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Abstract

Interest in the consumption of gum acacia (GA) has been associated with beneficial health effects, which may be mediated in part by prebiotic activity. Two doses of GA and fructooligosaccharide (FOS) (1% and 2%) were tested for their efficacy over 48 h in pH- and temperature-controlled anaerobic batch cultures inoculated with human faeces. Samples were taken after 0, 5, 10, 24, and 48 h of fermentation. The selective effects of GA (increases in *Bifidobacterium* sp. and *Lactobacillus* sp.) were similar to those of the known prebiotic FOS. The 1% dose of substrates showed more enhanced selectivity compared to the 2% dose. The fermentation of GA also led to SCFA production, specifically increased acetate after 10, 24, and 48 h of fermentation, propionate after 48 h, and butyrate after 24 and 48 h. Additionally, FOS led to significant increases in the main SCFAs. These results suggest that GA displays potential prebiotic properties.

Key words

Gum acacia (GA); Prebioitcs; Intestinal bacteria; *In vitro* fermentation.

Introduction

The colon has the most abundant and diverse population of bacteria in the human body and is inhabited by around 1000 different bacterial species, which can reach 10^{14} colony-forming units CFU (Gibson and Roberfroid 1995). The human microbiome is a complex and dynamic system that plays an important role in human health (Eckburg et al. 2005, Kaiko and Stappenbeck 2014). By interacting with consumed material, colonic inhabitants ferment undigested food and secrete end products such as gases and SCFA (Gibson and Roberfroid 1995). Moreover, the composition of the bacterial population may shift, resulting in increases in bacteria associated with beneficial effects. Inulin and FOS are known prebiotics that are commercially used worldwide (Mandalari et al. 2008, Steer et al. 2003). A prebiotic is defined as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (Gibson et al. 2010).

GA is derived from acacia trees of the *Leguminosae* family. It is an arabinogalactan protein complex with an approximate molecular weight of 350-850 K Da (Mahenran.T et al. 2008,, Williams and Phillips 2000.). It is a polysaccharide consisting of branched chains of (1-3) linked β-D galactopyranosyl units. Side chains, 2 to 5 units in length, are attached by (1-6) units to the main chain. Both the main and side chains contain α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl, and 4-O-methyl-β-D-glucuronopyranosyl units (Bliss et al. 2013). GA is widely used in the pharmaceutical and food industries as an additive , a stabilising, thickening, and an emulsifying agent (Dauqan and Abdullah 2013) (Verbeken et al. 2003).

GA is not digestible in the small intestine and is fermented in the large intestine, and has been observed to lead to increases in *Bifidobacterium* spp. (Calame et al. 2008) (Terpend et al. 2013) (Marzorati et al. 2015). *Bifidobacterium* spp. have been shown to inhibit the growth of

pathogenic bacteria, modulate the immune system, and produce SCFAs, which reduce the pH in the colon, imparting antimicrobial activity against pathogens (Gibson and Roberfroid 1995). A variety of GA doses ranging from 5 to 40 g/d have been reported to be effective in increasing *Bifidobacterium* spp. and *Lactobacillus* spp. populations (Calame, Weseler, Viebke, Flynn and Siemensma 2008, Cherbut et al. 2003). These lines of evidence indicate that GA has bifidogenic potential in healthy humans. However optimal effective doses have not been defined. The results from most previous studies cannot be directly compared, as different methodologies, population groups, and types of GA have been studied. In addition, high daily doses of GA could result in the manifestation of adverse effects such as mild diarrhoea and bloating (Babiker et al. 2012).

In vitro batch culture systems are used to simulate the main physiological and microbiological processes in the distal colon and can be combined with metabolic and molecular analyses. Therefore, the aim of the following study was to investigateof the impact of two GA doses on the faecal microbiota following 48 h fermentation in pH-controlled batch culture systems, as compared to the known prebiotic FOS.

Material and methods

Substrates

GA (KLTA-MF-Kerry Ingredients, U.K.) was in spray dried form as a water soluble, free-flowing powder (food-grade). **Table (1)** shows the composition of GA used in this study, dietary fibre were analysed by Campden BRI Laboratories (AOAC method 991·43). The FOS used was Orafti® P95 (Beneo, Belgium) extracted from chicory root.

98 [Table 1 near here].

