An exploratory study into the putative prebiotic activity of fructans isolated from *Agave angustifolia* and the associated anticancer activity

Philip Allsopp, Sam Possemiers, David Campbell, Iván Saldaña Oyarzábal, Chris Gill, Ian Rowland

**ABSTRACT**

Linear inulin-type fructan (ITF) prebiotics have a putative role in the prevention of colorectal cancer, whereas relatively little is known about branched fructans. This study aims to investigate the fermentation properties and potential prebiotic activity of branched fructans derived from *Agave angustifolia* Haw, using the Simulator of Human Intestinal Microbial Ecosystem (SHIME) model. The proximal, transverse and distal vessels were used to investigate fructan fermentation throughout the colon and to assess the alterations of the microbial composition and fermentation metabolites (short chain fatty acids and ammonia). The influence on bioactivity of the fermentation supernatant was assessed by MTT, Comet and transepithelial electrical resistance (TER), respectively. Addition of Agave fructan to the SHIME model significantly increased (\(P < 0.05\)), bifidobacteria populations (proximal and transverse), SCFA concentrations (proximal, transverse and distal) and decreased ammonia concentrations in the distal vessel. Furthermore, the fermentation supernatant significantly increased in the proximal colon sample containing fructan to the SHIME model. Agave fructans. To conclude, branched Agave fructans show indications of prebiotic activity, particularly in relation to colon health by exerting a positive influence on gut barrier function, an important aspect of colon carcinogenesis.

**Keywords:** Agave, Fructan, Prebiotic, SHIME, Short chain fatty acids, Transepithelial electrical resistance, Colon cancer, Comet assay

**1. Introduction**

The colonic microbiota is continuously gaining prominence as a source of factors contributing to the etiology and pathogenesis of a range of diseases, including colon cancer [1]. Identification of nutritional constituents that positively influence colonic health through modification of the microbiota offers a useful strategy for reducing the risk of colon cancer [2,3]. Prebiotics have emerged as food ingredients with beneficial health promoting activity through stimulation of beneficial bacteria (bifidobacteria/lactobacilli) and their associated saccharolytic metabolites (SCFA's, particularly butyrate) [4]. Putative mechanisms responsible for prebiotic-mediated anticancer activity include improved colonic barrier function and enhanced geno-protection which have been partly attributed to the potent activity of butyrate [4,5].

Inulin-type fructans (ITF) are unquestionably the most studied prebiotic candidates to date with research demonstrating a broad range of health benefits [6]. Linear chained ITF prebiotics have consistently exhibited stimulatory effects on bifidobacterial populations alongside an increase in associated saccharolytic fermentation products such as short chain fatty acids in both animal and human studies [7]. The rate and extent of ITF fermentation appears to be strongly influenced by the degree of polymerisation (DP). Fructooligosaccharides - FOS (low DP), are rapidly fermented in the proximal colon [8], whereas inulin (high DP) appears to have a more sustained fermentation profile potentially enabling it to exert protective effects in the distal regions of the colon [9,10]. Ideally, prebiotic supplementation should provide uniform stimulation of gut bacterial activities throughout the entire colon, in particular the distal colon where proteolytic fermentation predominates and is
associated with the production of toxic metabolites e.g. ammonia, hydrogen sulphide, and cresol [11,12]. It has been suggested that other fructan types, in particular Agave derived fructans (AGV), which historically have been consumed by indigenous Central American populations, could potentially offer similar or enhanced benefits to human colon health [13]. AGV have a branched fructan structure with both β(1-2) and β(2-6) linked fructosyl chains attached to the sucrose start unit, whereas ITF are limited to a linear structure with β(1-2) linkages [14]. It has been postulated that the structural branching of the Agave fructans may result in an alternative and more sustained fermentation pattern than linear ITF leading to enhanced saccharolytic fermentation at the expense of proteolytic fermentation in the distal colon. Furthermore, this would enable the protective effects exerted by prebiotics in the proximal colon to also be exhibited in the distal colon. A recent study by Gomez et al. has demonstrated prebiotic activity of fructans from Agave tequiliana in batch culture studies and this current study aims to provide information on the fermentation dynamics of fructans from Agave angustifolia in the different regions of the colon [15]. In this study the prebiotic efficacy will be determined using the continuous culture Simulated Human Intestinal Microbial Ecosystem (SHIME) model which has previously been used to characterise the fermentation profile of other prebiotic carbohydrates – inulin and FOS [10,16]. Furthermore, we will investigate the anticancer activity of the fermentation supernatant using a range of biomarkers that have been implicated as having a role in colon carcinogenesis.

