Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

In vitro evaluation of the fermentation properties and potential prebiotic activity of Agave fructans

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Keywords
Agave, batch culture system, gut microflora, inulin, prebiotic activity.

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2009/1363: received 30 July 2009, revised 23 October 2009, accepted 26 October 2009

doi:10.1111/j.1365-2672.2009.04617.x

Abstract

Aims: This study was carried out to evaluate *in vitro* the fermentation properties and the potential prebiotic activity of Agave-fructans extracted from *Agave tequilana* (Predilife).
Methods and Results: Five different commercial prebiotics were compared using 24-h pH-controlled anaerobic batch cultures inoculated with human faecal slurries. Measurement of prebiotic efficacy was obtained by comparing bacterial changes, and the production of short-chain fatty acids (SCFA) was also determined. Effects upon major groups of the microbiota were monitored over 24 h incubations by fluorescence *in situ* hybridization. SCFA were measured by HPLC. Fermentation of the Agave fructans (Predilife) resulted in a large increase in numbers of bifidobacteria and lactobacilli.
Conclusions: Under the *in vitro* conditions used, this study has shown the differential impact of Predilife on the microbial ecology of the human gut.
Significance and Impact of the Study: This is the first study reporting of a potential prebiotic mode of activity for Agave fructans investigated which significantly increased populations of bifidobacteria and lactobacilli compared to cellulose used as a control.

Introduction

Agave is a crassulacean plant and in adaptation to its arid or semi-arid environment uses crassulacean acid metabolism to fix CO_2 during photosynthesis. Fructans are the principal photosynthetic products (López *et al.* 2003). These fructans are classed in the *graminans* group, as ß-fructofuranosyl linkages are present, in addition to nonbranched fructofuranosyl moieties called agavins (Mancilla-Margalli and López 2006). Specifically, the molecular structure of *Agave tequilana* Weber var. *azul* shows complex and highly branched fructans with both ß (2-1) and ß (2-6) linkages that are resistant to hydrolysis by human digestive enzymes and can be fermented by colonic microflora producing short-chain fatty acids (SCFA) (López *et al.* 2003).

Gibson and Roberfroid (1995) first described selectively metabolized dietary carbohydrates as prebiotics and defined them as 'non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health'. In this manner, a 'healthier' microbiota composition is obtained whereby bifidobacteria and/or lactobacilli increase in the intestine and affect physiological, immunomodulatory and biochemical processes in humans and animals, resulting in better health and reducing the risk of many diseases (López and Urías-Silvas 2007). They may also act as a barrier against gastrointestinal pathogens, influence appetite regulation, improve intestinal absorption of minerals and decrease serum lipids concentration (Nyman 2002).

The prebiotic effect of a substrate can be measured as a selective effect upon growth of major bacterial groups commonly found in human gut, in particular a selection for increased numbers of bifidobacteria and lactobacilli in comparison with 'undesirable' micro-organisms, such as

certain clostridia and bacteroides (Vulevic *et al.* 2004; Mandalari *et al.* 2007).

There are a number of methods currently in use to determine the prebiotic properties of a substrate (from pure cultures studies to human trials). However, a defining characteristic of prebiotic is the selective nature of certain groups of colonic bacteria seen as beneficial towards human health. This can only be determined in studies using mixed microbial culture which mimic the microbial ecology of the human intestinal tract. For a rapid comparative evaluation, anaerobic batch fermentations inoculated with faecal slurries are used. Because they represent the diverse gut microflora, but are completed rapidly with several sets running simultaneously, these anaerobic batch fermentations present an excellent mode for small-scale screening of novel substrates. Until recently, growth of specific bacteria in such fermentations was measured through colony counting on selective agars. This approach, however, suffers from several drawbacks (time-consuming, labour-intensive and nonrecovery of uncultivable organisms). As a result, molecular techniques such as fluorescence *in situ* hybridization (FISH) have been developed to study microbial communities (Rycroft *et al.* 2001; Olano-Martin *et al.* 2005).

The aim of this study was to evaluate the potential prebiotic properties of Agave fructans in batch culture fermentation systems. The objective was to compare the ability of Predilife extracted from *Agave tequilana* Weber var. *azul* to selectively increase the number of bifidobacteria and alter colonic metabolic output (short-chain fatty acid profiles with four different commercial prebiotic brands and cellulose as a control).

