Vol. 19, No. 2 (2020) 827-838
Revista Mexicana de Ingeniería Química

Assessment of intermediate- and long- chains agave fructan fermentation on the growth of intestinal bacteria cultured in a gastrointestinal tract simulator

Evaluación de la fermentación de fructanos de agave de cadena media y larga sobre el crecimiento de bacterias intestinales utilizando un simulador de tracto digestivo humano

R. García-Gamboa[1], M.S. Gradilla-Hernández[2], R.I. Ortiz-Basurto[3], R.A. García-Reyes[1] and M. González-Avila[1]*

[1]*Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C. Av. Normalistas #800, col. Colinas de la Normal, C.P. 44270, Guadalajara, Jalisco, México.*
[2]*Tecnológico de Monterrey, Escuela de Ingeniería y Ciencias, Av. General Ramón Corona No. 2514, Col. Nuevo México, C.P. 45138 Zapopan Jalisco, México.*
[3]*Instituto Tecnológico de Tepic, Laboratorio Integral de Investigación en Alimentos, División de Estudios de Posgrado, Av. Tecnológico No. 2595, Col. Lagos del Country, C.P. 63175, Tepic, Nayarit, México.*

Received: September 4, 2019; Accepted: October 24, 2019

Abstract

The prebiotic activities of agave fructans are well documented; however, little is known about the effects of agave fructan fractions in particular. The aim of this study was to evaluate two *Agave tequilana* var. cenizo fructan fractions corresponding to intermediate and high polymerization degree, on the growth of probiotic and pathogenic bacteria cultured in a human gastrointestinal tract simulator. The growth was analyzed by plate count using media selective for *Lactobacillus* spp., *Bifidobacterium* spp. and *Clostridium* spp., in the case of *Salmonella* spp. a differential media was used. In addition, the metabolites generated via fructan metabolism were analyzed by ultra-high performance liquid chromatography. These results suggest that these fructan fractions possess prebiotic activities and have beneficial effects via inhibition of intestinal pathogen growth. These effects particularly depend on the length of the selected fructan fraction administration period. This knowledge is important for enhancing the selective use of prebiotics in functional foods.

Keywords: Prebiotic, agave fructan, polymerization degree, *ex-vivo* system, short- chain fatty acid.

Resumen

La actividad prebiótica de fructanos de agave ha sido ampliamente reportada; sin embargo, existen pocos estudios que demuestren esta actividad en función del grado de polimerización. El objetivo de este estudio fue evaluar dos fracciones de fructanos de *Agave tequilana* var. cenizo correspondientes a grado de polimerización intermedio y alto en el crecimiento de bacterias probióticas y patógenas cultivadas en un simulador de tracto digestivo humano. El crecimiento fue analizado mediante conteo en placa utilizando medios de cultivo selectivos para *Lactobacillus* spp., *Bifidobacterium* spp., y *Clostridium* spp., y medio diferencial para *Salmonella* spp. Adicionalmente, los metabolitos generados a partir de la digestión de fructanos, fueron analizados mediante cromatografía líquida de alta resolución. Los resultados sugieren que las fracciones de intermedio y alto grado de polimerización demostraron actividad prebiótica, además, mostraron inhibición en el crecimiento de bacterias patógenas intestinales. Estos efectos benéficos dependieron del grado de polimerización de los fructanos y del periodo de administración. Estos resultados proporcionan importante información para el uso selectivo de prebióticos en el área de alimentos funcionales.

Palabras clave: Prebiótico, fructanos de agave, grado de polimerización, sistema *ex-vivo*, ácidos grasos de cadena corta.

1 Introduction

The use of fructans has increased because of health benefits relating to their prebiotic effects (Acosta-Domínguez *et al*., 2018; Garcia-Curbelo *et al*., 2015; Gómez-Angulo *et al*., 2018; Wilson & Whelan, 2017). In Mexico, the main source of fructans is the agave plant since these molecules are its main photosynthetic product (Moreno-Vilet *et al*., 2014). Due to the structure of these molecules (β 2-1 and 2-6 chemical bonds), fructans are not digested by the human gastrointestinal tract (GIT) enzymes and are capable of reaching the section of the colon in which these molecules are fermented by bacteria, thus favouring the growth of these microorganisms (Fernandes *et al*., 2012).