2. Material and methods

2.1. Agave fructan extraction

The Agave fructans were obtained from Mercantil Orgánica S.A de C.V., a fructan distributor in Mexico. The food grade fructan powder product was obtained from the matured stems of 7–8 year old A. angustifolia Haw. plants. The stems were mechanically sliced into smaller pieces, pressed to extract their juices, and finally washed with abundant hot water (60°C) to maximise recovery. The extracted juice was then clarified, filtered, deionised, concentrated and lastly, spray dried to obtain the final product. This commercial product has a carbohydrate composition of >96% fructans plus <4% of monosaccharides (fructose/glucose) and an average DP of 16 which was kindly donated by Dr. Iván Saldaña Oyarzábal.

2.2. SHIME culture system

The SHIME model, adapted from Molly et al. [17], is a dynamic, 5 vessel model of the human adult gastrointestinal tract with a total retention time of 76 h. Vessels 1 and 2 are intended to model the stomach and small intestinal processes, with vessels 3, 4 and 5 modelling the proximal, transverse and distal colon respectively. The SHIME colonic vessels were inoculated with a faecal sample of a young adult male volunteer (following written and informed consent) with no history of antibiotic treatment or colonic disorders 6 months prior to the study. The study was approved by the Ethical Committee of Ghent University Hospital (Belgian registration number B670201214538). The freshly voided faecal sample was diluted and homogenised with phosphate buffer (0.1 mol/L, pH 7), (10% w/v) and following the removal of particulate material by centrifugation (at 50,000 g for 5 min), 50 ml was introduced into each of the SHIME colonic vessels. The microbial culture was stabilised over a period of 2 weeks on a carbohydrate-based medium [17] and allowed to adapt to the specific environmental conditions of the ascending, transverse and descending colon in terms of pH range, retention time and available carbon sources. During the pre-treatment control period (PRE) the SHIME was supplemented with the standard nutritional media for the first 2 weeks of the experiment. Subsequently a three week Agave fructan (AGV) treatment period was initiated whereby the standard media (which provided 2 g/d starch) was replaced with an experimental medium in which starch was substituted with AGV (2 g/day) (Fig. 1). The Agave experimental media was equivalent to a human intake of 4 g/day, a dose that has been shown not to exert any negative effects in human studies. The replacement of starch with fructans in the nutritional media ensured the amount of available carbohydrates for the microorganisms remained unaltered throughout the SHIME experimental period. Following the AGV treatment period, the starch based nutritional media was re-introduced for a 2 week post-treatment control period (POST) to investigate whether the microbial and metabolic parameters returned to PRE levels.

2.3. SHIME collection

AGV fermentation was modelled using the SHIME and samples were obtained from vessel 3 (proximal colon PV), vessel 4 (transverse colon TV) and vessel 5 (distal colon DV) during the PRE, AGV and POST periods. Samples were taken and directly used for microbial and metabolic analysis or stored at −80°C for subsequent testing.

2.4. Microbiota and metabolic activity analysis

2.4.1. Microbial community analysis

The quantification of bacteria groups using plate counting was adapted from Van de Wiele et al. [16], with the enumeration of colony forming units following growth on specific media (Oxoid, Hampshire, UK): lactobacilli (Rogosa agar), bifidobacteria (Raffinose Bifidobacterium agar), enterococci (Enterococcus agar), enterobacteria (MacConkey agar) and clostridia (tryptose sulphate cycloserin agar).

