72 hours of fermentation by rat fecal culture

Time (h)	Blank	Glucose	Native Starch	Sago RS	FOS	Inulin
	Lactic acid					
0	0c	0g	0d	0f	0g	0g
4	0c	18.56±0.10f	0.58±0.03b	12.80±0.24e	16.24±0.26f	10.99±0.13f
8	1.95±0.10a	37.15±0.23e	1.96±0.03a	20.82±0.04d	30.20±0.64e	14.02±0.60e
12	1.42±0.10b	59.73±0.5d	0.28±0.01c	27.48±0.05a	38.66±0.23d	20.74±0.55d
24	0c	68.20±1.03c	0.00d	25.65±0.52b	56.20±0.44c	34.37±0.67c
48	0c	75.60±0.45b	0.00d	24.80±1.01b	64.73±0.09b	50.69±1.69b
72	0c	79.08±1.37a	0.00d	23.27±0.64c	75.42±1.15a	55.34±0.73a
	Acetic Acid					
0	0g	0f	0f	0e	0g	0f
4	0.79±0.04f	4.90±0.05e	1.25±0.02e	4.97±0.17b	3.62±0.08f	1.24±0.04e
8	1.38±0.03e	5.40±0.07d	8.08±0.05d#	5.17±0.10ab	4.47±0.17e	3.57±0.15d
12	3.80±0.06d	5.96±0.03c	8.10±0.03d#	5.42±0.05a	5.91±0.06d	4.89±0.07c
24	4.17±0.13c	6.77±0.15b	11.84±0.14c*#	4.50±0.13c	6.86±0.09c	6.01±0.27b
48	4.84±0.22b	6.74±0.15b	16.90±0.38b*#	3.38±0.40d	7.94±0.08b	6.04±0.12b
72	5.42±0.19a	7.02±0.17a	17.46±0.43a*#	3.08±0.08d	8.97±0.07a	6.54±0.18a
	Propionic Acid					
0	0e	0g	0g	0f	0g	0n
4	0e	1.28±0.04f	1.77±0.06f	2.37±0.09e	1.09±0.04f	0.66±0.04f
8	1.23±0.04d	4.28±0.07e	3.89±0.11e	6.38±0.26d	2.14±0.02e	1.62±0.10e
12	2.00±0.06c	4.54±0.05d	5.01±0.06d	9.98±0.47c	3.85±0.09d	3.11±0.15d
24	2.48±0.09b	8.89±0.16c	9.17±0.25c	18.87±0.80b*	7.07±0.08c	10.85±0.43c
48	2.47±0.07b	12.15±0.12b	9.48±0.06b	20.16±0.58a*	13.73±0.52b	16.39±0.39b
72	3.39±0.20a	13.93±0.24a	11.55±0.04a	20.84±0.26a*	16.60±0.22a	26.74±0.10a
	Butyric Acid					
0	0g	0g	0g	0f	0g	0f
4	0.12±0.01f	0.03±0.00f	0.04±0.00f	0.02±0.00f	0.74±0.01f	0.11±0.00e
8	0.17±0.01e	0.05±0.00e	0.20±0.01e	0.32±0.01e	0.87±0.00e	0.20±0.02d
12	0.29±0.01d	0.34±0.01d	0.23±0.01d	1.98±0.08d	1.66±0.01d	0.27±0.01d
24	0.42±0.02c	0.97±0.02c	0.80±0.01c	2.92±0.04c*#	1.70±0.01c	1.56±0.09c
48	0.78±0.00b	1.50±0.01b	1.83±0.01b	4.08±0.02b*#	1.86±0.02b	1.98±0.02b
72	0.82±0.00a	1.61±0.02a	2.21±0.01a*	4.21±0.12a*#	1.90±0.03a	2.47±0.06a

Results are expressed as means with standard deviation (N=3). Mean values in the same column followed by different lower case alphabets within a particular substrate are significantly different (p<0.05) throughout the fermentation. Statistical Significance of Effect: Effect of time, t: p<0.05; Effect of substrate, S: p<0.05; Interaction, t x S: p<0.05. Within a particular type of short chain fatty acid, *values are significantly higher (p<0.05) than the highest concentration of acid in media containing FOS at 72 h while #values are significantly higher (p<0.05) than the highest concentration of acid in media containing inulin at 72 h.