Materials and methods

Substrates

Agave Fructans as a white crystalline powder consisting of fructose-based polymers were obtained from *Agave tequilana* Weber var. *azul* harvested in the zone of Los Altos, Jalisco, Mexico. The leaves were cut off, keeping the stems and base leaves, a part usually called the 'head' because of its similarity with a pineapple fruit. For the preparation of Predilife (Agave), the head was ground, diluted with water at high temperature (80–99°C) and treated with activated carbon and ion exchangers to eliminate calcium and chelates by a Mexican company (Agro Corona, SA de CV; Usmajac, Jalisco, Mexico). The following commercial preparations of prebiotic oligosaccharides were also investigated: Inulin (Orafti®HP, 100% inulin/oligofructose content and 0% glucose/fructose/sucrose, Orafti); Synergy1 (Orafti® Synergy1, 92% inulin/oligofructose content and 8% glucose/fructose/sucrose, Orafti); Oligofructose (Orafti®Raftilose95, 95% inulin/oligofructose content and 5% glucose/fructose/sucrose, Orafti); and scFOS (Actilight 950P; Beghin Meiji, Neuilly, France). Cellulose (Sigma-Aldrich, Poole, UK) was used as a control.

Batch culture fermentations

Sterile stirred batch culture fermentation vessels (280 ml working volume) were prepared and aseptically filled with 135 ml of sterile basal nutrient medium. This medium comprised peptone water (2 g l^{-1}), yeast extract (2 g l^{-1}), NaCl (0·1 g l^{-1}), KH_2PO_4 (0·04 g l^{-1}), K_2HPO_4 (0·04 g l^{-1}), $MgSO_4 \times 7H_2O$ (0·01 g l^{-1}), $CaCl_2 \times 6H_2O$ (0·01 g l^{-1}), $NaHCO_3$ (2 g l^{-1}), Tween 80 (2 ml l^{-1}), haemin (0·05 g l^{-1}), vitamin K (10 μl l^{-1}), L-cysteine hydrochloride (0·5 g l^{-1}) and bile salts (sodium glycocholate and sodium taurocholate) (0·5 g l^{-1}). The medium was adjusted to pH 7·0, and 4 ml of 0·025% (w/v) resazurin solution added prior to autoclaving. All media and chemicals were purchased from Oxoid and Sigma, respectively. Once in the fermentation vessels, the sterile medium was sparged with O_2-free N_2 (15 ml min^{-1}) overnight to maintain anaerobic conditions. The following day, a faecal slurry was prepared with pre-reduced sterile phosphate-buffered saline (PBS, pH 7·0) (to give a 10%, w/v, faecal slurry). This experiment was carried out in triplicate using faecal samples from three different volunteers (one faecal donor for each experimental set up). The faecal samples were obtained fresh at the premises of the department from three healthy human donors (one women, two men; average 30 ± 4·36 years of age, omnivores) who were free of any known metabolic and gastrointestinal diseases; were not taking probiotic or prebiotic supplements and had not taken antibiotics 6 months before faecal sample donation. Each vessel was inoculated with 15 ml of freshly prepared faecal slurry. Each carbohydrate was immediately added at a concentration of 1% (w/v) to one of the six vessels. The temperature of the fermentation vessels was held at 37°C using a circulating water bath. The pH of 6·7–6·8 was controlled via pH meter controllers (Electrolab260; Electrolab Ltd, Tewkesbury, UK) and adjusted by the addition of 0·5 mmol l^{-1} NaOH or HCl to the vessels when required. Anaerobic conditions were maintained by sparging the vessels with O_2-free N_2 (15 ml min^{-1}). Batch culture fermentations were ran for 24 h, and samples were taken at 0, 5, 10 and 24 h for analysis of SCFA by high-performance liquid chromatography and for analysis of bacterial populations by FISH.

Change in bacterial populations by FISH

FISH was performed as described by Martín-Peláez *et al.* (2008). Briefly, aliquots (375 μl) of batch culture samples

were fixed in three volumes of ice-cold 4% (w/v) paraformaldehyde for 4 h at 4°C. They were then centrifuged at 13 000 ***g*** for 5 min and washed twice in 1 ml of sterile PBS. The cells were pelleted by centrifugation and resuspended in 150 μl of sterile PBS, to which 150 μl of ethanol was added. The samples were then vortexed and stored at −20°C until used for hybridizations.