![Fig. 1. An overview of the SHIME experiment protocol from faecal inoculation and stabilisation of the 3 colonic vessels and subsequent treatment periods.](image-url)
2.4.2. Short chain fatty acids

Samples from the 3 ‘colonic’ vessels were collected and frozen at −20 °C for subsequent analysis. The short-chain fatty acids (SCFA) were extracted from the samples with diethyl ether (Sigma) and determined with a Di200 gas chromatograph (GC; Shimadzu’s-Hertogenbosch, The Netherlands) as described in Possemiers et al. [18].

2.4.3. Ammonia

The protocol was adapted from Van de wiele et al. [17]. In brief, using a 1026 Kjeltec Auto Distillation unit (FOSS Benelux, Amersfoort, The Netherlands), ammonium in the sample was liberated as ammonia by the addition of an alkali (MgO). The released ammonia was distilled from the sample into a boric acid solution. The solution was backtitracted using a 665 Dosimat (Metrohm, Berchem, Belgium) and 686 Titroprocessor (Metrohm).

2.5. Fermentation supernatant preparation for colon cancer bioassays

The SHIME fermentation cultures from the proximal colon vessel (PV), transverse colon vessel (TV) and distal colon vessel (DV) sampled during the PRE, AGV and POST were stored at −80 °C. Fermentation supernatants were prepared by centrifugation of the thawed fermentation slurries at 50000 g (Beckman Ultracentrifuge XL-70, Beckman Instruments, Inc., California, USA) at 4 °C for 2 h. Supernatants were filter sterilised using 0.45 μm and 0.2 μm syringe filters (Minisart, Vivasience AG, Hannover, Germany) and stored at −80 °C.

2.5.1. Cell culture

The human colon cancer cell line Caco-2 (colorectal adenocarcinoma) was obtained from the European Collection of Cell Cultures and used between passages 50–60 (Salisbury, UK). Caco-2 cells were routinely cultured in minimum essential media (MEM), 10% foetal bovine serum, 1% penicillin streptomycin and 1% non-essential amino acids. All tissue culture materials were purchased from Gibco (Paisley, UK).

2.5.2. Cell cytotoxicity (MTT) assay

The SHIME fermentation supernatants were assessed for cytotoxicity in Caco-2 cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. This method was adapted from Gill et al. [19] and enabled toxicity of test samples to be compared to media (untreated) and positive controls (deoxycylic acid 250/500 μM). Each treatment was carried out in 8 wells and the experiment repeated independently 3 times.

2.5.3. Transepithelial electrical resistance (TER) assay

The method used in this study was adapted from Gill et al. and McGilligan et al. [20,21]. The 0.4 μm cell culture inserts (Becton Dickinson) were coated with 0.01% type I rat tail collagen (Sigma) and UV sterilised. The Caco-2 cells were cultured for 21 days to enable enterocytic differentiation. The electrical resistance of the monolayer (TER) was measured using an EVOM epithelial voltmeter with chopstick electrodes (World Precision Instruments Ltd., Aston, UK). Upon stabilisation of TER, the media in the apical layer was removed and replaced with fluorescein enriched media containing the range of SHIME supernatant treatments and controls. Concentrations of ethanol (5.7%) and propionate (10 μM), that were known to decrease and increase TER, respectively, were used as positive controls. TER and basolateral fluorescein measurements were recorded at 0 h and 24 h to determine monolayer resistance and paracellular permeability, respectively. Each treatment was carried out in duplicate and the experiment repeated independently 3 times.

