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In vitro investigation of orange fleshed sweet potato prebiotic potential and its implication on human gut health

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ABSTRACT

Background: Some food ingredients (prebiotics) have been shown to promote a healthy gut by selectively stimulating growth/activity of beneficial gastrointestinal microbes and metabolites such as short chain fatty acids (SCFA) while inhibiting pathogens. Orange fleshed sweet potato (*Ipomoea batatas* Lam; OFSP) root tuber is a starchy tropical crop and highly nutritious in terms of pro-vitamin A (beta carotene), dietary fibre, and natural sugars, with negligible amount of fats and cholesterol.

Purpose of study: The aim of the study was to investigate using simulated human gut system whether OFSP may have prebiotic activity derived from their fibre, resistant starch, and/or the sugars.

Methodology*: In vitro* pH controlled stirred batch culture fermentation system was used to compare the effect on human gut microbiota of four substrates: two varieties of OFSP (SPK 004 and Tainung), FOS and sucrose known for positive prebiotic and non-selective change respectively. The system was inoculated with faecal slurry from six different human healthy donors from different ethical backgrounds, age, and the effectual change recorded over 24 hours by monitoring bacterial counts (total bacteria, *Bacteroides* and *Bifidobacterium*) using qPCR molecular technique and SCFA profiles by gas chromatography.

Results: The total bacteria count increased by (0.92-1.7 $\log_{10}$) and *Bacteroides* genus (1.03-1.8 $\log_{10}$) throughout the experimental period but with no significant differences ($p<0.05$) between the four substrates. However, there were significant differences ($p<0.05$) in the beneficial *Bifidobacterium* (1.66-2.66 $\log_{10}$) between the 2 varieties of OFSP and the two controls (FOS and sucrose). The levels of SCFA increased, with acetate as the predominant acid and lactic acid being the least. The OFSP purees elicited high butyric acid levels, which were comparable to those of positive control FOS.

Conclusions: The study demonstrated that OFSP purees may have prebiotic potential that can positively modulate gut microbiota by promoting growth of beneficial bacteria, *bifidobacterium* genus, and stimulating production of SCFA especially butyric acid which is the favourable in human gut health. However, further research using more probiotic and pathogenic microbes in addition to *in vivo* clinical studies and compositional analysis of OFSP is needed to confirm prebiotic activity.

Key words: Orange fleshed sweet potato, prebiotic, human gut microbiota

BACKGROUND

Consumption of foods rich in whole-grains, wild species of fruit, vegetables, seeds, root tubers, and gums which are rich in dietary fibre, oligosaccharides, inulin, and other complex polysaccharides have been shown to promote a healthy gut by increasing the relative abundance of *bifidobacteria/lactobacillus* and other butyrate-producing bacteria. While diets high in animal protein (particularly red and processed meats), eggs, protein, alcohol, energy, fat, sulphur, and sulphate have been linked to the negative modulation of colon microbial population and/or metabolisms by increasing the concentration of pathogenic microbes and toxinogenic metabolites [1-5]. Dietary prebiotic, the term used to define 'food ingredient(s) that are selectively fermented in the gut resulting in specific changes in the composition and/or activity of the gastrointestinal microbiota, thereby conferring benefit(s) upon host health,' are resistant to digestion by human enzymes and absorption in the small intestines but are fermentable/metabolized by some colon bacteria to produce beneficial metabolites. Fructooligosaccharide (FOS) is one of the prebiotic carbohydrates that have been proven *in vitro* and *in vivo* to have prebiotic activity by increasing levels of beneficial microbes especially *bifidobacteria*, thereby causing a healthier human colon [6-7]. Synbiotic, mixtures of prebiotics and probiotics (live beneficial microorganisms), in functional foods have been developed to improve the survival and implantation of probiotics in the human gut system [8].

From a nutritional point of view and perspective, orange fleshed sweet potato (OFSP) tubers were ranked from as number one among all vegetables [9-10], although they are also known to cause flatulence in some individuals when consumed in large quantities like other well-known prebiotic whole foods. This may be due to digestion resistance of some of its constituents by enzymes in human upper gastrointestinal tract which on proceeding to the colon are fermented by microbes producing the flatulence gases [11-14]. Several variety/cultivar of OFSP tubers have

been developed [15] whose composition vary significantly depending on age of root [16], climatic conditions [17], and soil fertility [18].