99

100 ***In vitro Upper Gut Digestion***

101 Upper gut digestion was performed according to the protocol of Mills *et al.*(2008) (Mills et
102 al. 2008). Briefly, 60 g of GA powder was added to 150 ml of distilled water and the solution
103 mixed with 20 mg α–amylase in 6.25 ml $CaCl_2$ (1 mM) and incubated on a shaker at 37 °C
104 for 30 minutes. This simulated the initial oral digestion. Subsequently, 2.7 g of pepsin in
105 25ml of HCl (0.1M) was used to facilitate gastric breakdown of the sample. The pH was then
106 reduced progressively to 2 by adding 6 M HCl, before incubating on a shaker at 37°C for 2
107 hours. A further 560mg of pancreatin and 3.5g of bile, in 125 ml of $NaHCO_3$ solution, was
108 added to simulate the effect of the small intestine on the gum sample. The pH was increased
109 to 7 by adding NaOH (6M) and the resulting suspension incubated on a shaker at 37°C for 3
110 hours. Samples were transferred to cellulose dialysis membrane (1 KDa molecular weight),
111 purchased from Cheshire biotech Cheshire, UK, and dialysed against 10Mm of NaCl solution
112 at 5°C to remove low molecular mass digestion products. After 15 hours, the dialysis fluid
113 was changed and dialysis continued for additional 2 h. The sample within the dialysis tubing
114 was freeze dried (5 days) prior to use in batch culture systems. The final sample was weighed
115 and GA loss in the upper gut was subsequently calculated.

116 ***Faecal sample preparation***

117 Faecal samples were obtained from three healthy volunteers (31- 35 years of age), who had
118 not been consuming antibiotics for at least 6 months before the study and had no history of
119 gastrointestinal disease. Volunteers were not consumers of probiotic or prebiotic
120 supplements. Samples were prepared on the day of the experiment and within 1 hour of
121 production and were diluted to 1:10, w/v in anaerobic phosphate buffer (0.1 M; pH7.4).

Samples were homogenised in a stomacher for 2 min, the resulting slurry was inoculated into batch culture fermenters.

Batch cultures

Three independent batch culture experiments were carried out using faeces from a different donor each time. Vessels were autoclaved and then aseptically filled with 135 ml of basal medium (peptone water (2 g/l), yeast extract (2 g/l), NaCl (0.1 g/l), K_2HPO_4 (0.04 g/l), KH_2PO_4 (0.04 g/l), $NaHCO_3$ (2 g/l), $MgSO_4 \cdot 7H_2O$ (0.01 g/l), $CaCl_2 \cdot 6H_2O$ (0.01 g/l), tween 80 (2 ml/l), hemin (50 mg/l), vitamin K1 (10 ml/l), L-cysteine (0.5 g/l), bile salts (0.5 g/l), 0.5 ml/l of 10% cysteine –HCl, resazurin (1 mg/l)). Vessels were left overnight with nitrogen pumping (15mL/min) through the vessel to provide an anaerobic environment. Before addition of faecal slurry, temperature of basal medium was set at 37 °C and pH was maintained at 6.7-6.9 using a pH meters (Electrolab pH controller, Tewksbury, UK) by the addition of 0.5 M HCl or 0.5M NaOH. The vessels were stirred using magnetic stirrers. 1.5g, 3g (1% w/v) of FOS and 0.6 g and 0.3 g (1:10 w/v) of pre-digested GA (taking to account the loss of substrate in the upper gut from the predigestion) were added to the vessel 1% and doubling the dose 2% just prior to the addition of 15 ml of faecal slurry (10%w/w). The vessels were left for 48h, with samples taken at 0, 5, 10, 24 and 48h. Samples were centrifuged in preparation for GC analysis, or prepared for microbial enumeration by FISH.

Florescence in situ hybridisation (FISH) analysis

To asses diffrences in bacterial population, samples hybridised as described by Daims et al., 1999 (Daims et al. 1999). A sample of 375-µl obtained from each vessel was fixed for four hours 4˚C in 1125 µ L (4% w/v) paraformaldehyde. Fixed samples were then centrifuged at 11,337g (Eppendorf centrifuge minispin, Eppendorf, UK) at room temperature for 5 minutes. The supernatant removed and discarded. The pellet was resuspended in 1 ml of cold 1×PBS

by aspirating carefully using a pipette. This step was conducted twice. The washed cells were suspended in 150 µL of cold 1×PBS, then 150 µL of ethanol (99%) was added and the samples were stored at -20˚C.