2.5.4. The Comet assay (single cell gel electrophoresis)

The antigenotoxicity of all fermentation supernatant samples were assessed by their ability to decrease DNA damage in Caco-2 cells induced by oxidative challenge with H2O2 (75 μM). This method was adapted from Gill et al. [19]. In brief, Caco-2 cells were seeded for 24 h followed by treatment with SHIME supernatant for 24 h. The cells were then trypsinised, counted, exposed to PBS or H2O2 (75 μM) for 5 min on ice, centrifuged and subsequently immersed in low melting point agar and placed on a bed of normal melting point agar gel. The cells were then placed in lysis buffer (1 h), immersed in electrophoresis buffer for 20 min followed by electrophoresis (26 V, 300 mA) for 20 min. The gels were then washed in neutralising buffer for 5 min (∗× 3) and stained with ethidium bromide. DNA damage, as measured by tail intensity, was subsequently quantified using an epi-fluorescent microscope and Komet 3.0 image analysis software (Kinetic Imaging Ltd, UK). Each treatment was carried out in triplicate and the experiment repeated independently 3 times.

3. Results

3.1. SHIME

3.1.1. Bacterial populations

Viable counting on selective growth media was used to investigate the microbial composition of the SHIME liquid from the different vessels throughout the SHIME experiment. During the studies with AGV there were no marked changes in the number of total anaerobic bacteria between the 3 phases of the SHIME runs PRE, AGV or POST in any of the colonic vessels (Table 1).

AGV treatment increased bifidobacteria populations in all 3 vessels, with significant increases in the ascending and transverse SHIME vessels (P < 0.05). In contrast to the other groups of organisms investigated, the number of bifidobacteria returned to PRE values when the AGV was removed and significant differences were noted between AGV and POST periods in all 3 vessels (P < 0.05). Lactobacilli populations were also stimulated during Agave treatment in all 3 vessels, with numbers declining again during the POST period, however no significant differences were noted (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Period</th>
<th>Proximal colon</th>
<th>Transverse colon</th>
<th>Distal colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacteria</td>
<td>Pre-treatment 6.86 ± 0.25*</td>
<td>6.70 ± 0.01*</td>
<td>6.74 ± 0.28ab</td>
<td>6.49 ± 0.01*</td>
</tr>
<tr>
<td></td>
<td>Treatment 3.75 ± 0.26*</td>
<td>7.26 ± 0.03*</td>
<td>7.09 ± 0.12ab</td>
<td>7.14 ± 0.12*</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>Pre-treatment 3.78 ± 0.20*</td>
<td>2.46 ± 0.28</td>
<td>1.96 ± 0.00ab</td>
<td>2.00 ± 0.00ab</td>
</tr>
<tr>
<td></td>
<td>Treatment 4.19 ± 0.11*</td>
<td>2.92 ± 0.38</td>
<td>2.80 ± 0.32ab</td>
<td>2.80 ± 0.32ab</td>
</tr>
<tr>
<td>Clostridia</td>
<td>Pre-treatment 7.51 ± 0.01*</td>
<td>7.04 ± 0.39</td>
<td>7.07 ± 0.05</td>
<td>7.14 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Treatment 7.73 ± 0.13</td>
<td>7.44 ± 0.17</td>
<td>7.20 ± 0.11</td>
<td>7.14 ± 0.15</td>
</tr>
<tr>
<td>Enterococci</td>
<td>Pre-treatment 6.61 ± 0.31</td>
<td>6.38 ± 0.21</td>
<td>6.14 ± 0.01ab</td>
<td>6.14 ± 0.01ab</td>
</tr>
<tr>
<td></td>
<td>Treatment 6.54 ± 0.15</td>
<td>6.12 ± 0.16ab</td>
<td>5.89 ± 0.31*</td>
<td>5.89 ± 0.31*</td>
</tr>
<tr>
<td>Total aerobes</td>
<td>Pre-treatment 7.70 ± 0.67</td>
<td>7.62 ± 0.51</td>
<td>7.59 ± 0.17</td>
<td>7.14 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Treatment 7.84 ± 0.50</td>
<td>7.79 ± 0.19</td>
<td>7.54 ± 0.21</td>
<td>7.49 ± 0.22</td>
</tr>
<tr>
<td>Total anaerobes</td>
<td>Pre-treatment 8.12 ± 0.22</td>
<td>7.29 ± 0.03</td>
<td>7.93 ± 0.09</td>
<td>7.93 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Treatment 8.46 ± 0.28</td>
<td>8.32 ± 0.21</td>
<td>8.05 ± 0.29</td>
<td>8.05 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>Treatment 8.51 ± 0.10</td>
<td>8.31 ± 0.18</td>
<td>8.08 ± 0.31</td>
<td>8.08 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>Treatment 8.30 ± 0.08</td>
<td>8.14 ± 0.13</td>
<td>7.88 ± 0.15</td>
<td>7.88 ± 0.15</td>
</tr>
</tbody>
</table>