Saccharolytic and proteolysis are the two main types of colonic microbial fermentation which mainly occur in the proximal and distal colon respectively [19-22]. The principal substrates for saccharolytic metabolism are non-digestible carbohydrates including oligosaccharides, gums, resistant starches, cellulose, hemicellulose, pectins, unabsorbed sugars, and alcohols, which on fermentation increases the relative abundance of desirable butyrate and other short-chain fatty acids (SCFA), organic acids such as lactate, vitamins producing bacteria and gases (CO_2, CH_4, H_2 and H_2S) that acidify the colon [23-27]. The acidic environment promotes proliferation of beneficial bacteria population such as *bifidobacteria* and/or *lactobacilli* [28-29], in addition to increasing mineral bioactivity and absorption [30-31]. Proteolytic fermentation promote growth of pathogenic bacteria, such as some species of *Bacteroides* and *Clostridium*, which produce toxigenic microbial metabolites (nitrogenous compounds e.g. ammonia amines, phenol substituted fatty acids, and hydrogen sulphide) and/or carcinogenic/genotoxic comounds that cause molecular receptor decoying, thereby inhibiting bacterial adhesion and antibiotic-associated diarrhoea [32-33] The majority of SCFA (acetate, propionate, and butyrate) and lactate are absorbed into the blood stream and can be further metabolized in the liver, muscle, brain, or other peripheral tissues, contributing to host daily energy requirements [34-35]. Acetate is mainly metabolized in human muscle, kidney, heart, and brain, while propionate is utilized primarily in the liver and has been suggested to be a potential modulator of cholesterol synthesis and a precursor in liponeogenesis, which may influence body weight. Butyrate is the preferred energy source for colonic epithelial cells and has been shown to stimulate apoptosis in the colon and is suggested to have anti-tumor properties that provide protection against cancer [36-37]. Non-digestible carbohydrates have the added health benefit of increasing stool bulk, which influences colonic transit times by increasing peristalsis and preventing constipation [38]. Clinical studies show that certain illnesses or metabolic dysfunction such as type-2 diabetes, obesity, atherosclerosis/hypertension, ulcerative colitis, cardiovascular diseases, colorectal cancer, Crohn's disease, inflammatory bowel disease (IBD), and some allergies have been linked with alterations in composition and functions of the normal gut microbiota [39-42].

The study therefore investigated whether OFSP tuber has prebiotic activity that may positively influence human gut by modulating the microbiota towards proliferation of the healthy bacteria and production of SCFAs during metabolism.

MATERIALS AND METHODS

Two varieties of OFSP tubers, SPK 004 (Rp) and Tainung (Op) sourced from Kenya Agricultural Research Institute, Embu Center, were used to prepare the purees at University of Reading, Food Pilot Plant whereby approximately 500 g medium sized tubers were boiled at temperature below 70^{o}C for 30 minutes until soft, peeled, and pulped immediately while still warm [43]. Four 120 ml glass batch culture fermentation vessels each containing 90 ml of pre-reduced gut model media (GMM), set to simulate human gastrointestinal (GI) colon conditions in terms of temperature (37

ºC), pH (6.85) and maintained anaerobic by continuous sparging with oxygen-free nitrogen were inoculated with 10ml of human faecal homogenate slurry using sterile syringes. The pH of each vessel was controlled automatically by the addition of 1 M HCl or 1 M NaOH using Electrolab pH controllers and temperature by circulating water bath while continuously stirring the vessels. The GMM comprised of (g/440 ml heated distilled water): starch from potato, 2.2; pectin from citrus fruits, 0.88; gum arabic, 0.44; xylan from oatspelts, 0.88; arabinogalactan from larchwood, 0.88; inulin, 0.44; caesin from bovine salts, 1.32; peptone water, 2.2; tryptone 2.2; bile salts, 1.76; yeast extract, 1.98; iron sulfate heptahydrate, 0.0022; sodium chloride, 1.98; potassium chloride, 1.98; potassium phosphate monobasic, 0.22; magnesium sulfate heptahydrate, 0.55; calcium chloride hexahydrate, 0.066, sodium bicarbonate, 0.66; L-cysteine HCl, 0.352; porcine gastric mucin (type III), 1.76; and 4.4 ml of haemin solution (0.5 mg/ml). All components of the GMM were combined and autoclaved at 121 ºC for 15 minutes. The four substrates were each added to the 4 vessels: 1 g sucrose (non-selective control); 1 g FOS (positive control); and the rest 2 vessels 2.5 g of OFSP purees (Rp and Op) that achieves the required daily intake (RDI) of 600 RAE. The final working volume of each batch culture vessel was 100 ml [44]. Samples were collected (on ice) in triplicate immediately after inoculation (T_0) and after 5, 10, and 24 hours for bacterial enumeration and SCFA analysis. Six separate batch fermentation runs were performed with different healthy faecal donor for each run.