For the hybridizations, 20 μl of each sample was pipetted onto Teflon- and poly-L-lysine-coated, six-well (10 mm diameter each) slides (Tekdon Inc., Myakka City, FL, USA). The samples were dried onto the slides at 46°C for 15 min and afterwards dehydrated in an alcohol series (50, 80 and 96%, 3 min each). The ethanol was allowed to evaporate from the slides before the probes were applied to the samples. A probe/hybridization buffer mixture (5 μl of a 50 ng μl^{-1} stock of probe plus 45 μl of hybridization buffer) was applied to the surface of each well. Hybridizations were performed for 4 h in an ISO20 oven (Grant Boekel; Grant Instruments Ltd, Shepreth, UK). Slides were stored in the dark at 4 °C (for a maximum of 3 days) until cells were counted under a Nikon E400 Eclipse microscope.

The hybridization was carried out as previously described (Rycroft *et al.* 2001) using genus- and group-specific 16S rRNA gene-targeted oligonucleotide probes labelled with Cy3 (Sigma-Aldrich) or the nucleic acid stain DAPI for total cell counts.

The probes were Bif164, specific for the *Bifidobacterium* genus (Langendijk *et al.* 1995); Bac303, specific for the *Bacteroides-Prevotella* group (Manz *et al.* 1996); Chis150, for the *Clostridium histolyticum* subgroup; Erec482 for most members of *Clostridium* cluster XIVa (Franks *et al.* 1998); Lab158, for the *Lactobacillus–Enterococcus* group (Harmsen *et al.* 1999) and Ato291 for the *Atopobium* cluster, including most *Coriobacteriaceae* species (Harmsen *et al.* 2000).

Short-chain fatty acid (SCFA) analysis

Samples taken from the batch culture vessels were centrifuged at 13 000 ***g*** for 5 min to remove all particulate matter. Supernatants were then filtered using 0·2 -μm polycarbonate syringe filters (Whatman Ltd, Maidstone, UK) and injected (20 μl) into an HPLC system (Merck, NJ) equipped with refractive index (RI) detection. The column used was an ion-exclusion REZEX-ROA organic acid column (Phenomenex, Macclesfield, UK) maintained at 85°C. Sulphuric acid in HPLC-grade H_2O (0·0025 mmol l^{-1}) was used as the eluent, and the flow rate was maintained at 0·5 ml min^{-1}. Quantification of the samples was obtained through calibration curves of lactic, acetic, propionic, butyric and valeric acids and branched-chain fatty acids in concentrations ranging between 12·5 and 100 mmol l^{-1}.

Statistical analysis

Differences between bacterial counts and SCFA profiles at 0, 5, 10 and 24 h fermentation for each substrate were tested for significance using paired t-tests assuming equal variances and considering a two-tailed distribution. To determine whether there were any significant differences in the effect of the substrates, differences at each time were tested using two-way ANOVA with Bonferroni post test. All statistical analyses were performed using the SPSS package program ver. 15 (SPSS Inc., Chicago, IL), and differences were considered significant if $P < 0{\cdot}05$.

Results

Changes in bacterial populations in *in vitro* batch culture fermentations

Population levels of the dominant members of the human microbiota determined by FISH are shown in Table 1. Bifidobacteria numbers increased significantly over time for all fructan-type substrates and were apart from Synergy1 significantly higher after 24 h fermentation of fructan-type substrates compared to cellulose. Predilife and FOS-Raftilose 95 increased bifidobacteria counts already after 10 h fermentation compared to cellulose indicating that the fructans with a lower degree of polymerization (DP) facilitated more rapid changes in the microflora. Similarly, lactobacilli were increased by Predilife and FOS-Raftilose 95 at 10 and 24 h as well as by Synergy1 at 10 h when compared to cellulose. However, there were no significant differences between the different fructans neither for bifidobacteria nor for lactobacilli counts. FOS-Raftilose 95, Predilife, Actilight and Inulin HP induced the growth of bacteria detected by Bac303 (*Bacteroides-Provetella* group) over 24 h fermentation, but there was no significant difference compared to the control substrate cellulose. Also, counts of bacteria detected by the Erec482 probe decreased at 5 or 10 h to increase again later on. This effect was not specific to the fructans as such decrease was also observed for cellulose. No significant differences were detected for *Atopobium* cluster and most *Coriobacteriaceae spp.*, as detected by Ato291, and the *Clostridium hystolyticum* subgroup. While there was a trend of increased numbers of total bacteria for all tests during the fermentations, this effect was only significant for Predilife between 0 and 24 h.