Acetic, propionic and butyric acids are the predominant short chain fatty acids generated in the human colon, accounting for 85-95% of the total short chain fatty acids produced [19]. Accumulation of lactic acid was seen in media containing glucose, FOS and inulin throughout the fermentation period and reached the maximal level at 72 h of fermentation with the highest concentration of 79.08

mM, 75.42 mM and 55.34 mM, respectively. The concentration of acetic acid in media containing sago RS_3 increased significantly ($p<0.05$) before a depletion occurred at the 24 h of fermentation and the concentration of propionic acid in media containing sago RS_3 (18.87-20.84 mM) was significantly higher ($p<0.05$) after 24 h than that of by FOS (16.60 mM) at 72 h of fermentation. The production of butyric acid increased continuously throughout the fermentation period with the highest concentrations of 4.21 mM (sago RS_3), 2.47 mM (inulin), 2.21 mM (native starch), 1.90 mM (FOS) and 1.61 mM (glucose) at 72 h of fermentation. The highest concentration of butyric acid production by sago RS_3 was significantly higher ($p<0.05$) than that of by the commercial prebiotics, FOS and inulin.

3.5 β-glucuronidase activity

Media containing glucose, sago RS_3, FOS and inulin showed significant decrease ($p<0.05$) in the activity of β-glucuronidase throughout the fermentation period whereas blank medium and medium with native sago starch exhibited a significant increase ($p<0.05$) until 48 h of fermentation concomitant with the growth of bacteroides, clostridia and enterobacteria as they are the producers of β-glucuronidase (Figure 2).

Patients diagnosed with colon cancer were found to have a higher activity of fecal β-glucuronidase than that of healthy people [20]. Thus, lowering β-glucuronidase activity would be desirable as this enzyme plays a crucial role in promoting carcinogenesis.

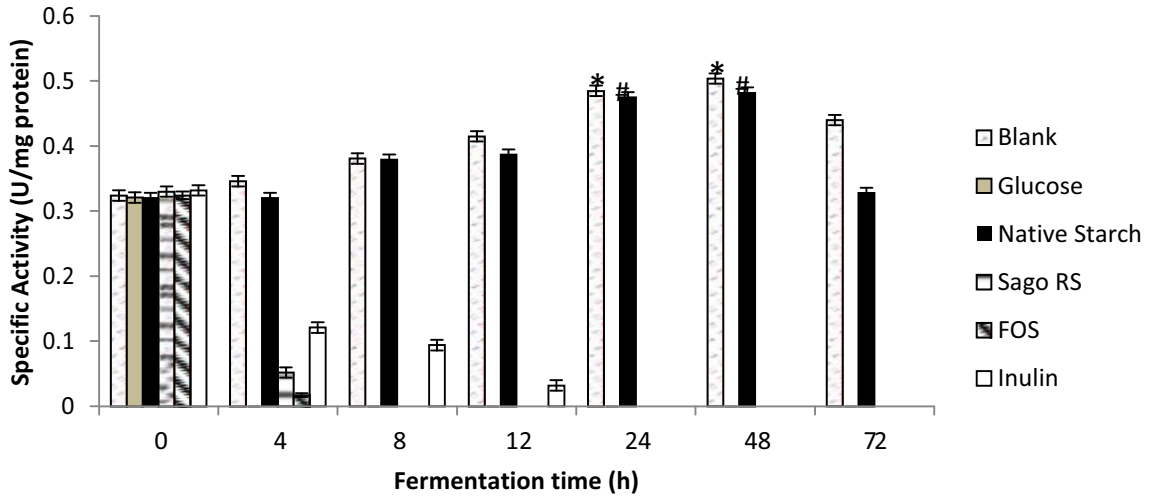

Figure 2. Activity of β-glucuronidase during fermentation of different substrates by rat fecal cultures. Error bars represent standard deviations of N=3. Bars bearing same symbols (*, #) within same substrate are not significantly different ($p>0.05$). Statistical Significance of Effect: Effect of time, t: $p<0.05$; Effect of substrates, S: $p<0.05$; Interaction, t x S: $p<0.05$.

4. Conclusions

Sago RS_3 exhibited comparable prebiotic effects with commercial prebiotics FOS and inulin, whereby sago RS_3 stimulated the growth of beneficial bacteria (lactobacilli and bifidobacteria) at the expense of detrimental bacteria (bacteroides, clostridia and enterobacteria). Sago RS_3 displayed the highest prebiotic index and fermentation of sago RS_3 produced the highest concentration of butyric acid while reduced β-glucuronidase activity.

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