Preparation of human faecal inoculum

Mixed microbial inoculum (faecal homogenate) were prepared from fresh faecal samples of six healthy human volunteers with no history of gastrointestinal disorders and who had not taken antibiotics for at least three months prior to the study. Donors were aged 20–55 yr; 2 males (1 Chinese and 1 Mexican) and 4 females (2 Chinese, 1 British, and 1 African). The volunteers were each supplied with a stool collection kit (white plastic pot containing stomacher bag, Anaerocult jar, and Anaerogen sachet [Oxoid Limited] which was used according the manufacturer's instructions to generate an anaerobic atmosphere). Fresh faecal samples were collected on the morning of the experiment, transported to the laboratory anaerobically and processed within 2 hours of defecation. The stool sample was manually kneaded, and faecal homogenate was then prepared by diluting a portion in 1:10 (w/w) in pre-reduced 0.1 M phosphate-buffered saline (PBS; pH 7.3) and stomaching in a filter bag for 2 min at 'high' speed (Stomacher 400 Lab System; Sewards).

Bacterial enumeration by qPCR

DNA extraction via phenol-chloroform method

Aliquots (1.5 ml) of each sample were immediately centrifuged (13000 rpm, 10 min) to collect cell pellets, which were washed with 1 ml PBS prior to storing in PBS:glycerol (1:1) at -20 °C for DNA extractions. PBS:glycerol samples were thawed on ice, centrifuged (13000 rpm, 5 min) and cell pellets washed with 1 ml of PBS prior to resuspension in 0.5 ml of TES buffer and treatment with lysozyme (8 μl, 10 mg/ml) and mutanolysin (2 μl, 1 mg/ml) at 37 °C for 30 min. Proteinase

K (10 µl, 20 mg/ml) and RNase (10 µl, 10 mg/ml) were added to the suspension which was vortexed and incubated at 65 °C for 1 hour before sodium dodecyl sulfate (SDS) treatment; 100 µl of 10 % SDS solution was added and the suspension gently mixed by inversion then incubated for a further 15 min at 65 °C. The suspension was cooled on ice for 30 min before phenol/chloroform extraction (1 volume phenol/chloroform/water [Applied Biosystems, UK] added, samples mixed by inversion for 2 min and centrifuged for 10 min at 6500 rpm). The upper aqueous layer was transferred to a clean, sterile microcentrifuge tube and 1 ml of ice-cold ethanol added. The samples were stored overnight at -20 °C before centrifugation (5 min at 13000 rpm) to collect the DNA pellet. The supernatant was carefully removed, and the DNA dried overnight at room temperature. DNA was eluted in 50 µl of sterile water subsequent to measuring concentration (ng/µl) by ND-1000 Nanodrop spectophotometer and storing at -20 °C.

Quantitative PCR

Quantitative PCR (qPCR) was performed on extracted DNA according to procedure [45] for universal (UniF–UniR), bacteroides (Bac303F–Bfr-Fmrev), and bifidobacteria (BifF–g-Bifid-R) using a BioRad miniOpticon and SsoAdvanced™ SYBR® Green Supermix (BioRad). Purified *Bacteroides thetaiotaomicron* DMS 2079^T 16S rRNA gene amplicons were used as the standard for universal and bacteroides qPCR and *Bifidobacterium bifidum* DMS 20456^T 16S rRNA gene amplicons was used as the standard for bifidobacteria qPCR.

Short chained fatty acids (SCFA) analysis by gas chromatography

Aliquots (1.5 ml) of fermentation samples were centrifuged (13000 rpm, 10 min) and filtered supernatants stored at -20 °C for SCFA analysis. SCFAs were measured by derivatization method of fatty acids using gas chromatography equipped with a flame ionisation detector (HP-1 column (10 mx 0.53 mm IDx2.65 mm), carrier gas helium with a total flow rate of 37 mL/min and pressure of 7 kPa, temperature 255–260 °C and holding for 5 min, pressure 15 kPa and holding for 4 min. The filtrates were thawed on ice and 1 ml was mixed with 50 µl of internal standard (0.1 M 2-ethylbutyric acid) prior to derivatization. Standard organic acids mixtures as external standards were derivatized alongside the samples [46].

Statistical analysis

Genstat 16th Edition statistical package (VSN International Ltd, University of Reading) was used for data analysis with substrates (FOS, sucrose, Op and Rp OFSP purees) and time (0, 5, 10 and 24 hr) as treatment factors. Means of analysis of variance (ANOVA) of SCFA concentration (mM/ml) and bacterial count which was transformed to $\log_{10}$ were conducted and Duncan's multiple range test was used for separation of means at (p<0.05).