SCFA production

Figure 1 shows the profiles of the SCFA in the batch cultures at 24 h of fermentation. Total SCFA production with Predilife was similar to all the substrates tested,

Table 1 Bacterial populations (log10 cells ml^{-1} ± SD) in pH-controlled and stirred batch cultures at 0, 5, 10 and 24 h in presence of different substrates

	Synergy1™				FOS-Raftilose 95™				Predilife				Actilight™				Inulin HP™				Cellulose			
	0 h	5 h	10 h	24 h	0 h	5 h	10 h	24 h	0 h	5 h	10 h	24 h	0 h	5 h	10 h	24 h	0 h	5 h	10 h	24 h	0 h	5 h	10 h	24 h
DAPI	8·95 ± 0·12	8·87 ± 0·16	9·15 ± 0·25	9·13 ± 0·22	8·87 ± 0·28	8·97 ± 0·17	9·26 ± 0·19 *	9·27 ± 0·07	8·86 ± 0·17	8·99 ± 0·19	9·09 ± 0·32	9·52† ± 0·14	8·95 ± 0·43	9·10 ± 0·28	9·41 ± 0·21 **	9·46 ± 0·07	8·74 ± 0·37	9·02 ± 0·31	9·44 ± 0·28 **	9·38 ± 0·19	8·82 ± 0·33	9·08 ± 0·25	8·72 ± 0·19	9·04 ± 0·04
Lab158	7·97 ± 0·55	7·83 ± 0·29	8·12 ± 0·31 *	8·37§ ± 0·25	8·09 ± 0·33	7·68 ± 0·43	8·13 ± 0·36 *	8·43 ± 0·14 *	7·75 ± 0·31	7·72 ± 0·07	7·52 ± 0·49 *	8·43 ± 0·31 *	7·95 ± 0·22	7·66 ± 0·18	7·73 ± 0·19	8·23 ± 0·16	7·94 ± 0·32	7·87 ± 0·14	7·73 ± 0·19	8·19 ± 0·15	7·92 ± 0·37	7·60 ± 0·22	7·51 ± 0·09	7·82† ± 0·07
Bif164	7·81 ± 0·26	8·17 ± 0·24	8·53‡ ± 0·27	8·37 ± 0·71	7·81 ± 0·22	8·54 ± 0·59	8·71† ± 0·40 *	8·80 ± 0·37 **	7·79 ± 0·25	8·27† ± 0·15	8·92†,‡ ± 0·18 *	8·99†,‡ ± 0·23 **	7·81 ± 0·02	8·02 ± 0·50	8·69 ± 0·39	9·03† ± 0·25 ***	7·95 ± 0·32	8·38† ± 0·23	8·29 ± 0·80	8·77 ± 0·28 *	7·90 ± 0·15	7·97 ± 0·09	7·99 ± 0·33	7·84 ± 0·47
Ato291	8·14 ± 0·31	8·01 ± 0·32	8·17 ± 0·59	8·08 ± 0·68	8·26 ± 0·30	7·93 ± 0·64	7·98 ± 1·08	7·99 ± 0·70	8·17 ± 0·31	7·98 ± 0·35	8·15 ± 0·91	8·05 ± 0·61	7·77 ± 0·26	7·65 ± 0·64	7·63 ± 1·19	7·13 ± 0·29	7·92 ± 0·45	8·10 ± 0·16	8·01 ± 0·21	7·43 ± 0·24	7·61 ± 0·77	7·83 ± 0·59	7·49 ± 0·39	7·74 ± 0·30
Erec482	7·92 ± 0·35	7·49† ± 0·25	7·34† ± 0·45	7·73 ± 0·63	7·86 ± 0·31	7·72 ± 0·37	7·61 ± 0·50	7·95 ± 0·41	7·80 ± 0·48	7·76 ± 0·21	7·06 ± 0·75	7·83 ± 0·31	7·95 ± 0·35	7·25 ± 0·79	7·01† ± 0·05	7·74 ± 0·05	8·00 ± 0·21	7·54† ± 0·37	7·23†,‡ ± 0·37	7·96 ± 0·34	7·74 ± 0·17	7·30† ± 0·21	7·45 ± 0·27	7·97 ± 0·29
Chis150	6·89 ± 0·61	7·33 ± 0·09	7·37 ± 0·09	6·95 ± 0·32	7·05 ± 0·52	7·31 ± 0·24	7·41 ± 0·14	6·99 ± 0·10	6·99 ± 0·51	7·28 ± 0·48	7·06‡ ± 0·55	6·88 ± 0·13	7·00 ± 0·50	7·42 ± 0·31	7·47 ± 0·11	6·91 ± 0·26	6·96 ± 0·46	7·37 ± 0·14	7·07 ± 0·56	6·71 ± 0·71	6·63 ± 0·71	7·11 ± 0·49	6·94 ± 0·44	7·37 ± 0·26
Bac303	7·87 ± 0·09	7·88 ± 0·29	8·18 ± 0·32	8·08 ± 0·34	7·75 ± 0·31	7·67 ± 0·42	8·47†,‡ ± 0·16	8·44†,‡ ± 0·33	7·79 ± 0·25	7·91† ± 0·22	8·72†,‡ ± 0·20	8·27†,‡,§ ± 0·24	7·81 ± 0·13	7·66 ± 0·03	8·13‡ ± 0·11	8·16‡,§ ± 0·12	7·98 ± 0·27	7·93 ± 0·47	8·28 ± 0·77	8·70† ± 0·35	7·93 ± 0·27	7·96 ± 0·29	8·25 ± 0·43	8·23 ± 0·51