The oligonucleotide probes used were commercially synthesised and labeled with the fluorescent dye Cy3 (Sigma Aldrich Co. Ltd. UK). These were: Bif164 for *Bifidobacterium spp.* (BIF), Lab158 for *Lactobacillus/enterococcus* (LAB), Ato291 for *Atopobium cluster* (*Atopobium, Coriobacterium, Collinsella spp.)* (ATO), Chis 150 for *Clostridium histolyticum* group (CHIS) Erec 482 for *Eubacterium rectale – Clostridium coccoides* group (EREC), Bac 303 for *Bacteroides–Prevotella* group (BAC). EUB 338 mixture consisting of EUB338, EUB338II and EUB338III for total bacteria (Total) see **Table (2)**. For the hybridisation 20 µ L of diluted sample was pipetted onto a teflon poly-L-lysine-coated six-well slide (Tekdon Inc., Myakka City, FL). The samples were dried onto the slides at 46-50˚C for 15 minutes and after that dehydrated in an alcohol series 50, 80, and 96%. The ethanol was allowed to evaporate from the slides before hybridisation buffer was added. A probe/hybridization buffer mixture (5 µL of a 50 ng/µL stock of probe plus 50 µL of hybridization buffer). To permeabilise the cells for use with probes Bif164 and Lab158, samples were treated with 20 µ L of lysozyme at room temperature for 15 min before being washed briefly for 2−3 seconds in water and then dehydrated in the ethanol series. Then slides were placed in hybridisation oven for 4 hours (ISO 20 oven, Grant Boekel). For the washing step, slides were placed in 50 ml of washing buffer (0.9 M NaCl, 0.02 M Tris/HCl (pH 8.0), 0.005 M ethylenediaminetetraacetic acid (EDTA) solution (pH 8.0, **Table (2)**, warmed at the appropriate temperature for each probe and 20 µL of 4;6-diamidino-2-phenylindole di hydrochloride (DAPI) was added to the washing buffer for 15 min. They were then briefly washed (2−3 s) in ice-cold water and dried under a stream of compressed air. Five microliters of ProLong Gold antifade reagent (Invitrogen) was added to each well and a coverslip

applied. Slides were stored in the dark at 4 °C until cells were counted under a Nikon E400 Eclipse microscope. DAPI stained cells were examined under UV light, and a DM510 light filter was used to count specific bacteria hybridised with the probes. For each slide, 15 random different fields of view were counted.

[Table 2 near here].

Preparation sample for short chain fatty acids

Samples were extracted and derivatised as previously described (Richardson et al. 1989). Samples were defrosted and 1ml of each sample or standard solution was transferred into a labeled 100 mm × 16mm glass tube with the internal standard of 50µl of 2- ethyl butyric acid (0.1M). 0.5 ml concentrated HCl and 2 ml of diethyl ether was added to each glass tube and samples vortexed for 1 min. samples were centrifuged at 2000 g for 10 min (SANYO MSE Mistral 3000i; Sanyo Gallenkap PLC, Middlesex, UK). The diethyl ether (the upper layer) was transferred in a new glass tube. A second extraction was conducted by adding 1 ml of diethyl either to the sample followed by vortex and centrifugation. 400 µl of pooled ether extract and 50 µl N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) was added in a GC screw-cap vial. Samples were heated at 80°C for 20 minutes and then left at room temperature for 48 hours to allow lactic acid in the samples to completely derivatise.

A 5890 SERIES II Gas Chromatograph (Hewlett Packard, UK) using an Rtx-1 10m×0.18mm column with a 0.20µm coating (Crossbond 100% dimethyl polysiloxane; Restek, Buckinghamshire, UK) was used for analysis of SCFA. Temperatures of injector and detector were 275°C, with the column programmed from 63°C for 3 minutes to 190°C at 10°C min-1 and held at 190°C for 3 minutes. Helium was the carrier gas (flow rate 1.2 ml min-1; head pressure 90 MPa). A split ratio of 100:1 was used. The standard solution contained (mM): sodium formate, 10; acetic acid, 30; propionic acid, 20; isobutyric acid, 5; n-butyric acid, 20;

iso-valeric acid, 5; n-valeric acid, 5; sodium lactate, 10; sodium succinate, 20. The sample was injected onto the column, which was maintained at 140 ^{o}C for the first 5 minutes, temperature of the column was increased over 5 minutes to 240 ^{o}C. To maintain appropriate calibration after injection of every 20 samples an external standard solution, with known concentrations of SCFAs was injected. Peaks and response factors within samples were calibrated and calculated using ChemStation B.03.01 software (Agilent Technologies, Cheshire, UK).