RESULTS AND DISCUSSIONS

pH controlled in vitro batch fermentation of OFSP puree

Overall variations were observed across the 6 different faecal donor runs, with relatively large standard deviations in bacterial counts and SCFA (Figure 1 and Table 1) due to broad differences

in their ethnic backgrounds (British, 3 Chinese, Mexican and African), age (20–55 yr) and gender, thereby introducing variations in diet and phenotype/genotype. No consistent pattern was seen in relation to changes in qPCR bacterial populations, even between runs 3 and 4, which were inoculated with faecal from 2 Chinese females of the same age. The result is consistent with recent studies that reported variations of the subsequent metabolic fermentation trajectory due to differences of donors' inoculums, with high specificity at both species and strain level [47-48].

Effects of OFSP puree batch fermentation on bacteria

Total bacteria: Total bacteria increased throughout the fermentations by the end of the 24 hours fermentation period in all the 6 runs (Table 1). The maximum average change in total bacteria varied for the 4 different substrates (Table 3), but it remained relatively stable (0.92–1.7 $\log_{10}$[copies/ml]) with no significant differences (p>0.05).

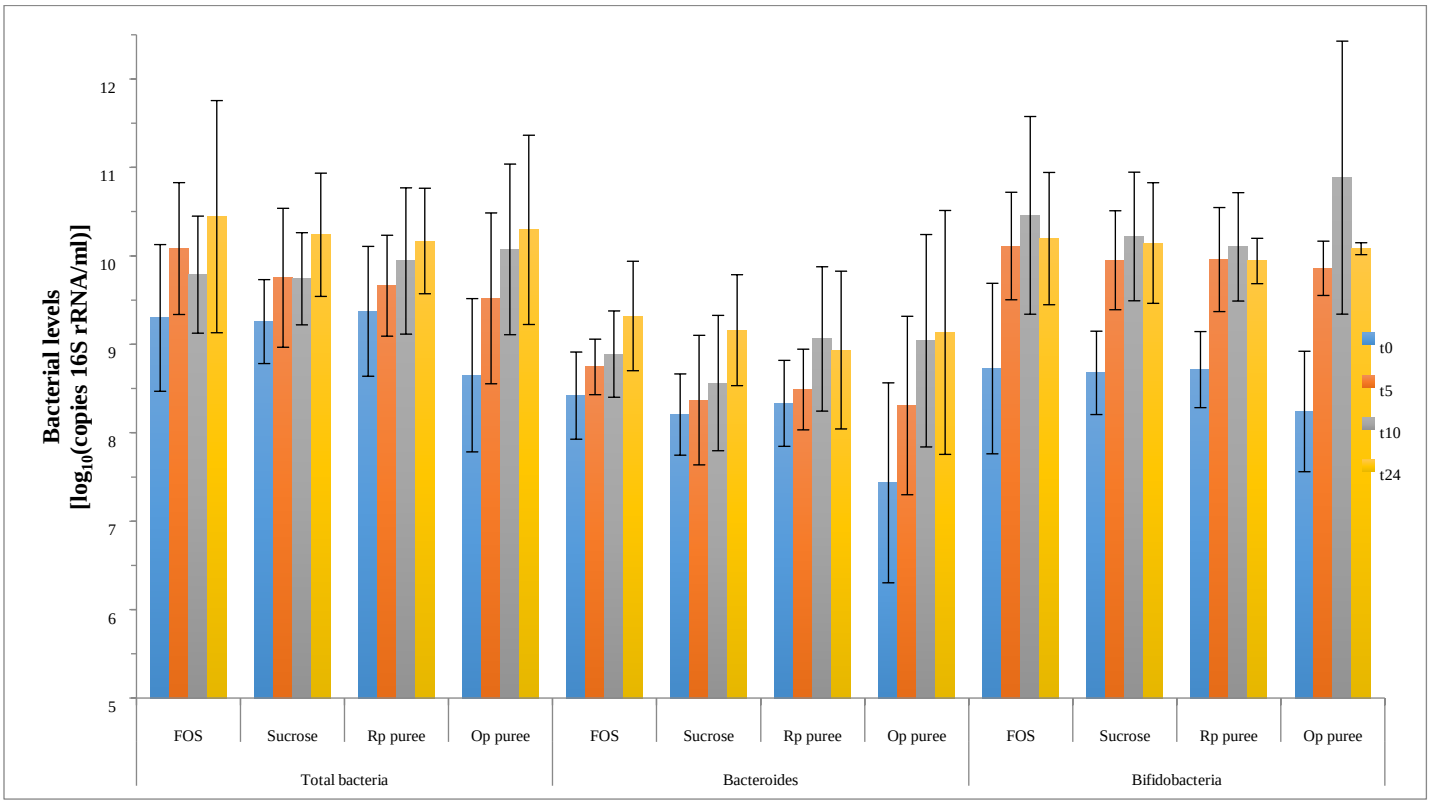

Figure 1: Investigation of the effects of OFSP purees on total bacteria, *Bacteroides* and *Bifidobacterium* populations during pH controlled in vitro batch fermentations. Data are presented as averages with SD (error bars) of six runs, each using a different donor. FOS was used as prebiotic control and sucrose as non-selective control for 24 hr fermentations – blue, baseline (t0); red, t5; green, t10; and purple, t24. Rp, SPK 004; and Op, Tainung OFSP.