* *Corresponding author. E-mail*: mgavila@ciatej.mx
Tel. 33-33-45-52-00, Ext. 1312
https://doi.org/10.24275/rmiq/Bio842
issn-e: 2395-8472

The growth of specific colonic bacteria, mainly *Lactobacillus* spp. and *Bifidobacterium* spp., has some human health benefits, such as producing a decrease in blood glucose, improvement of mineral absorption, lipid homeostasis regulation, and immune system modulation (Krumbeck *et al*., 2016; Márquez-Aguirre *et al*., 2013). Furthermore, the GIT microbiota is capable of producing a wide range of metabolites, including short-chain fatty acids (SCFAs); these molecules are absorbed in the colon, are generally composed of 1-6 carbon units, and their production is influenced by the consumption of prebiotics (Morrison & Preston, 2016) and dietary fibers (Smith *et al*., 2013), which are fermented by microbiota.

SCFAs exert diverse effects on human physiology by contributing to intestinal environment regulation, influencing colonocyte physiology, and participating in different host signaling mechanisms. Nevertheless, reports regarding the prebiotic activities of agave fructans in relation to their polymerization degree (PD) are insufficient. There are different methods for evaluating the prebiotic effects of Agave ingredients, which may be via *in-vitro* studies (Arrizon *et al*., 2014; González-Avila *et al*., 2014; Moreno-Vilet *et al*., 2014), in-vivo studies (Márquez-Aguirre *et al*., 2016), and/or clinical trials (Ramnani *et al*., 2015). One prebiotic characteristic is selective metabolism by certain bacterial groups, and this can only be determined in studies utilizing mixed microbial cultures that mimic the GIT microbial environment (Gomez *et al*., 2010). It is important to highlight that various existing approaches for gut microbiota studies are based on the use of faecal samples because they are easy to obtain and by non-invasive methods. However, these approaches do not simulate the various parts of the GIT; furthermore, during colonic fermentation, there are substances produced that can be absorbed or metabolized. These disadvantages may be overcome with the use of GIT simulators (Olivarez-Romero *et al*., 2018; Marzorati *et al*., 2015). The Automatic and Robotic Intestinal System (ARIS) is a scientific tool that mimics the human GIT. ARIS represents five sections of the GIT (stomach, small intestine [duodenum, jejunum, and ileum], and ascending, transverse, and descending colon sections) and can be adapted for the study of any population parameter, including physiological and dietary parameters. The colonic sections are inoculated with microbiota obtained from different subjects. This system allows for assessment of the effects of bioactive ingredients, functional foods, and food ingredients on intestinal microbiota along with the associated health impacts (Ruiz-Alvarez *et al*., 2014). The aim of this study was to evaluate two *Agave tequilana* var. cenizo fructan fractions corresponding to intermediate and high polymerization degree, on the growth of probiotic and pathogenic bacteria cultured in a human gastrointestinal tract simulator.

2 Materials and methods

2.1 *Agave fructan fractions*

Two agave fructan fractions were used in this study: intermediate PD (IPD) (10-22 fructose molecules) and high PD (HPD) (22-60 fructose molecules). These were extracted from *Agave tequilana* var. cenizo harvested in the state of Jalisco, Mexico. Aqueous preparations of these extracts were used, and their preparation was carried out as previously described (Lopez *et al*., 2003). The fructans were then subject to fractionation utilizing ultrafiltration membranes. The fractions were freeze-dried and kept frozen (-20ºC) until further evaluation.

2.2 *Conditioning of the GastroIntestinal Tract Simulator*

ARIS was used to evaluate the effect of two agave fructan fractions on intestinal microbiota. Before the evaluation, the pH in the complete system was adjusted; the pH in each section was automatically adjusted by the addition of 3 M NaOH or 0.5 M HCl by pH titrators (EZO-PH, Atlas Scientific LLCTM). The reactor vessels were stirred continuously with a magnetic stirrer at 120 rpm, and the temperature was kept at 37 °C. Reactor volumes and residence times in each GIT section were established according to human physiology as shown in Fig. 1 (Macfarlane *et al*., 1998). For this study, the latter three sections were inoculated with fresh faecal microbiota from 20 healthy volunteers between 18 and 55 years who had no history of antibiotic treatment in the three months prior to the faecal sample collection. Aliquots of 2 g of faecal samples from each subject were diluted with 20 mL of a sterile phosphate buffer solution (0.1 mol/L, pH 7), homogenized, and then centrifuged at 5000 rpm for 10 min at 4 ° C. Five milliliters of each supernatant with bacteria were inoculated into each colonic compartment in the simulator system.