Statistical analysis

GA and FOS both doses were tested in batch cultures inouculated with faecal samples collected from three individial donors in three separate experiments. The log $_{10}$ numbers of specific bacteria were expressed as mean values and standard diviation. Statistical tests were performed using SPSS, (SPSS Statistical Software, Inc., Chicago, IL, USA), version 18.0 A repeated measures one-way analysis of variance ANOVA to test the effect of time with the factor subjects, with five levels (0 h, 5 h, 10 h, 24 h and 48 h) and to assess the significant differences between the two subjects in the same time points. Significant differences between times point were represented by "*" $p < 0.05$, "**" $p < 0.01$ and "***" $p < 0.001$.

Results

Effects of different doses of GA and FOS on human faecal bacteria

To assess the impact of GA on the intestinal microbiota composition, pH-controlled, anaerobic, faecal batch cultures were conducted using FOS as a positive prebiotic control. Samples were taken after 0, 5, 10, 24, and 48 h of fermentation. Bacteria were enumerated by FISH.

The fermentation of GA at the 1% dose led to increased numbers of *Bifidobacterium* spp. after 5, 10, and 24 h of fermentation and of *Lactobacillus* spp. after 5 and 24 h compared with the levels at 0 h, as shown in **Tables (3a, 3b)**. However, a small but statistically significant drop in *Bifidobacterium* spp. compared with the negative control was seen after 24 h (7.53±0.10, 7.22±0.37 GA 1%) (7.46±0.21, 7.64±0.29 control).

Fermentation of 2% GA a led to a significant increase in *Bifidobacterium* spp. after 5 and 10 h compared with the baseline levels (time 0 h). Total bacteria increased after the fermentation of 2% GA for 48 h compared with the baseline levels.

The 1% dose of FOS led to a significant increase in *Bifidobacterium* spp. after 5 h compared with baseline (7.39± 0.21, 7.83± 0.06). *Lactobacillus* spp. increased following fermentation of FOS at a dose of 1% at 5, 10, and 24 h. The 2% FOS dose significantly increased *Bifidobacterium* spp. numbers after 24 h compared with the negative control (8.12±0.16, 7.78±0.17) and after 5 h compared with baseline levels ($p = 0.03$), whereas an increase in *Lactobacillus* spp. was observed after 5 h (7.75±0.14, 7.29± 0.07) and 24 h (7.51± 0.10, 7.29± 04) ($p = 0.01$ and 0.02, respectively) compared with the negative control.

The number of bacteria in the *C. histolyticum* group decreased after fermentation of 1% FOS and 1% GA for 5 and 10 h, respectively, compared with the baseline levels. Additionally, GA and FOS enhanced the growth of *Atopobium* spp. after 5 h of fermentation compared with the baseline levels. The *C. coccoides-Eubacterium rectale* group did not change with any of the tested substrates. Additionally, *Atopobium* also increased after 5 h of fermentation of 2% FOS.

In the current study both substrates led to increases in *Bacteroides* spp.; these changes occurred after 5 h of fermentation of GA and FOS at the 1% dose ($p = 0.01$ and $p = 0.02$, respectively) and with the 2% dose of GA and FOS compared with the baseline levels ($p =$

241 0.02 and p = 0.00, respectively). Moreover, 1% FOS increased *Bacteroides* spp. after 24 h (p
242 = 0.01). On the other hand, the prebiotic FOS at the 1% dose enhanced the growth of total
243 bacteria, achieving statistical significance after 10 h and 24 h. Additionally, total bacterial
244 growth was enhanced with 2% FOS after 5 h compared with the negative control.

245 [Tables 3a & 3b near here].

246 ***Impact of GA and FOS on SCFA production***

247 **Table (4)** shows that within 1% GA, the concentration of acetate significantly increased after
248 24 h of fermentation ($p \leq 0.05$) and exhibited an increasing trend after 5, 10, and 48 h (p =
249 0.06, 0.06, and 0.08, respectively). Acetate levels were elevated at all time points after FOS
250 fermentation compared with the levels at 0 h (p = 0.00, 0.00, 0.04, and 0.00, respectively),
251 and 1% FOS led to an increase after 5 h compared with the negative control (p = 0.01).
252 Butyrate production was significantly enhanced following the fermentation of FOS between 0
253 and 24 h (p = 0.02) and following GA 1% fermentation after 24 and 48 h (p = 0.03, 0.02);
254 this was also the case at 24 h when compared with the negative control (p = 0.04). Compared
255 with the levels at 0 h, propionate production increased following the fermentation of GA1%
256 for 48 h (p = 0.03) and the fermentation of 1% FOS for 24 h (p = 0.01).

