Effect of agave fructans on the production of short chain fatty acid in mice

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Abstract The aim of this study was to evaluate the effect of agave fructans on short chain fatty acid (SCFA) production in mice (C57BL/6). Animals were randomly divided into three groups (n = 8): control group (CG), fructans from *Agave tequilana* group (FG) (55 g per day/kg of body weight), and oligofructose group (OG) (55 g per day/kg of body weight). Treatments were administered oral via for 6 weeks. After 24 days of treatment (phase 1), both treated groups exhibited elevated levels of acetic (12%) and

propionic (FG 66% and OG 38%) acids in comparison with the CG. After 42 days of treatment (phase 2), FG had higher concentrations of acetic (33%) and propionic (73%) acids than the CG; while OG exhibited higher levels of butyric (12%) and propionic (35%) acids compared with the CG. Fructans from *Agave tequilana* improve the production of SCFA in mice, suggesting a prebiotic activity.

Keywords Agave fructan · Oligofructose · Short chain fatty acid · Mice · Prebiotic activity

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Introduction

Prebiotics are non-digestible carbohydrates that selectively stimulate the growth and activity of specific bacteria in the human large intestine influencing host metabolism (Gibson et al., 2010). Through the variety of reactions and metabolic processes of non-digestible carbohydrates, the colonic microorganisms produce several metabolites such as CO₂, CH₄, H₂, and short chain fatty acids (SCFA), which are the main end products of colonic fermentation (Topping and Clifton, 2001). SCFA are carboxylic acids with aliphatic tails less than six carbons (acetate: C_{2:0}, propionate: C_{3:0}, and butyrate: C_{4:0}) (Layden et al., 2013). The most SCFA are absorbed in cecum and colon, while only a small percentage is excreted through the feces (5–10%). Once absorbed, SCFA are metabolized in ceco-colonic epithelium cells, hepatocytes, and muscle cells. SCFA have important biological functions including maintenance-energy, modulation of colonic and intracellular pH, cell volume ion transport, regulation on proliferation, and differentiation of gene expression (Meijer et al., 2010). Therefore, the production of SCFA is closely linked with intestinal homeostasis and host health (Wang et al., 2017).

It has been observed that some oligosaccharides act as prebiotics modulating microbiota composition and the production of SCFA. Wang et al. (2017) found that the treatment with oligosaccharides increased the levels of *lactobacillus* and *bifidobacterium* and elevated the concentration of SCFA in BALB/c mice. Nonetheless, there are other fibers such as agave fructans that have also shown prebiotic potential (Roberfroid et al., 1998). Agave fructans are carbohydrates with linear or branched fructose (oligo) polymers (β -2, 1-linked inulins or β -2, 6-linked levans), which confers resistance to hydrolysis by human digestive enzymes (Márquez-Aguirre et al., 2013), reaching the large bowel without structural changes, serving as fermentative substrates for the colonic microflora (López and Urías-Silvas, 2007). In this regard, a previous study investigated the prebiotic potential of fructans extracted from five different species of *Agaves* spp. on strains of *bifidobacteria* and *lactobacilli*, which revealed that fructans from *Agave tequilana* Weber increase the growth of both bacteria genera accompanied by the fermentation of acetic, formic and lactic acids (Urías-Silvas and López, 2009). Additionally, Márquez-Aguirre et al. (2013) also reported an increased count of fecal *bifidobacteria* in diet-induced obesity model receiving *Agave tequilana* Weber var. azul. In consistency, a clinical trial in healthy human subjects showed that supplementation with agave fructans from *Agave tequilana* Weber increases fecal *bifidobacteria* and *lactobacilli* (Ramnani et al., 2015). However, the effect of fructans from *Agave tequilana* Weber on SCFA production in mice has not been clarified. Therefore, the aim of the present study was to assess the effect of agave fructans on *bifidobacteria*, *lactobacillus*, and SCFA concentrations in C57BL/6 mice.

Materials and methods

Materials

The tested commercial prebiotics were: agave fructans from *Agave tequilana* Weber (90% inulin and 10% glucose/fructose/sucrose content. American Foods, Jalisco, Mexico), citric acid, glucose, fructose and sucrose from Sigma Aldrich (St. Louis, MO.) and oligofructose (93% oligofructose and 7% glucose/fructose/sucrose content. Raftilose P95 Beneo e Orafiti, Tienen Belgium) and donated by MEGAFARMA S.A. de C.V. México. This latter was used as an active control.

Animals and experimental design

Female C57BL/6 mice (Rismart SA de CV, México City, México), 12 weeks old, were housed in a light and

temperature-controlled room (12–12 h light–dark cycle; 25 ± 1 °C). The protocol was approved by the animal ethics committee of the TECNM/Instituto Tecnológico de Durango, Durango, Mexico and all experiments on animals were performed in accordance with the animal care and use protocol of the Norma Oficial Mexicana NOM-062-ZOO-1999, as recommended by NIH Guidelines.