Bars represent mean and SD values of the different treatments. One-way ANOVA and Dunnett T test, values sharing a superscript are not significantly different whilst differing superscripts denotes a significant difference (P < 0.05).
3.2. Short chain fatty acids

3.2.1. Proximal colon vessel (Table 2)

Supplementation with AGV resulted in an increase of total SCFA concentration from 42.73 mM during PRE to 49.84 mM (P < 0.01) which persisted throughout the POST period at 52.51 (P < 0.05). Acetic acid concentrations significantly increased from 28.94 mM in PRE to 31.74 mM during treatment (P < 0.05) which remained elevated during the POST period at 33.40 mM (P < 0.01). Propionate showed an increase from 4.98 mM in PRE to 7.53 mM during treatment (P < 0.05) which remained relatively stable throughout the POST period (16.48 mM (P < 0.01) which remained relatively high at 7.77 mM (P < 0.01) compared to PRE.

3.2.2. Transverse colon vessel (Table 2)

An increased total SCFA was observed in the transverse colon, rising from 51.03 mM during the PRE period to 61.02 mM (P < 0.01) during the POST period. Acetic acid was increased from 28.65 mM during the PRE to 31.74 mM during treatment (P < 0.01), and continued to rise throughout the POST period to 33.57 mM (P < 0.01). Propionic acid concentrations slightly increased from PRE values of 14.19 mM – 16.48 mM during treatment (P < 0.01) and remained relatively stable throughout the POST period (15.59 mM). Butyric acid increased from 5.59 mM during PRE to 7.77 mM (P < 0.01) during treatment and continued to increase throughout the POST period (P < 0.01) reaching 8.81 mM.

3.2.3. Distal colon vessel (Table 2)

Total SCFA concentrations increased from 57.54 mM to 63.05 mM during treatment and continued to increase during the POST period peaking at 65.88 mM (P < 0.05). Acetic acid slightly increased from 33.40 mM to 34.90 mM and remained high during the POST period 36.62 mM (P < 0.01). Propionic acid increased from 15.21 mM during the PRE to 16.48 mM (P < 0.05) and regressed slightly to 16.30 mM (P < 0.01). Butyric acid concentrations increased from 6.12 mM during PRE to 9.56 mM (P < 0.01) during treatment and increased further to 9.56 (P < 0.01) during the POST period.

3.2.4. Ammonia (Table 2)

AGV treatment did not significantly change ammonia concentration in the PV or TV, however it significantly decreased ammonia concentration in the distal colon (27.00 mM – 25.60 mM, P < 0.01) with both returning to close to PRE levels during the POST period.

3.3. Anticancer bioactivity of SHIME supernatants

3.3.1. TER assay (Fig. 2)

The control treatments for the TER experiments gave the expected results: propionate increased TER at 24 h (20.31 ± 3.3, P < 0.01) in relation to its value at 0 h, whereas ethanol decreased TER (−54.67 ± 4.0, P < 0.001) which was significantly different from the media control.