257 Additionally, with 2% FOS, acetate increased after 24 and 48 h (p = 0.00 and 0.01,
258 respectively), and with 2% GA, acetate increased after 48 h compared with the baseline levels
259 (p = 0.02). Butyrate production increased following the fermentation of 2% GA for 10 and 48
260 h compared with the levels at 0 h (p= 0.01 and 0.03, respectively). In addition, propionate
261 increased after 10 h of fermentation of 2% FOS compared with the negative control (p =
262 0.04) and the baseline values (p = 0.01).

263 [Table 4 near here].

Discussion

Prior studies have noted the importance of the effect of GA on improving human health (Terpend, Possemiers, Daguet and Marzorati 2013). GA is not digestible in the upper gastrointestinal tract, therefore it can reach the large intestine where it is fermented by intestinal bacteria (Adiotomre et al. 1990),(Annison et al. 1995), (Bourquin et al. 1996). Therefore this study aimed to determine the effects two doses of GA on human intestinal bacteria and to assess prebiotic potential as compared to prebiotic FOS. As such pH-controlled batch culture fermentation systems were used to evaluate the selectivity of GA when fermented with healthy human gut microbiota compared with FOS at two different doses.

Several studies have shown that GA can undergo a slow fermentation, specifically a more distal fermentation, whereas existing prebiotics typically undergo proximal fermentation (Macfarlane et al. 1992), as proteolytic fermentation develops in the distal colon; therefore, this substrate may be able to be saccharolytically fermented in this part of colon. In this present study GA fermentation shows selectivity in bifidobacteria and *Lactobacillus* spp at time 10 h and continues to 24 h which could indicate slower fermentaion and is inline with others work (Cherbut, Michel1, Raison1, Kravtchenko and Severine 2003). However, it is worth noting that the bifidogenic effect of the 1% was not maintained at 48 hours in current study.

In the present study, GA significantly enhanced the growth of *Bifidobacterium* spp.. *Bifidobacterium* is considered an important group related to human health, having a favourable impact in the large intestine (Gibson and Wang 1994),(Russell et al. 2011). These results are consistent with those of Calame *et al* (2008) in which the consumption of 10 g of GA daily by healthy adults had a beneficial effect on the gut microbial composition, and increases in bifidobacteria (Calame, Weseler, Viebke, Flynn and Siemensma 2008).

289 Furthermore, the 1% dose also led to increases in lactobacilli. Lactobacilli has long been
290 considered a positive microbial group; as such, stimulation of this genera offers potential
291 benefits to the host (Ouwehand et al. 2009). The 2% dose did not lead to the same lactobacilli
292 impact. The use of a higher doasge could have impacted on selectivity through a cross
293 feeding network (Guiot 1982).

294 Furthermore, after 10 h, the numbers of bacteria in the *C. histolyticum* group decreased
295 following the fermentation of 1% GA, which also agreed with results of *in vivo* studies
296 (Wyatt et al. 1986), (Calame, Weseler, Viebke, Flynn and Siemensma 2008), (Cherbut,
297 Michel1, Raison1, Kravtchenko and Severine 2003). This group of bacteria has sometimes
298 been associated with inflammation and large bowel disease (Hughes 2008), (Gibson and
299 Roberfroid 2008). It is thought that increased numbers of beneficial bacteria could lower the
300 pH within the colon, therefore making the environment unfavourable for pathogenic groups;
301 the results of the present study indicate that the fermentation of GA selectively increased the
302 number of beneficial bacteria and reduced the number of harmful bacteria. This result further
303 suggests the lower dose to offer improved selective potential.

304 In the current study GA fermentation resulted in a similar bacterial profile to FOS. Several
305 *in vivo* and *in vitro* studies have confirmed that FOS can regulate the gut through the selective
306 stimulation of the gut microbiota (Hidalgo et al. 2012), (Palframan et al. 2002), (Cueva et al.
307 2013), (Tuohy et al. 2001), therefore, GA could has potentially prebiotic properties. GA can
308 be incorporated into baked goods, therefore could provide an alternative prebiotic source for
309 inclusion in the diet.

310 Moreover, an increase in *Bacteroides* spp. was observed with in GA and FOS, this group is
311 associated with a range of colonic activities (Pool-Zobel et al. 2002), (Nakamura et al. 2002).
312 *Bacteroides* spp. constitute a large proportion of the microbial population in the healthy adult
313 gastrointestinal tract (Walton 2006). In previous *in vivo* and *in vitro* studies on GA

fermentation, increased propionate levels were associated with *Bacteroides* spp. and *Prevotella* spp. which has relevance to the improving in lipid metabolism (Frost et al. 2014),(Tulung et al. 1987).