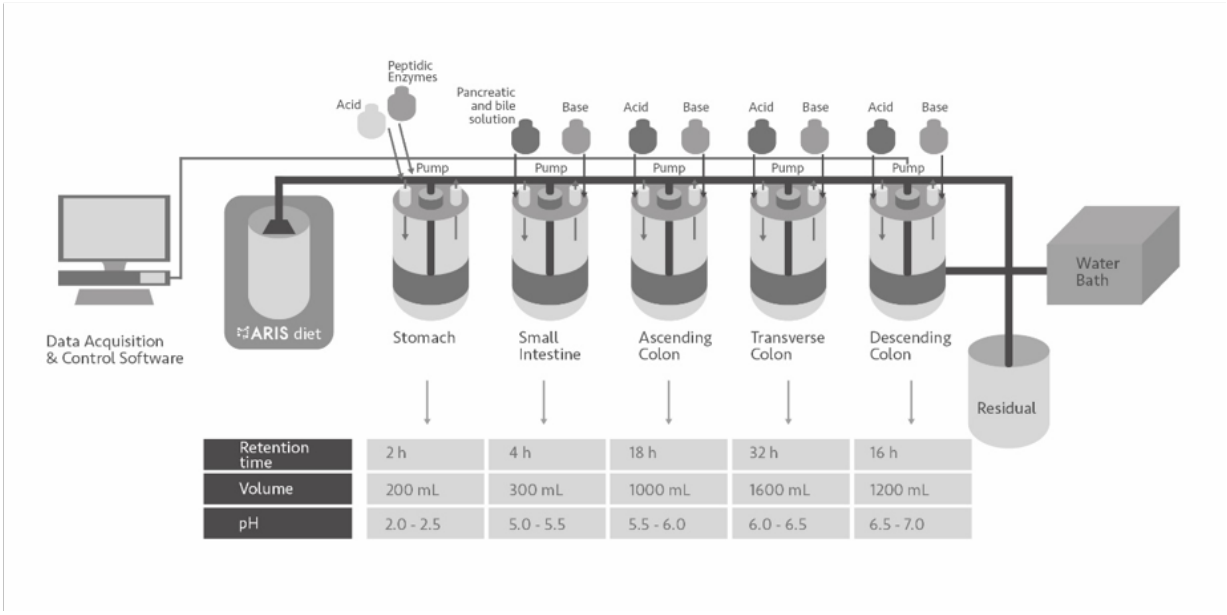

Fig. 1. Schematic representation of the human gastrointestinal tract simulator ARIS. ARIS: Automatic Robotic Intestinal System; Reactor 1: stomach; Reactor 2: small intestine (SI); Reactor 3, 4, and 5 represent the ascending colon (AC), transverse colon (TC), and descending colon (DC), respectively.

Previously, each reactor contained 100 mL of a growth medium for the microbial inoculum according to (Molly *et al*., 1993). After inoculation, a 2-week stabilization period was required to allow the microbial community to differentiate in the distinct colon sections, depending on their environmental conditions (Terpend *et al*., 2013).

2.3 *Ex-vivo fermentation of agave fructans*

Two fractions were studied separately; 1 g/day was employed because the ARIS system was downscaled to 10% of the recommended supplementation of 10 g (Fedewa & Rao, 2013). Fructan IPD and HPD fractions were added for nine consecutive days to the ARIS diet. The dietary composition was established through dietary questionnaires in order to mimic the diet of the population that donated the faecal samples. The diet was composed of 65% carbohydrates, 15% protein, and 20% lipids. The process of fructan digestion was carried out according to Hernández-Moedano *et al*., 2014, following the patent procedure (González-Avila and collaborator Mx 354295 2018).