Bacteroides: Generally, the *Bacteroides* increased throughout the fermentation period although the highest levels were seen at t5 or t10 for some vessels/runs (Table 1). The highest count in the 6 runs was recorded in Op puree, run 1 (3.3 $\log_{10}$[copies/ml]) which also recorded the highest average maximum change, although it was not significantly different between the substrates (Table 3). *Bacteroides* have been reported to have a diverse array of substrate (polysaccharides) utilized

during metabolism [49], which most likely explains the increase in levels on all substrates in this study. Our data concur with the findings from a previous study of green and gold kiwifruit, which showed the *Bacteroides* population increased between t5 and t24 although it subsequently dropped by t48 [50].

Table 2: SCFA concentrations (mmol/ml) during pH controlled batch fermentation of OFSP puree and control substrates

SCFA	Time (hr)	Substrate			
		FOS	**Sucrose**	**Rp puree**	**Op puree**
Total	0	5.07±0.88	5.12±0.89	4.83±0.79	5.07±0.88
	5	43.13±7.79	41.36±6.75	51.59±7.89	46.32±7.07
	10	75.84±12.92	68.30±11.04	66.12±10.54	67.64±11.71
	24	103.13±16.60^{a}	93.24±14.95^{b}	94.93±14.23^{b}	92.58±14.76^{b}
Formate	0	1.87±0.35	1.77±0.22	1.54±0.44	1.73±0.62
	5	8.63±0.92	8.79±2.26	8.06±1.68	7.69±1.57
	10	6.45±4.45^{a}	7.65±4.58^{b}	5.30±2.39^{c}	3.13±1.85^{d}
	24	2.91±2.24^{a}	2.89±2.22^{a}	3.31±1.93^{a}	1.83±0.64^{b}
Acetate	0	2.29±2.56	2.40±2.79	2.16±3.16	2.41±3.16
	5	23.83±5.44^{a}	20.15±7.98^{b}	23.31±9.14^{a}	19.56±6.44^{b}
	10	36.10±5.29^{a}	31.99±12.94^{b}	31.34±8.08^{b}	32.77±6.68^{b}
	24	41.95±3.74	37.44±11.33	40.08±10.51	38.85±5.29
Propionate	0	0.16±0.05	0.17±0.05	0.16±0.05	0.14±0.04
	5	0.16±3.09^{a}	0.14 ±2.14^{a}	3.52±2.69^{b}	3.32 ±1.50^{b}
	10	4.35±7.87^{a}	3.32±4.62^{a}	11.98±5.39^{b}	12.31±4.25^{b}
	24	18.28±6.09^{a}	12.31±6.74^{b}	18.28±8.77^{a}	18.52±4.49^{a}
Butyrate	0	ND	0.03±0.07	ND	ND
	5	6.61±5.97^{a}	7.19±4.69^{a}	2.85±10.30^{b}	12.63±12.45^{c}
	10	24.61±6.03^{a}	19.58±4.18^{b}	14.75±11.46^{c}	17.15±7.79^{b}
	24	33.91±6.25^{a}	29.88±10.19^{b}	25.64±4.45^{c}	27.00±7.93^{bc}
Lactate	0	0.08±0.05	0.06±0.02	0.08±0.04	0.05±0.01
	5	2.21±1.32^{a}	4.35±2.03 ^{b}	2.75±2.71^{a}	2.26±0.70^{a}
	10	1.93±1.05^{a}	4.01±2.42^{b}	1.65±0.85^{a}	1.24±0.33^{a}
	24	1.99±1.69	0.80±1.24	2.15±1.90	1.38±0.30

ND, not detected. Data are presented as means ± SD (n=6). Different superscripted letters across rows indicate statistically significant differences (p<0.05).