The samples from the proximal SHIME vessel, after AGV treatment caused an increase in TER over 24 h (12.7 ± 3.13, P < 0.05) which was significantly different from both PRE (4.5% ± 1.25% and POST (7.9% ± 1.62%). AGV treatment showed a significant decrease in fluorescein flow compared to PRE at 24 h (−19.3 ± 8.55, P < 0.05) which is in agreement with the changes in TER. Despite an elevated TER after AGV treatment of 11.06% ± 2.76% in the transverse vessel this did not reach statistical significance compared to the changes apparent in the PRE (4.55% ± 2.75%) or post treatment (4.64% ± 4.34%). However, AGV treatment supernatant from the distal SHIME vessel significantly increased TER (17.2% ± 5.60, P < 0.05) relative to corresponding PRE (10.0% ± 4.07) and POST (9.0% ± 11.93) samples. The TER changes observed in the media control after 24 h (6% ± 5.23) and 48 h (−1.24% ± 0.91) did not significantly differ from the TER at 0 h.

3.3.2. Cytotoxicity and Comet assays

The MTT assay showed no cytotoxic effects of any of the SHIME supernatants from any of the vessels. When challenged with 75 μM H2O2, the DNA damage (% tail DNA) induced in Caco-2 cells averaged 52.93% ± 0.92 in the negative control. No significant anti-genotoxic effect was observed in any of the treatment supernatant samples when pre-incubated for 24 h with Caco-2 cells. Genotoxicity was not significantly different in any of the SHIME treatment samples compared to the negative media control (data not shown).

4. Discussion

The high mortality and risk of colorectal cancer and the modifiable nature of the disease particularly through diet highlights a need to identify dietary components to decrease risk [22]. Prebiotics, inulin-type prebiotics in particular, are emerging as a food ingredient which can positively influence the intestinal microbiota composition and metabolism and have been shown to beneficially alter biomarkers of colon cancer in in-vitro and in-vivo studies [6]. Recent studies have also implicated faecal transplantation as a method to alter the gut microbiota to benefit health, however this research approach is in its infancy and usually only applicable for high risk individuals [23]. Continuous culture systems simulate the...
environmental conditions of the different colonic segments found in-vivo and enable changes of regional luminal contents to be investigated in a manner which is not possible in normal human intervention studies [10,16].

Several studies have investigated the relationship between DP and fermentation dynamics of linear inulin [9,10], however there is a paucity of data on the influence of branched inulin on microbiota composition and fermentation and the associated health benefits. The findings from our study have demonstrated that branched Agave fructans exert significant bifidogenic activity alongside significantly elevated SCFA production (butyrate in particular) and reductions in ammonia production. Moreover, significant increases in bifidobacteria were noted in the simulated PV and TV of the SHIME and although there was an increase in the DV, it failed to reach significance. The results obtained from this continuous culture study confirm the stimulatory effect of AGV on bifidobacteria populations alongside the increase in short chain fatty acids observed in the batch culture study by Gomez et al. [15]. However, the increases of lactobacilli populations in our study in all 3 SHIME vessels failed to reach the statistical significance, unlike the Gomez et al. study.

The current study has provided additional information on the fermentation activity along the simulated colon. Previous studies have consistently shown prebiotics to selectively increase bifidobacteria and lactobacilli and these bacteria populations and their fermentative activities have been suggested to be responsible for modulation of the microbiota leading to a range of health benefits [24]. The noted population increases of these groups following AGV treatment would implicate Agave as an alternative source to chicory for inulin-type prebiotics. However, more work is needed to investigate the particular species that benefit from AGV supplementation and future work using pyrosequencing technologies could provide pivotal information regarding the more intricate changes of the microbiota [25].