Digestibility in stomach was initiated by adjusting the pH to 2.3 with 5 M HCl and adding 20 mL of pepsin solution (0.7 Ug/L porcine gastric enzyme, Sigma-Aldrich, St. Louis MO, USA) fallowing the procedure of (Gutiérrez-Zamorano *et al*., 2019). In this GIT section, the sample was continually stirred for 2 h. Immediately after stomach digestion finished, the pH of sample was adjusted to 5.5 with a 3 M NaOH solution and 9 mL of a pancreatic enzyme mixture (25,000 Ug/L lipase, 100,000Ug/L amylase, and 60,000 Ug/L protease, Sigma Aldrich St. Louis MO. USA), and 20 mg/mL of bile salts extract (Sigma Aldrich, St. Louis MO, USA) were added (Santiaguín-Padilla *et al*., 2019). The mixture was stirred at 60 rpm for 4 h (Molly *et al*., 1993). After the digestive process in the upper part of the GIT, food with each type of fructan passed into each colon section. The food retention time through the different colon sections was 66 h and included the time required to ferment complex carbohydrates and form products in addition to allowing bacterial populations to grow. The total transit of the whole GIT system was set to 72 h in order to mimic the microbiota colonization of the intestine *in vivo* (Molly *et al*., 1993). Samples were taken at the following different times: (1) day 0 (control/baseline period); (2) on day 4 of administration; and (3) on day 9 of administration. All three colon segments, including ascending, transverse, and descending, were evaluated. Colony forming units (CFU) from bacteria of the genera *Lactobacillus* spp., *Bifidobacterium* spp., (probiotics), *Clostridium* spp., and *Salmonella* spp. (opportunistic pathogens) were quantified using plate counts. Bacterial counts

were performed using DeMan, Rogosa, and Sharpe (MRS) media (BIOXON) for *Lactobacillus* spp. (24 h, microaerophilic), Bifidus Selective Medium (BSM) (Sigma Aldrich) for *Bifidobacterium* spp. (48 h, anaerobic), Tryptose Sulfite Cycloserine (TSC) (Sigma Aldrich) for Clostridia (48 h, anaerobic) and for *Salmonella* spp. (24 h, microaerophilic), Lysine Iron Agar (LIA) (Sigma Aldrich) was used. LIA is a differential media that differentiates *Salmonella* colonies from Enterobacteriaceae based on color change from purple to yellow, if lysine is not decarboxylated and lactose or sucrose or both are fermented. Typical salmonellae decarboxylate lysine and do not ferment lactose or sucrose and thereforethe purple color of the medium is maintained (Atlas & Snyder, 2006; Procura *et al*., 2019).

2.4 *Ultra-High Pressure Liquid Chromatography*

Samples of each fructan fraction were taken from ARIS colon sections during the control period and after four and nine days. These were analyzed using the ultra-high performance liquid chromatography (UHPLC) Acquitic Arc System with refractive index (RI) detection (Waters Corp., Milford, MA, USA). Samples were rendered cell-free through centrifugation (5,000 RPM, 10 min, 4°C) and filtered through 0.22-μm filters prior to injection (10 μL) into the column. All samples were analyzed in triplicate. Calibration curves were prepared for each metabolite in order to quantify each metabolite.

2.4.1 *Sugars, lactic acid, and short-chain fatty acid determinations*

Residual concentrations of sucrose, fructose, and glucose were determined using the Aminex HPX-87C (250 mm × 4.0 mm) column. Acetonitrile (1.0%) in water was the mobile-phase reagent with a flow rate of 0.2 mL min^{-1}. Lactic, acetic, propionic, butyric, and isobutyric acids were analyzed using the SHODEX KC-811 (7 μm, 8 × 300 mm) column. Phosphoric acid (0.1%) in water comprised the mobile-phase reagent with a flow rate of 1.0 g/L.

2.5 *Statistical analysis*

Analytical data were expressed as the mean ± standard error of the mean (SEM) of the three separate CFU plate counts and short-chain fatty acid (SCFA) and carbohydrate determinations. One-way analysis of variance (ANOVA) with post-hoc Tukey HSD tests were conducted to compare microbial counts, SCFAs, and carbohydrate results. All of the statistical analyses were carried out using the STATGRAPHICS Centurion ver. XVI.II statistical software.

3 Results and discussion

Both fructan fractions have been previously tested *in vitro*, and they showed high prebiotic activity with respect to specific bacteria. The majority of these bacteria pertained to the *Lactobacillus* genus (Gamboa *et al*., 2018). Hence, the objective of this work was to evaluate these two fractions and compare their capability to be fermented by the colonic microbiota in a human digestive tract simulator. By means of the dynamic multireactor GIT simulator, which is representative of human ascending, transverse, and descending colon conditions, it was possible to demonstrate that the two fructan fractions exhibited different fermentation profiles and induced different specific effects in the different colon regions.