†Significantly different from 0 h for the same substrate.

‡Significantly different from 5 h for the same substrate.

§Significantly different from 10 h for the same substrate (paired *t*-test, $P < 0·05$).

$*P < 0·05$, $**P < 0·01$, $***P < 0·001$ Significantly different from control (cellulose) using two-way ANOVA with Bonferroni post test.

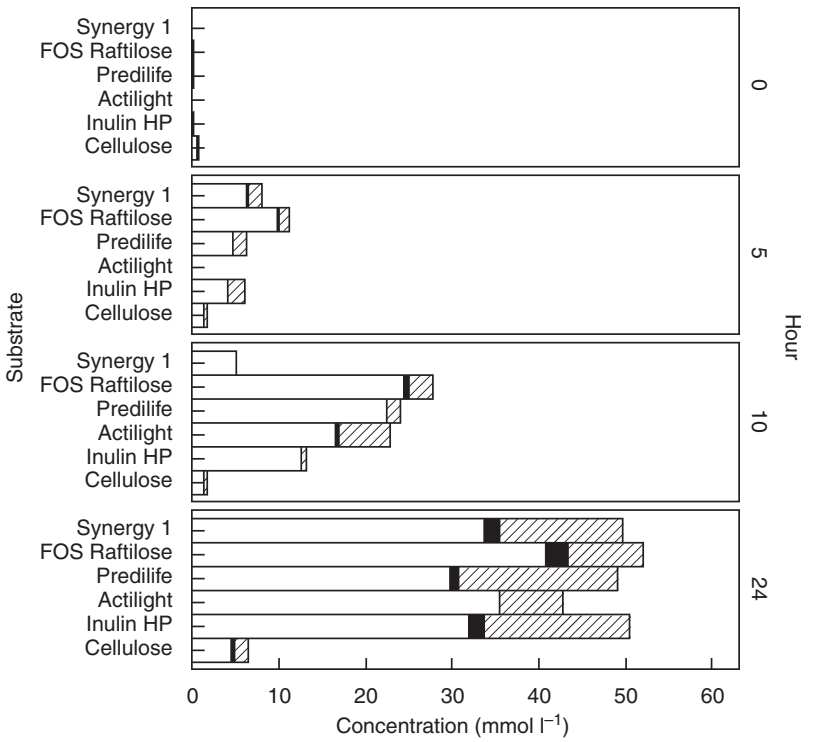

Figure 1 Total short-chain fatty acids production by substrates during 24 -h fermentation in pH-controlled and stirred batch cultures. (▨ propionic, ■ butyric, □ acetic acid). Bars represent means of triplicate cultures from three different faecal donors.

except for cellulose which was significantly lower than all of them (Table 2).

