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

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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 ($P < 0.05$) increased the TER of a Caco-2 cell monolayer (%) and decreased fluorescein-based paracellular flux, suggesting enhanced barrier function and reduced epithelial barrier permeability (proximal and distal vessel). While cytotoxicity and genotoxicity remained unaltered in response to the presence of *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.

1. Introduction

The colonic microbiota is continuing to gain prominence as a source of factors contributing to the aetiology 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

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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 sulfite cycloserin agar).

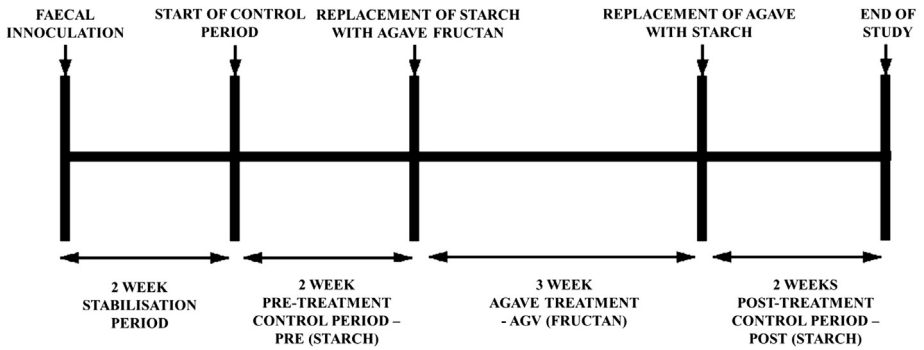

Fig. 1. An overview of the SHIME experiment protocol from faecal inoculation and stabilisation of the 3 colonic vessels and subsequent treatment periods.