The bacterial counts are presented in Fig. 2. Counts from the control period were utilized as a reference point in order to assess changes in bacterial populations. When the fructan IPD fraction was administered, significant increases in *Lactobacillus* spp., *Clostridium* spp., and *Salmonella* spp. ($p < 0.05$) were observed on day 4 of administration in the transverse colon section (+1.84, +1.32, and +2.7 log CFU/mL, respectively), whereas by day 9 of administration, *Lactobacillus* spp. had increased in the ascending colon (+1.99 log CFU/mL), *Bifidobacterium* spp. had increased in the descending colon (+0.86 log CFU/mL), and *Salmonella* spp. had decreased in the transverse colon (-1.24 log CFU/mL) ($p < 0.05$).

With respect to the administration of HPD fructans, no significant differences ($p < 0.05$) in the *Lactobacillus* and *Salmonella* groups were observed by day 4 of administration in any colon section. Also, on day 4, bifidobacteria strains had increased in the transverse colon (+0.95 log CFU/mL), while Clostridia showed a decreased in the ascending colon (-1.30 log CFU/mL) compared to the control period. On day 9 of fructan HPD administration, a significant change ($p < 0.05$) was exhibited regarding the population of the four bacterial genera compared to the control period (day 0). *Lactobacillus* spp. increased in the ascending and in the transverse colon sections (+1.77 and +0.82 log CFU/mL, respectively).