Table 1: *In vitro* investigation of the impact of OFSP purees on the human faecal microbiota using pH controlled batch fermentations

Substrate	Total bacteria					Bacteroides					Bifidobacteria				
	0	5	10	24	$\Delta_{max(t0)}$	0	5	10	24	$\Delta_{max(t0)}$	0	5	10	24	$\Delta_{max(t0)}$
FOS	**10.07**	**11.16**	**11.19**	**11.43**	**1.36**	**8.55**	**8.70**	**9.04**	**9.90**	**1.35**	**8.29**	**10.55**	**10.90**	**10.69**	**2.61**
Sucrose	**10.65**	**10.61**	**10.73**	**11.39**	**0.74**	**8.09**	**7.19**	**9.30**	**9.90**	**1.82**	**8.63**	**10.25**	**10.47**	**10.52**	**1.89**
Rp puree	**10.43**	**11.21**	**11.55**	**11.52**	**1.12**	**7.63**	**8.89**	**9.78**	**10.23**	**2.60**	**8.21**	**10.67**	**10.85**	**10.76**	**2.63**
Op puree	**10.46**	**11.08**	**11.33**	**11.40**	**0.94**	**6.94**	**8.68**	**9.60**	**10.24**	**3.30**	**8.32**	**10.54**	**10.91**	**10.59**	**2.59**
FOS	**8.63**	**10.20**	**9.27**	**11.99**	**3.36**	**7.78**	**8.33**	**8.63**	**9.63**	**1.85**	**9.29**	**10.06**	**10.13**	**10.73**	**1.44**
Sucrose	**8.46**	**8.06**	**8.33**	**10.66**	**2.20**	**7.34**	**7.47**	**7.28**	**9.67**	**2.33**	**8.94**	**8.71**	**10.04**	**11.18**	**2.24**
Rp puree	**9.18**	**8.10**	**9.72**	**9.56**	**0.54**	**8.33**	**7.08**	**9.84**	**8.77**	**1.51**	**9.44**	**9.26**	**10.76**	**9.50**	**1.32**
Op puree	**7.60**	**8.09**	**9.52**	**10.48**	**2.88**	**6.73**	**7.43**	**8.40**	**8.66**	**1.93**	**7.66**	**9.08**	**12.82**	**9.55**	**5.17**
FOS	**8.69**	**8.77**	**8.53**	**8.80**	**0.11**	**8.52**	**8.34**	**8.55**	**8.60**	**0.07**	**8.90**	**9.97**	**11.71**	**9.02**	**2.81**
Sucrose	**8.48**	**9.17**	**8.46**	**8.72**	**0.69**	**8.15**	**8.58**	**8.01**	**8.51**	**0.43**	**8.72**	**10.26**	**10.67**	**9.46**	**1.95**
Rp puree	**8.54**	**9.28**	**8.49**	**9.86**	**1.32**	**8.19**	**8.78**	**8.74**	**8.20**	**0.59**	**8.83**	**10.11**	**9.69**	**9.20**	**1.27**
Op puree	**7.33**	**8.36**	**8.47**	**8.65**	**1.32**	**7.24**	**8.04**	**8.76**	**8.35**	**1.52**	**8.58**	**9.07**	**9.53**	**9.07**	**0.95**
FOS	**8.87**	**10.53**	**10.45**	**10.44**	**1.66**	**8.14**	**9.18**	**9.88**	**9.66**	**1.74**	**7.77**	**10.30**	**10.43**	**10.46**	**2.69**
Sucrose	**9.75**	**10.34**	**10.34**	**10.10**	**0.59**	**9.00**	**9.54**	**9.84**	**9.35**	**0.84**	**8.65**	**10.39**	**9.82**	**10.06**	**1.74**
Rp puree	**9.77**	**ND**	**10.23**	**10.00**	**0.45**	**9.03**	**9.53**	**9.52**	**9.40**	**0.49**	**8.53**	**10.22**	**9.83**	**10.28**	**1.75**
Op puree	**9.37**	**ND**	**10.45**	**10.19**	**1.08**	**8.65**	**9.23**	**9.46**	**9.39**	**0.81**	**8.26**	**10.05**	**10.32**	**10.39**	**2.13**
FOS	**9.40**	**9.27**	**9.21**	**10.00**	**0.60**	**8.81**	**9.11**	**8.76**	**9.35**	**0.54**	**8.84**	**9.56**	**9.27**	**9.95**	**1.12**
Sucrose	**9.36**	**9.63**	**9.81**	**9.78**	**0.45**	**8.73**	**9.19**	**9.17**	**9.26**	**0.53**	**8.66**	**9.74**	**9.87**	**9.70**	**1.21**
Rp puree	**9.24**	**9.95**	**9.19**	**9.77**	**0.70**	**8.69**	**8.43**	**9.05**	**9.59**	**0.90**	**8.55**	**9.83**	**9.09**	**9.84**	**1.29**
FOS	**10.14**	**10.57**	**10.08**	**10.01**	**0.44**	**8.71**	**8.82**	**8.48**	**8.78**	**0.10**	**9.28**	**10.23**	**10.30**	**10.32**	**1.05**
Sucrose	**8.84**	**10.71**	**10.77**	**10.78**	**1.95**	**7.92**	**8.24**	**7.77**	**8.26**	**0.34**	**8.46**	**10.35**	**10.44**	**9.95**	**1.98**
Rp puree	**9.08**	**9.78**	**10.48**	**10.30**	**1.40**	**8.12**	**8.23**	**7.43**	**7.43**	**0.11**	**8.72**	**9.66**	**10.40**	**10.08**	**1.68**
Op puree	**8.49**	**10.55**	**10.60**	**10.76**	**2.27**	**7.61**	**8.16**	**8.97**	**9.03**	**1.42**	**8.38**	**10.56**	**10.85**	**10.81**	**2.47**

