

# *In vitro* fermentation of gum acacia - impact on the faecal microbiota

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Accepted Version

Alarifi, S., Bell, A. and Walton, G. (2018) In vitro fermentation of gum acacia - impact on the faecal microbiota. *International Journal of Food Sciences and Nutrition*, 69 (6). pp. 696-704. ISSN 1465-3478 doi:  
<https://doi.org/10.1080/09637486.2017.1404970> Available at  
<https://centaur.reading.ac.uk/75203/>

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To link to this article DOI: <http://dx.doi.org/10.1080/09637486.2017.1404970>

Publisher: Informa Healthcare

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1 **Title page**

2 ***In vitro* fermentation of gum acacia - impact on the faecal microbiota**

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23 ***In vitro* fermentation of gum acacia - impact on the faecal microbiota**

24 **Abstract**

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

36 **Key words**

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

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## 50 **Introduction**

51 The colon has the most abundant and diverse population of bacteria in the human body and is  
52 inhabited by around 1000 different bacterial species, which can reach  $10^{14}$  colony-forming  
53 units CFU (Gibson and Roberfroid 1995). The human microbiome is a complex and dynamic  
54 system that plays an important role in human health (Eckburg et al. 2005, Kaiko and  
55 Stappenbeck 2014). By interacting with consumed material, colonic inhabitants ferment  
56 undigested food and secrete end products such as gases and SCFA (Gibson and Roberfroid  
57 1995). Moreover, the composition of the bacterial population may shift, resulting in increases  
58 in bacteria associated with beneficial effects. Inulin and FOS are known prebiotics that are  
59 commercially used worldwide (Mandalari et al. 2008, Steer et al. 2003). A prebiotic is  
60 defined as “a selectively fermented ingredient that results in specific changes in the  
61 composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon  
62 host health” (Gibson et al. 2010).

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

72 GA is not digestible in the small intestine and is fermented in the large intestine, and has been  
73 observed to lead to increases in *Bifidobacterium* spp. (Calame et al. 2008) (Terpend et al.  
74 2013) (Marzorati et al. 2015). *Bifidobacterium* spp. have been shown to inhibit the growth of

75 pathogenic bacteria, modulate the immune system, and produce SCFAs, which reduce the pH  
76 in the colon, imparting antimicrobial activity against pathogens (Gibson and Roberfroid  
77 1995). A variety of GA doses ranging from 5 to 40 g/d have been reported to be effective in  
78 increasing *Bifidobacterium* spp. and *Lactobacillus* spp. populations (Calame, Weseler,  
79 Viebke, Flynn and Siemensma 2008, Cherbut et al. 2003). These lines of evidence indicate  
80 that GA has bifidogenic potential in healthy humans. However optimal effective doses have  
81 not been defined. The results from most previous studies cannot be directly compared, as  
82 different methodologies, population groups, and types of GA have been studied. In addition,  
83 high daily doses of GA could result in the manifestation of adverse effects such as mild  
84 diarrhoea and bloating (Babiker et al. 2012).

85

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

91

## 92 **Material and methods**

### 93 ***Substrates***

94 GA (KLTA-MF-Kerry Ingredients, U.K.) was in spray dried form as a water soluble, free-  
95 flowing powder (food-grade). **Table (1)** shows the composition of GA used in this study,  
96 dietary fibre were analysed by Campden BRI Laboratories (AOAC method 991.43). The  
97 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  $\alpha$ -amylase in 6.25 ml  $\text{CaCl}_2$  (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  $\text{NaHCO}_3$  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).