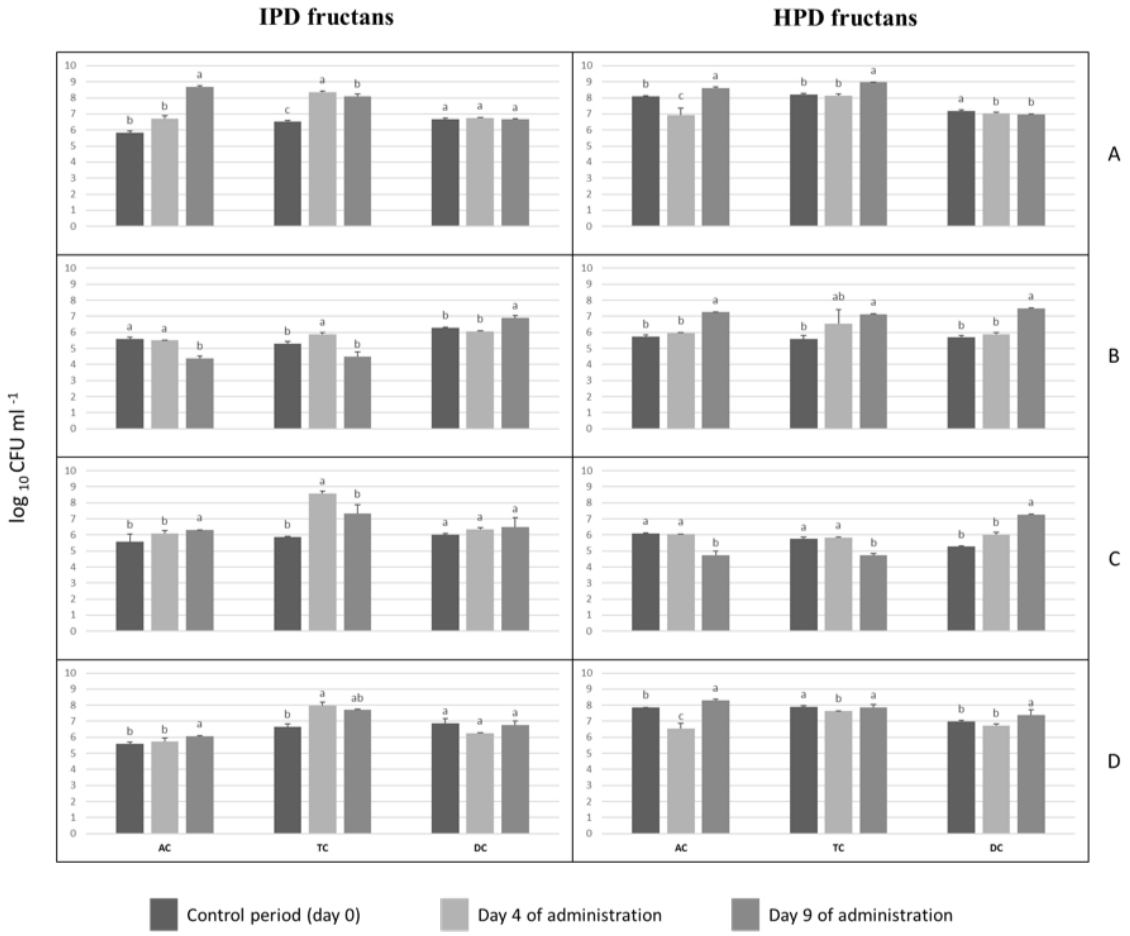

Fig. 2. Bacterial count results of the metabolization of agave fructans in ARIS. (A) *Lactobacillus* spp.; (B) *Bifidobacterium* spp.; (C) *Salmonella* spp., and (D) *Clostridium* spp. Samples were taken in triplicate; the standard error is indicated in each bar. Different lowercase letters indicate significant differences ($p < 0.05$) among day 0 (control period), day 4 and day 9 for a colon section. Ascending Colon (AC); Transverse Colon (TC), and Descending Colon (DC).

Bifidobacterium spp. increased in the ascending and the descending colon sections (+1.30 and +1.59 log CFU/mL, respectively). *Salmonella* spp. decreased in the ascending and transverse colon sections (-1.29 and -1.03 log CFU/mL, respectively), and *Clostridium* spp. increased in the ascending colon (+1.75 log CFU/mL).

An analysis was also performed to compare each fructan PD with respect to the growth of each bacterial group. Lactobacilli revealed growth in both fructan fractions, but no significant differences ($p < 0.05$) were found between them. Bacteria of the species *Bifidobacterium* spp. and *Clostridium* spp. demonstrated higher growth following fructan HPD administration. On the other hand, *Salmonella* spp. showed higher growth when IPD fractions of *Agave tequilana* var. cenizo were administrated ($p < 0.05$). According to the definition of prebiotics (Rastall & Gibson, 2015) and the great interest in prebiotic development that stimulates growth of probiotics and inhibits pathogen growth (Marzorati *et al.*, 2015), these two analysed fractions (IPD and HPD) could be used as prebiotics. They were selectively fermented by probiotic bacteria, and there was a decrease in the growth of pathogens in the GIT after their administration, thus they fulfilled the prebiotic definition. This process was also related to the time of the fructan fraction administration and the colon section.

Intestinal microbiota analysis (plate counts) confirmed a different modulating effect of both fructan fractions on the simulated colon compartments. *Lactobacillus* spp. showed an increase on day 4 of fructan IPD administration. This suggests that the bacteria of this genus possess the capability to metabolize these polymers upon first contact; therefore, no prolonged administration is necessary to provide a prebiotic effect as the literature has suggested (Santos *et al*., 2006). In addition, on day 9 of administration, both fructan fractions demonstrated a beneficial prebiotic effect with respect to *Lactobacillus* spp. and *Bifidobacterium* spp. According to the previous results, long-term administration causes an increase in beneficial bacteria in the different sections of the colon as has been suggested in the literature (Santos *et al*., 2006). These two genera have been reported as potentially beneficial bacteria, which shows that these two fractions have a potential health-promoting effect (Zhang *et al*., 2015).

On the other hand, both fractions exhibited an inhibitory effect on pathogen growth. The HPD fraction presented inhibition of the Clostridia genus on day 4 of administration. Furthermore, on day 9 of administration, the HPD fraction showed an inhibitory effect on *Salmonella* spp. The IPD fraction produced inhibition for *Salmonella* strains after nine days of administration. This decrease in the presence of pathogens could be due to inhibition of pathogen growth by probiotic bacteria and also because the beneficial microbiota play an important role in the production of metabolites with antimicrobial activity, such as butyric acid, which is an SCFA that possesses the capability of suppressing intestinal bacterial expansion (Melgar-Lalanne *et al*., 2019; Nagao-Kitamoto, Kitamoto, Kuffa, & Kamada, 2016). Furthermore, pathogens are responsible for the generation of gas, intestinal infections, diarrhea, and abdominal bloating (Ringel-Kulka *et al*., 2016); therefore, it is possible that the IPD and HPD fractions will also help with these symptoms.

A wide range of bacterial hydrolytic enzymes are capable of causing complex molecule depolymerization (Macfarlane & Macfarlane, 2003). Agave fructans are included in the group of polysaccharides that bypass small intestinal digestion and may be used as a substrate for these bacteria and for the production SCFAs as a result of this fermentation.