Data are presented as $\log_{10}$(copies of 16S rRNA/ml sample); $\Delta_{max(t0)}$, Maximum change in population from t0. ND, none detected. Run 5 (Op) had experimental error

Table 3: Maximum change in population levels compared to baseline (t_0) levels during pH controlled batch fermentations

Bacteria	Substrate	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Average±SD
Total	FOS	1.36	3.36	0.11	1.66	0.60	0.44	1.25±1.18
	Sucrose	0.74	2.20	0.69	0.59	0.45	1.95	1.10±0.76
	Rp puree	1.12	0.54	1.32	0.45	0.70	1.40	0.92±0.41
	Op puree	0.94	2.88	1.32	1.08	ND	2.27	1.70±0.84
Bacteroides	FOS	1.35	1.85	0.07	1.74	0.54	0.10	0.94±0.81
	Sucrose	1.82	2.33	0.43	0.84	0.53	0.34	1.05±0.83
	Rp puree	2.60	1.51	0.59	0.49	0.90	0.11	1.03±0.90
	Op puree	3.30	1.93	1.52	0.81	ND	1.42	1.80±0.93
Bifidobacterium	FOS	2.61	1.44	2.81	2.69	1.12	1.05	$1.95±0.83^{a}$
	Sucrose	1.89	2.24	1.95	1.74	1.21	1.98	$1.84±0.35^{a}$
	Rp puree	2.63	1.32	1.27	1.75	1.29	1.68	$1.66±0.52^{a}$
	Op puree	2.59	5.17	0.95	2.13	ND	2.47	$2.66±1.54^{b}$

Data are presented as $\log_{10}$(copies of 16S rRNA/ml sample). Different superscripted letters between substrates indicate statistically significant differences ($p<0.05$).

Bifidobacteria: Although the bifidobacterial levels were exceptionally high, probably due to the probes inability to differentiate the species, the white milky morphological shape characteristic of bif bacteria was clearly observable, demonstrating the ability of OFSP purees to support the growth of bifidobacteria (Table 1). On average, bifidobacterial counts increased by 1.66–2.66 ($\log_{10}$[copies/ml]), with significant differences ($p<0.05$) between the substrates; Op puree recording the highest maximum change (Table 3). Studies with other non-digestible carbohydrates reported increases in bifidobacteria up to 24 hr of fermentation [51-54]. *Bifidobacterium* species have been reported to be greatly beneficial to gastrointestinal health by inhibiting pathogen growth, stimulating the immune system and production of vitamin B complex [55-56].

Further investigation of other bacterial populations (i.e. different *Clostridium* clusters, lactobacillus, fusobacteria, *Coriobacteriia*, and *Faecalibacterium*) is required to better understand the overall impact of OFSP purees on the faecal microbiota, and thereby their comprehensive potential in gut modulation/prebiotic activity and possible health benefits. In addition, future work should compare different universal primers and/or develop more robust universal primer sets for accurately determining the total bacterial load using qPCR.

Effects of OFSP puree batch fermentation on production of SCFA

Total SCFA: Total SCFA significantly ($p<0.05$) increased throughout the 24 hr fermentation in all vessels (Table 2). However, the only statistically significant difference ($p<0.05$) was observed between FOS and the other different substrates at t24. The predominant SCFA seen during the batch fermentations were acetate and butyrate (t10 and t24). High levels of organic

acid increase the acidity of the colon and have been reported to have various health benefits such as inhibiting growth of pathogen and increasing bioavailability of minerals [57-58].

Acetic acid: Acetate levels increased significantly over time in all the four substrates (Table 2), by eight to ten-fold during the first 5 hours. Concentrations were significantly higher for FOS and Rp puree at t5 compared to Op puree and sucrose ($p<0.05$). All four substrates resulted in ~ 40 mmol/ml levels of acetate at the end of fermentation (t24). Acetic acid is not only beneficial as a strong organic acid for reduction of pH that causes inhibition of growth of pathogens, as it is also metabolized systemically in brain and muscle tissues generating energy for host cells [59].