Mice were randomly divided into three groups of eight animals each: (1) control group (CG): base diet + water, (2) fructans from *Agave tequilana* group (FG): base diet + 55 g per day/kg of body weight in water, and (3) oligofructose group (OG) base diet + 55 g per day/kg of body weight in water. Groups were fed with a base diet (proteins 23%, lipids 4.5% [1% saturated fat], and carbohydrates 64%) for 7 weeks. Water and diets were provided ad libitum. After 1 week of acclimatization, treatments were administered for 6 weeks using oral via. Measurement of food and water intake were obtained daily, whereas body weight was performed weekly.

Sample collection

Feces of 24 h were collected in four periods: baseline, phase 1 (24 days of intervention), phase 2 (42 days of intervention), and washing phase (8 days after intervention). Phase 1 and 2 were conducted to observe early changes in bacterial and SCFA concentrations following treatments, and the washing phase was performed to evaluate the remaining effect of the treatments after intervention period.

Feces were weighed and divided in two: one for bacterial quantification (*lactobacilli* and *bifidobacteria*) and the other was lyophilized and stored at -20 °C for SCFA analysis.

Bacterial quantification

Anoxic one-fourth strength peptone-water were used for serially diluted 10-fold of 100 mg/mL of feces. Then, 100 μ L of appropriated dilutions were inoculated (in triplicate) using selective media for the different bacteria: *lactobacilli* (Man-Rogosa-Sharpe (MRS) Difco, Detroit, MI USA) and *bifidobacteria* (Man-Rogosa-Sharpe (MRS) broth (Difco, Detroit, MI) supplemented with 0.05% (w/v) L-cysteine). After incubation, single colonies were quantified. Results were expressed as the log values of the colony-forming unit (CFU) per gram of wet weight of fecal content.

Feces sample processing for SCFA analysis

Feces samples (40 mg) were mixed with 1 mL of formic acid (1%) using an Ultra-Turrax homogenizer (IKA, Lab.

Equip., Germany) for 2 min. The samples were centrifuged at $14,000\times g$ 4°C for 10 min. The clear supernatants were collected. Then, 500 μL of ethyl acetate was added, and vortex mixing for 1 min. Feces samples were centrifuged again at $10,000\times g$ for 5 min. The extraction was done in duplicate.

Chemical derivatization

Feces extract were derivatized according the methodology described by Han et al. (2015) with little modifications. Briefly, 20 μL of extracts were mixed with 10 μL of 40 mM 3NPH-HCl solution and 10 μL of a mixed 37.5 mM EDC-HCl 1.5% pyridine solution. The samples were reacted at 40°C for 30 min, then were cooled on ice for 1 min. Finally, 960 μL of 10% aqueous acetonitrile (50:50) were added. Injection into the ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS) instrument was performed.

Ultra-performance liquid chromatography coupled with tandem mass spectrometry analysis

Acquity UPLC system (Waters Corp., Milford) coupled with a tandem Xevo TQ-S triple quadrupole mass spectrometer (Waters Corp., Wexford) were used for sample analysis. The sample manager (20°C) and a binary solvent manager respectively for LC system. The column used to determine SCFA was an Acquity UPLC HSS C18 SB, 100 mm $\times$ 2.1 mm, 1.8 μm (Waters Corp., Wexford) operated at 40°C . The elution profile included two solvents (LC-MS, both J.T. Baker): acidified water with 0.01% formic acid (solvent A) and acetonitrile with 0.01% formic acid (solvent B), flow rate was 0.350 mL/min: initial 85% A, 0–2 min, 45% A, 2–9 min, 0% A, 9–10 min, 0% A, 10–13 min, and 85% A (linear gradient) for column stabilization. Multiple reaction monitoring (MRM) data were collected from 0 to 15 min. For MS assay a negative ionization was used. Electron spray ionization (ESI) conditions were as follows: capillary voltage 1.5 kV, cone 2 V, desolvation temperature 350°C , source temperature 150°C , desolvation and cone gas 350 and 151 L/h, respectively; collision gas 0.13 mL/min and nebulizer gas flow 7.0 Bar. For identification and quantification, a multiple reaction-monitoring mode with standards (acetate acid, propionic acid, and butyric acid from Sigma-Aldrich, USA) was used. The UPLC and tandem Xevo TQ-S triple quadrupole mass spectrometer control and data were processed using MassLinx v. 4.1, Waters Corp.) Software.

Statistical analysis

Data were expressed as mean values $\pm$ standard error (SE). Statistical significance was determined by one-way variance analysis (ANOVA) ($p < 0.05$) followed by the Tukey's test. Statistical analysis was conducted using the Sigma Plot software version 13.0 (Systat Software, Inc., San Jose, CA).

Results and discussion

Animal model

There were no significant differences between the study groups for body weight, water and food consumption (data not showed). Given that we used a healthy animal model, these results were expected.

Bacterial concentrations in mice feces

The major fermentation products of prebiotic metabolism in large bowel are SCFAs; these have different effects on colon morphology and function such as regulation of the pH, absorption of water and sodium, and energy supply to the intestinal mucosa (Scheppach, 1994). Previous studies have reported that, at least in part, specific SCFA reduce the risk of developing some chronic gastrointestinal disorders such as inflammatory bowel disease, irritable bowel, and colorectal cancer (Jenkins et al., 1999; Scheperjans et al., 2015). In this regard, the purpose of this study was to evaluate the effect of fructans from *Agave tequilana* on the production of SCFA in mice.