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

#### 124 ***Batch cultures***

125 Three independent batch culture experiments were carried out using faeces from a different  
126 donor each time. Vessels were autoclaved and then aseptically filled with 135 ml of basal  
127 medium (peptone water (2 g/l), yeast extract (2 g/l), NaCl (0.1 g/l), K<sub>2</sub>HPO<sub>4</sub> (0.04 g/l),  
128 KH<sub>2</sub>PO<sub>4</sub> (0.04 g/l), NaHCO<sub>3</sub> (2 g/l), MgSO<sub>4</sub>•7H<sub>2</sub>O (0.01 g/l), CaCl<sub>2</sub>•6H<sub>2</sub>O (0.01 g/l), tween  
129 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  
130 ml/l of 10% cysteine –HCl, resazurin (1 mg/l)). Vessels were left overnight with nitrogen  
131 pumping (15mL/min) through the vessel to provide an anaerobic environment. Before  
132 addition of faecal slurry, temperature of basal medium was set at 37 °C and pH was  
133 maintained at 6.7-6.9 using a pH meters (Electrolab pH controller, Tewksbury, UK) by the  
134 addition of 0.5 M HCl or 0.5M NaOH. The vessels were stirred using magnetic stirrers. 1.5g,  
135 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  
136 loss of substrate in the upper gut from the predigestion) were added to the vessel 1% and  
137 doubling the dose 2% just prior to the addition of 15 ml of faecal slurry (10%w/w). The  
138 vessels were left for 48h, with samples taken at 0, 5, 10, 24 and 48h. Samples were  
139 centrifuged in preparation for GC analysis, or prepared for microbial enumeration by FISH.

#### 140 ***Florescence in situ hybridisation (FISH) analysis***

141 To asses differences in bacterial population, samples hybridised as described by Daims et al.,  
142 1999 (Daims et al. 1999). A sample of 375-µl obtained from each vessel was fixed for four  
143 hours 4°C in 1125 µ L (4% w/v) paraformaldehyde. Fixed samples were then centrifuged at  
144 11,337g (Eppendorf centrifuge minispin, Eppendorf, UK) at room temperature for 5 minutes.  
145 The supernatant removed and discarded. The pellet was resuspended in 1 ml of cold 1×PBS



146 by aspirating carefully using a pipette. This step was conducted twice. The washed cells were  
147 suspended in 150  $\mu$ L of cold 1 $\times$ PBS, then 150  $\mu$ L of ethanol (99%) was added and the  
148 samples were stored at -20°C.

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

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

175 [Table 2 near here].

#### 176 *Preparation sample for short chain fatty acids*

177 Samples were extracted and derivatised as previously described (Richardson et al. 1989).  
178 Samples were defrosted and 1ml of each sample or standard solution was transferred into a  
179 labeled 100 mm × 16mm glass tube with the internal standard of 50µl of 2- ethyl butyric acid  
180 (0.1M). 0.5 ml concentrated HCl and 2 ml of diethyl ether was added to each glass tube and  
181 samples vortexed for 1 min. samples were centrifuged at 2000 g for 10 min (SANYO MSE  
182 Mistral 3000i; Sanyo Gallenkamp PLC, Middlesex, UK). The diethyl ether (the upper layer)  
183 was transferred in a new glass tube. A second extraction was conducted by adding 1 ml of  
184 diethyl ether to the sample followed by vortex and centrifugation. 400 µl of pooled ether  
185 extract and 50 µl N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) was  
186 added in a GC screw-cap vial. Samples were heated at 80°C for 20 minutes and then left at  
187 room temperature for 48 hours to allow lactic acid in the samples to completely derivatise.

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

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

### 202 *Statistical analysis*

203 GA and FOS both doses were tested in batch cultures inoculated with faecal samples  
204 collected from three individual donors in three separate experiments. The log<sub>10</sub> numbers of  
205 specific bacteria were expressed as mean values and standard deviation. Statistical tests were  
206 performed using SPSS, (SPSS Statistical Software, Inc., Chicago, IL, USA), version 18.0 A  
207 repeated measures one-way analysis of variance ANOVA to test the effect of time with the  
208 factor subjects, with five levels (0 h, 5 h, 10 h, 24 h and 48 h) and to assess the significant  
209 differences between the two subjects in the same time points. Significant differences between  
210 times point were represented by “\*” p < 0.05, “\*\*” p < 0.01 and “\*\*\*” p < 0.001.

## 211 **Results**

### 212 *Effects of different doses of GA and FOS on human faecal bacteria*

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

217 The fermentation of GA at the 1% dose led to increased numbers of *Bifidobacterium* spp.  
218 after 5, 10, and 24 h of fermentation and of *Lactobacillus* spp. after 5 and 24 h compared with  
219 the levels at 0 h, as shown in **Tables (3a, 3b)**. However, a small but statistically significant  
220 drop in *Bifidobacterium* spp. compared with the negative control was seen after 24 h  
221 ( $7.53\pm 0.10$ ,  $7.22\pm 0.37$  GA 1%) ( $7.46\pm 0.21$ ,  $7.64\pm 0.29$  control).