Butyric acid: Butyrate levels also increased during the entire experimental fermentation period, from minimal or non-detectable levels at t0 to 25–34 mmol/ml at t24 (Table 2). Significant differences ($p< 0.05$) profiles were observed in production of butyrate in the different substrates (on average and between runs; e.g. greater variation was seen across different runs for OFSP purees during the first ten hours than for control substrates). Overall, FOS had the highest level of butyrate while Rp puree the lowest (Table 3). Recent studies have demonstrated the relationship between the gut microbiota and metabolites especially butyrate and some behavioural disorders (depression, anxiety, and schizophrenia [60]), obesity [61], and infectious diseases due to a disturbed gut barrier [62].

Propionic acid: Propionate levels increased significantly ($p<0.05$) for all the four substrates throughout the experimental period, although OFSP purees elicited significantly higher levels at t5 and t10 compared to the control substrates (Table 3). With the exception of sucrose (which was significantly lower), propionate levels averaged ~ 18 mmol/ml at the end of fermentation (t24). It is worth noting that the changes in propionate levels did not appear to correlate to changes in *Bacteroides* levels, as recently reported (2016) by Chung *et al.* [63]. Propionate is a substrate for energy production in the liver and has several proposed health benefits such as weight loss, anti-inflammatory, and cholesterol-lowering properties, immunity and brain development [64 -66] as well as associations with reduced risks of diseases, such as diabetes, obesity, and inflammatory diseases and autism spectrum disorders [67- 70].

Formic acid: Formate levels initially increased for all substrates, with maximum levels seen at t5 (Table 2). Afterwards, formate levels declined with significantly different concentrations ($p<0.05$) as recorded between the different substrates at t10 and levels similar to starting levels seen at t24. Op puree recorded the lowest t24 levels of formic acid, which was significantly different ($p<0.05$) to that of all other substrates. Formic acid is a strong organic acid that can impact on acidity level in the gut, thus killing pathogens.

Lactic acid: Lactate levels demonstrated the smallest changes in SCFA during the 24 hours fermentation of the SCFA detected (Table 2). Lactate increased in the first 5 hours, with significantly higher levels seen for sucrose compared to all other substrates. Similar to formate, lactate levels decreased (by varying levels) afterwards to t24. Although the amount of lactic acid is low in human blood (1–2 mmol/L) during rest, some studies suggests its importance during early stages of brain development and in the gut by reduction of infection/inflammation and lowering levels of lipopolysaccharides (LPS) in immunity enhancement and cancer prevention [71-72].

Confirmation of prebiotic activity by composition analysis and characterization of OFSP puree for ingredients such as dietary fibre, resistant starch, and oligosaccharides and/or use of different biomarkers such as immunological changes, inflammatory mediators, serum lipid levels, genotoxicity, toxicity, and cognitive function would provide a more accurate way of monitoring and relating prebiotic food ingredients to healthy [73]. However, the best selective validation method which requires vigorous ethical consensus is *in vivo* study using human or animal by administering sweet potatoes grown in stable ^{13}C isotope, followed by quantification of SCFA in plasma or faeces and molecular technology for profiling the microbes [74-75].

CONCLUSIONS

Bacterial profiles and SCFA levels varied over the entire experimental fermentation time in the two varieties of OFSP and controls. Since increases were observed in the two well-known biomarkers of a healthy gut, bifidobacteria, and SCFA metabolites (especially butyrate), the current research clearly suggests there is potential to promote gut health, although further investigation on bifidogenic effect and pathogen inhibition is warranted. Despite the inter-individual variation (whether it be age-related or inter-ethnical) of the gut microbiota and hence the baseline composition of bacterial population, the results suggest ability of the OFSP purees to positively modulate gut microbiota. However, further investigation is warranted for proving prebiotic activity of OFSP by profiling more beneficial gut bacteria (including lactobacilli and bifidobacteria) such as butyrate producers e.g. *Eubacterium rectale* while demonstrating inhibition of growth of pathogenic bacteria, such as clostridia (e.g., *Clostridium difficile* and *C. perfringens*) and *Enterobacteriaceae* in addition to possibly measuring prebiotic index (PI). It is also recommended to conduct biochemical analysis of OFSP to determine if ingredients with the potential to elicit prebiotic/beneficial effects (for example, non-starch polysaccharides) are present in addition to *in vivo* human feeding studies

List of abbreviations: Fructooligosaccharide (FOS), Gastrointestinal (GI), Gut model media (GMM), Lipopolysaccharides (LPS), Orange fleshed sweet potato (OFSP), Phosphate-buffered saline (PBS), Quantitative polymerase chain reaction (qPCR), Short chain fatty acid (SCFA)

Conflicts of interest: The authors declare that there is no conflict of interest associated with this manuscript.

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