The results show that both doses of GA were selectivly fermented. The higher dose was arguably less selective as the impact on lactobacilli and *C. histolyticum* groups were no longer apparent. In fact Calame et al (2008) noted that an increase in the concentration of substrates results in less selectivity (Calame, Weseler, Viebke, Flynn and Siemensma 2008). This might be explained by competiton for substrate, at higher dose other bacterial strains have easier access to the substrate and subsequently, become less selevtively than the lower dose.

GA fermentation induced modulation of the colonic microbiota, with increased levels of acetate, propionate, and butyrate. Acetate is produced mainly through the fructose-6-phosphate phosphoketolase pathway by bifidobacteria, and the increased production of this acid could be related to increased numbers of this group (Miller and Wolin 1996). Acetate plays an important role in controlling inflammation and resisting invasion by pathogens (Rigottier-Gois et al. 2003). Propionate may be produced by Cluster IX Clostridia groups; indeed an increase in this bacterial groups was observed during GA fermentation Hosseini *et al* (2011). Propionate may have a direct role in central appetite regulation; increasing satiety and reducing food intake by the host (Russell et al. 2013), (Brown et al. 2003), (Chambers et al. 2015), (Cherbut et al. 1998). Whilst butyrate is often used as an energy source by epithelial cells; as such . these SCFA increases could offer benefits to the host.

The pH-controlled stirred batch culture systems enabled rapid analysis of the effects of GA on the faecal microbiota. In the absence of absorption, colonic secretions, and epithelial interactions, the system has limitations. However, processes such as SCFA production can

still be monitored away from the impact of additionally dietary factors. Thus, batch culture systems provide an alternative way of assessing how bacteria ferment a substrate and the end products they produce (Ohashi et al. 2012).

The comparisons of the substrates in the pH-controlled batch cultures indicated that GA has selective abilities that are at least similar to those of the known prebiotic FOS, as indicated by the bacteriology results and increased concentrations of acetate, butyrate, and propionate. These results could be relevant to improving host health by increasing the levels of the bifidobacteria group, especially in individuals with lower numbers such as elderly population. Tuohy *et al* (2001) reported that prebiotics can alter the gut microbiota in those with initially low numbers of bifidobacteria (Tuohy, Kolida, Lustenberger and Gibson 2001). This may be particularly relevant in elderly people. Elderly are experiencing negative changes in their gut microbiota.

Conclusion

The aim of the current study was to use *in vitro* batch cultures to assess the effects of GA on the microbiota compared to FOS. Here, we showed that GA modulated the gut microbiota similarly to FOS, furthermore, the 1% dose showed additional selective potential. As such GA holds the potential to be used as a novel prebiotic source.

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Table 1: Composition and nutritional profile of GA used in the present study

Analysis	**Results**
Energy (kcal)	1205kJ/100g
Protein	2.1g/100g
Total carbohydrate (by difference)	82.6g/100g
Carbohydrate (avail)	56.5g/100g
Total Suger*	0.6g/100g
Fibre	26.1g/100g
Fat	0.1g/100g
Sodium	11.0mg/100g
Moisture	11.8g/100g
Ash	3.49g/100g

593 * Total sugars are the sum of glucose, sucrose and fructose expressed as monosaccharides

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609 **Table 2 :** Hybridisation and washing conditions for oligonucleotide probes

Probe name	Sequence (5' to 3')	Hybridisation pre-treatment	Formamide (%) in hybridisation buffer	Hybridisation temperature (°C)	Washing temperature (°C)	Reference
Ato 291	GGTCGGTCTCTCAACCC	Lysozyme	0	50	50	(Harmsen et al. 2000)
Lab 158	GGTATTAGCAYCTGTTTCCA	Lysozyme	0	50	50	(Harmsen et al. 1999)
Bif 164	CATCCGGCATTACCACCC	Lysozyme	0	50	50	(Langendijk et al. 1995)
Erec 482	GCTTCTTAGTCARGTACCG	None	0	50	50	(Franks et al. 1998)
Chis 150	TTATGCGGTATTAATCTYCCTTT	None	0	50	50	(Franks, Harmsen, Raangs, Jansen, Schut and Welling 1998)