Acetate was the most prevalent SCFA in all treatments, and all fructans produced significantly higher amounts at 24 h compared to cellulose. Fermentation of Predilife and FOS-Raftilose 95 resulted in significantly higher acetate amounts at 10 h compared to cellulose as well as to Synergy1, confirming the more rapid response already observed for bacterial changes. Only Predilife and Inulin HP produced significantly higher amounts of propionate than cellulose while Synergy1 increased butyrate production compared to cellulose. However, there were no significant differences between the different fructans.

Discussion

Inulins are common plant storage carbohydrates present in many human foods. They are also typical prebiotic oligosaccharides, being nondigestible in the upper gut, having proven microbiota modulatory ability (both *in vitro* and *in vivo*) and a range of health effects which have varying degrees of supportive scientific data (Salminen *et al.* 1998; Vulevic *et al.* 2008).

The health claims that are or might become scientifically substantiated are that inulin-type fructans help improve gut function, especially by improving regularity, by increasing stool frequency and by faecal bulking. Inulin-type fructans are thus classified as functional food ingredients that target gastrointestinal functionality but also, most likely via their effects on the gut and the gut microflora, systemic functions that are known to be closely related to health and well-being.

To date, much scientific focus has been placed on inulin and inulin-derived products of chicory origin, while inulin-type fructans are present in a range of other food plants including wheat, onion, banana, garlic and leek (Van Loo *et al.* 1995).

The effect of agave fructans on gut microbiota is less established compared to fructans isolated from chicory roots. Urías-Silvas *et al.* (2008) studied several species of agave fructans from *A. angustifolia Oax, Dasyrilion spp. and A. tequiliana Gto* and reported different effects *in vitro* towards the stimulation of *Bifidobacterium breve* and in the production of butyric acid compared with the effect of FOS-Raftilose 95 by gas chromatography mass spectrometry technique.

The work presented here is the first study to investigate the influence of the fermentation of Agave fructans on a complex faecal microbiota *in vitro*. The results indicate that inulin derived from Agave has a potential prebiotic effect as it increases the growth of bifidobacteria and lactobacilli. This effect is similar to the one observed for established inulin-type prebiotics derived from chicory root thus justifying the potential of Agave as a prebiotic.

Agave consumption represents the oldest evidence of prebiotics consumption in North America (dating back

Table 2 Short-chain fatty acids production ± SD by substrates fermentations in pH-controlled and stirred batch cultures

	Total production				Acetic				Propionic				Butyric			
Substrate	0 h	5 h	10 h	24 h	0 h	5 h	10 h	24 h	0 h	5 h	10 h	24 h	0 h	5 h	10 h	24 h
Synergy1™	0 ± 0·00	8·15 ± 10·3	5·08 ± 8·80	58·09† ± 4·66***	0 ± 0·00	6·34 ± 7·24	5·08 ± 8·81	33·62†,§ ± 7·24***	0 ± 0·00	1·59 ± 2·76	0 ± 0·00	14·33†,‡,§ ± 12·7	0 ± 0·00	0·22 ± 0·38	0·00 ± 0·00	1·80 ± 2·09
FOS-Raftilose 95™	0·22 ± 0·38	11·30 ± 15·5	27·85 ± 20·1**	51·97† ± 11·4***	0·22 ± 0·38	9·77 ± 13·2	24·41† ± 14·7**	40·74†,§ ± 4·76***	0 ± 0·00	1·26 ± 1·88	2·88 ± 4·48	8·54† ± 7·56	0 ± 0·00	0·27 ± 0·47	0·56 ± 0·97	2·69 ± 4·03*
Predilife	0·15 ± 0·26	6·24 ± 5·27	24·41† ± 10·2*	49·15† ± 15·6***	0·15 ± 0·26	4·67 ± 4·16	22·38†,‡ ± 9·19**	29·85†,‡ ± 4·53***	0 ± 0·00	1·57 ± 1·86	1·70 ± 1·68	18·32† ± 17·4*	0 ± 0·00	0 ± 0·00	0 ± 0·00	0·98 ± 0·85
Actilight™	0 ± 0·00	0·00 ± 0·00	22·87 ± 11·3*	42·80† ± 13·5***	0·00 ± 0·00	0 ± 0·00	16·54 ± 6·19	35·41†,‡ ± 6·51***	0 ± 0·00	0 ± 0·00	5·88 ± 10·2	7·21 ± 7·05	0 ± 0·00	0 ± 0·00	0·45 ± 0·78	0·16 ± 0·27
Inulin HP™	0·26 ± 0·40	6·01 ± 5·70	13·23 ± 16·8	50·83† ± 12·3***	0·26 ± 0·00	4·21 ± 3·66	12·62†,‡ ± 17·3	31·95†,‡,§ ± 12·9	0 ± 0·00	1·80 ± 3·12	0·60 ± 1·05	16·83† ± 17·4	0 ± 0·00	0 ± 0·00	0 ± 0·00	1·78 ± 1·99
Cellulose	0·68 ± 0·19	1·83 ± 1·80	1·70 ± 0·28	6·50† ± 1·63	0·52 ± 0·18	1·33 ± 1·22	1·27 ± 0·38	4·55†,‡,§ ± 0·80	0·05 ± 0·08	0·50 ± 0·61	0·42 ± 0·11	1·61 ± 0·59	0·11 ± 0·16	0 ± 0·00	0 ± 0·00	0·34 ± 0·31