Short chain fatty acids (SCFA) are a major product of prebiotic fermentation and increased colonic concentration of these compounds (acetate, propionate and butyrate) has been attributed to the health benefits of inulin [26]. This study demonstrated significant increases in SCFA’s with notable increases in the proportion of butyrate in all 3 vessels provide indications of enhanced saccharolytic fermentation. Butyrate is a compound of critical importance in the colon, with multiple roles including the provision of 70% of the colonocytes metabolic needs, controlling normal colonic mucosal homeostasis through its proliferative and apoptotic activities in healthy and transformed tissue, respectively [27], as well as its immunomodulatory activity through its histone deacetylase inhibitory activity on NFkB expression [28]. The butyrigenic effect of AGV is a desirable observation and has been suggested to be a major contributor to prebiotic-mediated health benefits on colon health [29]. Previous SHIME experiments investigating the prebiotic activity of linear inulin exhibited similar activity [8]. The reversible and significant decrease in ammonia alongside enhanced SCFA apparent in the distal vessel may be due to enhanced saccharolytic fermentation at the expense of proteolytic fermentation. The reduced exposure of ammonia to the colonocytes following prebiotic supplementation has been attributed to increased ammonia assimilation by bacteria, and the protective effects of butyrate have also been suggested to be responsible for reducing ammonia mediated toxicity [30,31]. The observed reduction in ammonia alongside the elevated bifidobacteria populations and
short chain fatty acid production during AGV treatment indicates a degree of shift from proteolytic fermentation to saccharolytic fermentation, this is particularly important in the distal vessel where proteolytic fermentation predominates leading to the accumulation of toxic metabolites. Barrier function of the colonic mucosa is critical to the maintenance of health and immunity of the human host [32]. Loss of barrier function has been attributed as a key factor in the pathogenesis of many gastrointestinal disorders including inflammatory bowel disease and the promotion of colon cancer [33–35]. Despite a range of suggested mechanisms to implicate a role of mucosal barrier function in colon cancer promotion, the exact pathological consequences of altered gut permeability on the process of carcinogenesis have not been fully elucidated [36,37].

Gill et al. showed using a caco-2 barrier function model that the faecal water of healthy adults improves barrier function whilst that of the elderly impaired it, suggesting faecal water as a putative marker to determine age-related alteration of barrier function [20]. Similar ex-vivo models have been used to investigate the influence of dietary components and metabolites on barrier function, such as probiotics, prebiotics, butyrate and vitamin D, which have all been shown to improve barrier function [38–42]. The use of these ex-vivo and in-vivo models could help in the identification of dietary components that alter barrier function to enable the development of dietary strategies towards preventing age-related barrier impairment and reducing the risk of associated diseases. The current study used the Caco-2 barrier function model to investigate the influence of AGV fermentation supernatant. This study demonstrated that AGV fermentation supernatant from all SHIME vessels resulted in elevated monolayer resistance although only significant differences were noted in the proximal and distal colon, and these results were corroborated, in part, by the fluorescein results. The complexity and diversity of metabolites within the SHIME supernatants makes it difficult to conclusively identify the contributing compounds associated with the observed bioactivity. However, a potential mechanism for the increase in barrier function is the elevated SCFA concentration (especially that of butyrate) seen in the AGV fermentation supernatants [38,43–45]. Butyrate is thought to act via up-regulating tight junction proteins or influencing their assembly. In contrast to the effect on barrier function, no protectve effects were observed against DNA damage, the other cancer biomarker tested. Interestingly, beneficial effects on genotoxicity have been reported for certain prebiotics in animal and human studies [45,46] and for probiotic/prebiotic mixtures in humans [42] with increased SCFA concentrations, particularly butyrate, implicated in antigenotoxic activity [44].

This study has demonstrated that AGV specifically stimulated bifidobacteria populations in a human colonic model, without changes in total bacterial numbers, as well as increasing SCFA concentrations. In combination with the demonstrated enhancement in barrier function in-vitro, these results strongly suggest that AGV have prebiotic potential, which needs to be confirmed in placebo controlled human dietary intervention studies.

Acknowledgements

This project was carried out with the financial support from the Northern Ireland Department of Education and Learning and The Rank Prize Fund travel grant.

References