Bac 303	CCAATGTGGGGGACCTT	None	0	46	48	(Manz et al. (1996))
EUB338*	GCTGCCTCCCGTAGGAGT	None	35	46	48	(Daims, Brühl, Amann, Schleifer and Wagner 1999)
EUB338II*	GCAGCCACCCGTAGGTGT	None	35	46	48	(Daims, Brühl, Amann, Schleifer and Wagner 1999)
EUB338III*	GCTGCCACCCGTAGGTGT	None	35	46	48	(Daims, Brühl, Amann, Schleifer and Wagner 1999)
* These probes are used together in equimolar concentrations (all at 50 ng μl^{-1})						

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	Bif164		Lab158		Ato291		Bac303		Erec482		Chis150		EubI-II-III	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Control 0h	7.46	0.21	7.24	0.09	7.40	0.18	7.85	0.24	7.96	0.20	6.91	0.24	8.26	0.35
Control 5h	7.76	0.20	7.29	0.07	7.60	0.15	8.04	0.20	7.36	0.07	6.42	0.38	8.45	0.08
Control 10h	7.75	0.24	7.47	0.07	7.56	0.02	8.25	0.28	7.51	0.07	6.99	0.16	8.44	0.04
Control 24h	7.78	0.16	7.29	0.04	7.54	0.14	8.12	0.21	7.54	0.08	5.73	0.47	8.53	0.36
Control 48h	7.64	0.29	7.14	0.50	7.50	0.61	7.76	0.17	7.51	0.06	5.68	0.73	8.42	0.18
FOS 0h	7.39	0.21	7.31	0.08	7.52	0.09	7.80	0.11	7.72	0.2	6.62	0.53	8.21	0.14
FOS 5h	7.83 *****	0.06	7.78 ******	0.04	7.83 *****	0.10	8.16 *****	0.09	7.41	0.16	5.71 *****	0.55	8.78	0.11
FOS 10h	8.00	0.07	7.53 ******	0.09	7.77	0.30	8.30	0.10	7.83	0.29	6.23	1.09	8.72 *****	0.08
FOS 24h	8.17	0.23	7.75 *****	0.14	7.79	0.31	8.24 *****	0.01	7.50	0.12	6.27	0.88	8.83 *****	0.14
FOS 48h	7.82	0.15	7.44	0.15	7.58	0.18	7.93	0.18	7.28	0.19	5.65	0.69	8.57	0.17
GUM 0h	7.53	0.10	7.00	0.16	7.43	0.05	7.70	0.22	7.81	0.37	6.08	0.50	8.29	0.22
GUM 5h	7.92 *****	0.11	7.32 *****	0.05	7.80 ******	0.06	7.97 *****	0.19	7.42	0.11	6.64	0.42	8.54	0.18
GUM 10h	7.90 *****	0.14	7.46	0.11	7.60	0.26	8.01	0.35	7.44	0.166	5.87 *****	0.82	8.79	0.71
GUM 24h	7.93 *****	0.06	7.59 ******	0.22	7.66	0.22	8.08	0.52	7.65	0.17	5.87	0.82	8.53	0.25
GUM 48h	7.22 **a**	0.37	7.39	0.11	7.53	0.40	8.20	0.24	7.39	0.49	6.28	0.86	8.49	0.56

Table 3-b: changes in the bacterial composition figures are presenting the mean bacterial populations in pH-controlled batch cultures at 0, 5, 10, 24, and 48h.Values are mean ± SD.*, significant differences from the 0 h value within the same treatment, p<0.05. small letters differences from the negative control. 1% faecal batch culture inoculated with vessel 1 negative control, vessel 2 FOS 1%, vessel 3 FOS 2%, vessel 4 GA 1%, vessel 5 GA 2%.