Values are mmol l^{-1} concentrations in batch culture at 0, 5 and 24 h of fermentation as means of three experiments with different faecal donors.
†Significantly different from initial concentration ($P < 0·05$).
‡Significantly different from 5 h concentration ($P < 0·05$).
§Significantly different from 10 h concentration ($P < 0·05$).
*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ Significantly different from control (cellulose) using two-way ANOVA with Bonferroni post test.

over 9000 years) (Leach *et al.* 2006). Mexico is considered the origin centre of *Agave* genus because of the 272 species that can be found within its territory, most of them are used for the production of popular ethnic alcoholic beverages such as sotol, mezcal and tequila, and as a source of food, fibre, medicine, shelter and tools (Gómez-Ayala *et al.* 2005). *Agave* genus tequilana Weber var. *azul* represents an economically important crop in Mexico, because it is the only plant allowed for the production of tequila, which is one of the most frequently consumed alcoholic beverages worldwide (López *et al.* 2003). *A. tequilana* is grown mainly in the state of Jalisco, Mexico and exhibits an average content of water soluble carbohydrates of 900 mg g^{-1} possessing a higher carbohydrate content (mostly fructans) compared to that reported for chicory (240 mg g^{-1}) (Gil-Vega *et al.* 2001). The molecular structure of fructans in *A. tequilana* Weber var. *azul* represents the three well-known fructans-based types: inulin, levan and neoinulin; and different ß-D-Fru*f* moieties, including (2 → 1), (2 → 6) and branched linkages, the former being the most abundant, with a molecular weight distribution of 527–4739 Da by MALDI-TOF-MS analysis, which corresponds to a DP ranging from 3 to 29. Additionally, agave fructans are very soluble in cold water, meaning that they can be easily incorporated into beverages, bakery products, dairy products like cheese and yogurts and used as fat replacers.

Currently, there is an overproduction of Agave plants in Mexico (Reported on Nutraingredients, 2005), and there is a high interest in its high fructan concentration to explore this natural resource as fibre, sweeteners and supplement ingredients. As Agave represents a very important agricultural resource in Mexico, industries could be encouraged to use it as a cheaper source of inulin compared with the derived from imported brands. Its ability to exert a prebiotic effect in a human study remains to be determined, however.

Acknowledgements

This study was supported by the Food microbial sciences unit at University of Reading, United Kingdom in cooperation with National Autonomous University of Mexico. R.E. Gómez was supported with a postgraduate scholarship from Consejo Nacional de Ciencia y Tecnología, CONACYT, México.

Conflict of interest, source of funding and authorship

The authors declare that they have no conflict of interests. The research project was supported by a postgraduate grant (No. 169721) from CONACYT, Food Microbial Science Unit at University of Reading and Faculty of Medicine at the National Autonomous University of Mexico. All authors made active contributions to the conceptions and design and analysis and interpretation of data. Also, all authors reviewed the final stage of the manuscript.

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