Previous studies have described that prebiotics such as oligofructose selectively stimulate the growth of *bifidobacteria* and *lactobacilli* in the gastrointestinal tract, and consequently the fermentation products including SCFAs (Roberfroid, 2000). With this respect, we found a prebiotic property of fructans from *Agave tequilana* by increase in the concentration of *bifidobacterium* and *lactobacillus*. In phase 1, mice treated with agave fructans exhibited a significant increase in the *bifidobacteria* concentration ($\log_{10}$ 1) compared with both OG and CG, whereas in phase 2, FG and OG had a higher elevation of these bacteria ($\log_{10}$ 0.47 and $\log_{10}$ 0.33, respectively) than the CG (Fig. 1). Moreover, only in phase 2, treatment with agave fructans increased the *lactobacillus* concentration ($\log_{10}$ 0.53) in comparison with both OG and CG (Fig. 1). Finally, bacterial concentrations of *bifidobacteria* and *lactobacillus* returned to basal conditions in both intervention groups after the washing period. In agreement with the above-mentioned, Pompei et al. (2008) observed similar changes on these bacteria species in favor of fructans compared

Fig. 1 *Bifidobacteria* and *lactobacillus* concentrations in feces of mice treated with agave fructans (FG) or oligofructose (OG), and control group without prebiotic (CG). Values are means of triplicate

determinations $\pm$ standard error. ^{a,b,c}Different letters are significantly different between groups for each phase ($p \leq 0.05$) by Tukey's test

with oligofructose. Thus, the greater prebiotic effect of agave fructans could be related to the fermentative metabolism, which seems to be faster in the oligofructose than in fructans by the different degree of polymerization distribution of the former. In this regard, Velázquez-Martínez et al. (2014) described the prebiotic activity of *Agave angustifolia* Haw fructans with different degrees of polymerization through fermentation of *bifidobacterium* and *lactobacillus* strains. Besides, they demonstrated that some species of bacteria exhibit preference for low molecular weight fructans resulting in a greater prebiotic effect of agave-derived fructans.

Nonetheless, we recognize that the plate counting analysis is not the most specific to determine the bacterial concentration nor identification of bacterial strain; however, this method may reflect close changes in the microbiota environment as a consequence of the intervention.

Short-chain fatty acid (SCFA) concentrations in feces of study groups

The results of SCFA concentrations are shown in Fig. 2. During phase 1, FG exhibited elevated levels of acetic (12%) and propionic (66%) acids in comparison with the CG; whereas OG showed increased concentrations on acetic (12%) and propionic (38%) acids compared with the controls.

With respect to phase 2, FG had higher concentrations of acetic (33%) and propionic (73%) acids than the CG; while OG exhibited higher levels of butyric (12%) and propionic (35%) acids than the CG. Finally, after washing period, concentrations of acetic (25%) and propionic (35%) acids remained higher in the FG compared with both OG and CG.

Even we observed a significant elevation in SCFA levels following treatment with either fructans from *Agave*

tequilana or oligofructose, being the effect most favorable for the former.

The production of SCFA is regulated by the chemical composition, physical form, and amount of substrate available. Also, types and numbers of bacterial species found in the gut and intestinal transit time are other factors involved in the regulation of SCFA (Macfarlane and Gibson, 1994; Macfarlane and Macfarlane, 2003). Thus, the greater effect of agave fructans on the production of SCFA could be linked to their chemical structure and the fermentation rate by the microbiota. In this context, an experimental study in mice revealed that agave fructans are easily fermentable probably due to the presence of branches, which might be more accessible to bacterial enzymes inducing the growth of endogenous bacteria (*bifidobacteria* and/or *lactobacilli*), and consequently an increase in the production of SCFA (Huazano-García and López, 2013; López and Urías-Silvas, 2007), which is in accordance with our findings.

On the other hand, treatment period (during 42 days) of agave fructans might have induced changes in other bacterial strains, in addition to *bifidobacterium* and *lactobacillus*, which also participate in SCFA regulation (Macfarlane and Macfarlane, 2003). The abovementioned could explain the high levels of SCFA after washing period even though the bacterial concentrations of *bifidobacterium* and *lactobacillus* returned to basal condition; hence, other additional mechanisms may have influenced our results.

Finally, the present study suggests that the consumption of agave fructans has a beneficial effect on SCFA concentrations by microbiota regulation. Thus, though agave fructans could be a therapeutic option to improve the host health, future studies are needed in this field to establish the biological actions induced by agave fructans on human gastrointestinal tract.

Fig. 2 Short chain fatty acids content (A): acetic acid, (B): propionate acid, and (C): butyrate acid) in feces of mice treated with agave fructans (FG) or oligofructose (OG), and control group without

prebiotic (CG). Values are means of triplicate determinations $\pm$ standard error. ^{a,b,c} Different letters are significantly different between groups for each stage ($p \leq 0.05$) by Tukey's test

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All institutional and national guidelines for the care and use of laboratory animals were followed.

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