	Bif164		**Lab158**		**Ato291**		**Bac303**		**Erec482**		**Chis150**		**EubI-II-III**	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Control 0h	7.46	0.21	7.24	0.09	7.40	0.18	7.85	0.24	7.96	0.20	6.91	0.24	8.26	0.35
Control 5h	7.76	0.20	7.29	0.07	7.60	0.15	8.04	0.20	7.36	0.07	6.42	0.38	8.45	0.08
Control 10h	7.75	0.24	7.47	0.07	7.56	0.02	8.25	0.28	7.51	0.07	6.99	0.16	8.44	0.04
Control 24h	7.78	0.16	7.29	0.04	7.54	0.14	8.12	0.21	7.54	0.08	5.73	0.47	8.53	0.36
Control 48h	7.64	0.29	7.14	0.50	7.50	0.61	7.76	0.17	7.51	0.06	5.68	0.73	8.42	0.18
FOS 0h	7.4	0.1	7.24	0.24	7.57	0.10	7.89	0.17	7.84	0.27	6.44	0.51	8.35	0.29
FOS 5h	7.93 *****	0.15	7.75 **a**	0.14	7.98 ******	0.12	8.38 *****	0.02	7.74	0.3	6.45	0.40	8.89 **a**	0.083
FOS 10h	8.02	0.11	7.58	0.14	7.89	0.18	7.8	0.32	7.88	0.19	6.25	0.94	8.76	0.26
FOS 24h	8.12 **a**	0.17	7.51 **a**	0.1	7.81	0.19	8.07	0.3	7.82	0.19	5.88	0.85	8.56	0.08
FOS 48h	7.88	0.11	7.43	0.15	7.67	0.10	7.95	0.34	7.62	0.18	5.87	0.15	8.69	0.29
GUM 0h	7.33	0.05	7.38	0.14	7.58	0.18	7.73	0.22	7.77	0.2	6.39	0.58	8.11	0.17
GUM 5h	7.78 ******	0.06	7.32	0.13	7.67	0.29	8.01 ******	0.23	7.6	0.16	6.31	0.67	8.44	0.16
GUM 10h	7.98 *****	0.13	7.6	0.31	7.70	0.25	8.21	0.54	7.58	0.2	6.09	0.75	8.74	0.47
GUM 24h	7.93	0.28	7.67	0.27	7.66	0.26	8.17	0.3	7.68	0.29	6.57	0.73	8.72	0.18
GUM 48h	7.69	0.149	7.34	0.12	7.78	0.27	8.2	0.25	7.46	0.3	6.04	0.91	8.66 *****	0.17

Table 4: changes in the SCFA concentration are presenting in table 3 in pH-controlled batch cultures at 0, 5, 10, 24, and 48h.Values are mean ± SD.*, significant differences from the 0 h value within the same treatment, $p<0.05$. small letters differences from the negative control. 1% faecal batch culture inoculated with vessel 1 negative control, vessel 2 FOS 1%, vessel 3 FOS 2%, vessel 4 GA 1%, vessel 5 GA 2%.

	ACETATE	**PROPIONATE**	**BUTYRATE**
Control			
0 h	1.68 ± 1.16	0.19± 0.12	0.15 ± 0.09
5 h	4.49 ± 0.99 a	0.82 ± 0.86	2.15 ± 3.19
10 h	6.47 ± 1.34	2.51 ± 1.19 a	1.47 ± 0.35
24 h	8.88 ± 1.71	4.50 ± 2.29	1.73± 0.85 a
48 h	10.57 ± 3.57	5.40 ± 3.11	3.10 ± 2.41
FOS 1%			
0 h	0.98 ± 0.04	0.10 ± 0.03	0.08 ± 0.02
5 h	8.84 ± 3.53 ** a	1.86 ± 0.31	0.66 ± 0.24
10 h	13.12 ± 2.02 **	8.45 ± 6.98	4.42 ± 2.02
24 h	11.89 ± 4.22 *	8.13 ± 2.10 *	8.26 ± 2.98 *
48 h	10.00 ± 1.11 **	4.32 ± 3.42	1.21 ± 1.58
FOS 2%			
0 h	1.35 ± 0.26	0.19 ± 0.10	0.13 ± 0.06
5 h	14.28 ± 4.93	4.24 ± 3.54	2.32 ± 2.10
10 h	15.08± 2.93	5.80 ± 1.04 * a	5.93± 4.34
24 h	13.00 ± 7.81 **	5.44 ± 2.56	6.21 ± 4.05
48 h	8.80 ± 0.32 *	2.31 ± 1.85	3.66± 1.52
GUM 1%			
0 h	2.01 ± 1.73	0.18± 0.10	0.16 ± 0.12
5 h	5.36 ± 0.37	4.79 ± 4.76	4.99 ± 7.61
10 h	12.72± 4.79	6.77 ± 4.78	6.96 ± 7.79
24 h	10.78 ± 8.27	3.28 ± 2.61	4.59 ± 1.49 * a
48 h	11.64 ± 4.10 *	4.79 ± 1.46 *	4.01 ± 0.88 *
GUM 2%			
0 h	2.37 ± 2.29	0.19±0.12	0.16 ± 0.12
5 h	5.24 ± 0.56	5.13 ± 5.93	0.73 ± 0.42
10 h	16.67 ± 7.59	12.03 ± 8.32	2.40 ± 0.51
24 h	11.41 ± 9.15	3.28 ± 2.51	3.09 ± 2.49 *
48 h	13.63 ± 3.17 *	6.15 ± 2.24	6.24± 2.22 *