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

225 The 1% dose of FOS led to a significant increase in *Bifidobacterium* spp. after 5 h compared  
226 with baseline ( $7.39\pm 0.21$ ,  $7.83\pm 0.06$ ). *Lactobacillus* spp. increased following fermentation  
227 of FOS at a dose of 1% at 5, 10, and 24 h. The 2% FOS dose significantly increased  
228 *Bifidobacterium* spp. numbers after 24 h compared with the negative control ( $8.12\pm 0.16$ ,  
229  $7.78\pm 0.17$ ) and after 5 h compared with baseline levels ( $p = 0.03$ ), whereas an increase in  
230 *Lactobacillus* spp. was observed after 5 h ( $7.75\pm 0.14$ ,  $7.29\pm 0.07$ ) and 24 h ( $7.51\pm 0.10$ ,  
231  $7.29\pm 0.04$ ) ( $p = 0.01$  and  $0.02$ , respectively) compared with the negative control.

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

238 In the current study both substrates led to increases in *Bacteroides* spp.; these changes  
239 occurred after 5 h of fermentation of GA and FOS at the 1% dose ( $p = 0.01$  and  $p = 0.02$ ,  
240 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 ( $p = 0.00, 0.00, 0.04, \text{ and } 0.00$ , 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].

264        **Discussion**

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

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

283        In the present study, GA significantly enhanced the growth of *Bifidobacterium* spp..  
284        *Bifidobacterium* is considered an important group related to human health, having a  
285        favourable impact in the large intestine (Gibson and Wang 1994),(Russell et al. 2011). These  
286        results are consistent with those of Calame *et al* (2008) in which the consumption of 10 g of  
287        GA daily by healthy adults had a beneficial effect on the gut microbial composition, and  
288        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 (Ouweland et al. 2009). The 2% dose did not lead to the same lactobacilli  
292 impact. The use of a higher dosage 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 Michell, Raison, 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 have 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

314 fermentation, increased propionate levels were associated with *Bacteroides* spp. and  
315 *Prevotella* spp. which has relevance to the improving in lipid metabolism (Frost et al.  
316 2014),(Tulung et al. 1987).

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

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

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



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

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

## 350 **Conclusion**

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

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## 357 ***Acknowledgements:***

358 We thank Saudi Arabian Ministry of Education, Kingdom of Saudi Arabia for their  
359 sponsorship of this study.

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

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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 $\mu\text{l}^{-1}$ )						

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	<b>Bif164</b>		<b>Lab158</b>		<b>Ato291</b>		<b>Bac303</b>		<b>Erec482</b>		<b>Chis150</b>		<b>EubI-II-III</b>	
	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>
<b>Control 0h</b>	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
<b>Control 5h</b>	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
<b>Control 10h</b>	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
<b>Control 24h</b>	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
<b>Control 48h</b>	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
<b>FOS 0h</b>	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
<b>FOS 5h</b>	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
<b>FOS 10h</b>	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
<b>FOS 24h</b>	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
<b>FOS 48h</b>	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
<b>GUM 0h</b>	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
<b>GUM 5h</b>	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
<b>GUM 10h</b>	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
<b>GUM 24h</b>	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
<b>GUM 48h</b>	7.22 <b>a</b>	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  $\pm$  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%.

	<b>Bif164</b>		<b>Lab158</b>		<b>Ato291</b>		<b>Bac303</b>		<b>Erec482</b>		<b>Chis150</b>		<b>EubI-II-III</b>	
	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>
<b>Control 0h</b>	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
<b>Control 5h</b>	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
<b>Control 10h</b>	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
<b>Control 24h</b>	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
<b>Control 48h</b>	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
<b>FOS 0h</b>	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
<b>FOS 5h</b>	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
<b>FOS 10h</b>	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
<b>FOS 24h</b>	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
<b>FOS 48h</b>	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
<b>GUM 0h</b>	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
<b>GUM 5h</b>	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
<b>GUM 10h</b>	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
<b>GUM 24h</b>	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
<b>GUM 48h</b>	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

618 **Table 4:** changes in the SCFA concentration are presenting in table 3 in pH-controlled batch  
619 cultures at 0, 5, 10, 24, and 48h. Values are mean  $\pm$  SD. \*, significant differences from the 0 h  
620 value within the same treatment,  $p < 0.05$ . small letters differences from the negative control. 1%  
621 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%.

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

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