Table 1. Lactic acid and Short-Chain Fatty Acids production by fermentation of agave fructans in a gastrointestinal tract simulator.

LACTIC ACID AND SCFAS/ COLON SECTION	FRUCTAN FRACTIONS					
	IPD			HPD		
	CONTROL PERIOD (MMOL L^{-1})	DAY 4 OF ADMINISTRATION (MMOL L^{-1})	DAY 9 OF ADMINISTRATION (MMOL L^{-1})	CONTROL PERIOD (MMOL L^{-1})	DAY 4 OF ADMINISTRATION (MMOL L^{-1})	DAY 9 OF ADMINISTRATION (MMOL L^{-1})
Lactic acid						
AC	527.48 ± 0.04aB	176.57 ± 0.04cC	411.60 ± 0.07bC	516.16 ± 0.03bC	522.05 ± 0.46aC	503.99 ± 0.14cC
TC	558.55 ± 0.13aA	522.06 ± 0.05cA	522.81 ± 0.10bA	568.59 ± 0.56cA	657.54 ± 0.09aA	645.75 ±0.32bA
DC	450.60 ± 0.53cC	484.56 ± 0.01bB	521.31 ± 0.10aB	599.71 ± 0.32cB	633.34 ± 0.01aB	628.52 ± 0.23bB
Acetic acid						
AC	44.80 ± 0.24aA	32.44 ± 0.22bC	11.80 ± 0.03cC	21.11 ± 0.34aC	15.84 ± 0.45bB	11.74 ± 0.14cC
TC	24.30 ± 0.00bB	36.42 ± 0.01aB	20.67 ± 0.57cB	30.63 ± 0.21aB	17.42 ± 0.12bB	14.82 ± 0.16cB
DC	16.05 ± 0.08cC	45.20 ± 0.09aA	28.66 ± 0.20bA	70.14 ± 0.27aA	48.87 ± 0.59bA	41.70 ± 0.17cA
Propionic acid						
AC	38.95 ± 0.71bA	65.45 ± 0.42aA	11.50 ± 0.28cC	35.96 ± 0.92aA	31.02 ± 0.14bA	17.83 ± 0.99cB
TC	27.99 ± 0.71bB	48.75 ± 0.05aB	17.27 ± 0.71cB	33.02 ± 0.14aB	26.21 ± 0.85bB	23.68 ± 0.80bA
DC	26.84 ± 0.71bB	37.39 ± 0.15aC	21.90 ±0.71cA	28.13 ± 0.71aC	26.33 ± 0.63aB	23.85 ± 0.89bA
Butyric acid						
AC	35.70 ± 0.02aA	35.41 ± 0.76aA	30.71 ± 1.07bA	34.77 ± 0.41bA	38.64 ± 0.98aA	32.07 ± 0.14cB
TC	36.11 ± 0.37aA	34.08 ± 0.95aA	31.84 ± 0.81bA	32.83 ± 0.33aB	40.16 ±3.79aA	38.22 ± 0.17aA
DC	34.96 ± 0.19aB	29.08 ± 2.53aA	24.47 ± 0.05bB	33.27 ± 0.06aB	31.05 ± 0.41bA	30.34 ± 0.63bC
Isobutyric acid						
AC	6.05 ± 0.24aA	4.97 ± 0.46aB	2.91 ± 0.33bB	4.66 ± 0.13aB	3.83 ± 0.52aA	3.59 ± 0.20aB
TC	3.90 ± 0.07bB	7.38 ± 0.68aA	4.44 ± 0.33bA	4.43 ± 0.09aB	3.88 ± 1.02aA	3.93 ± 0.27aB
DC	4.86 ± 0.01cB	7.24 ± 0.21aA	5.42 ± 0.01bA	5.90 ± 0.34aA	5.64 ± 0.35aA	6.45 ± 0.68aA

SCFAs: Short-Chain Fatty Acids; IPD: intermediate polymerization degree fructans; HPD high polymerization degree fructans; AC; Ascending Colon; TC: Transverse Colon; DC: Descending Colon. Different lowercase letters indicate significant differences in rows among administrations for a colon section. Different capital letters in columns indicate significant differences among colon sections for a certain time ($p < 0.05$).

Table 1 presents SCFA profiles generated during fructans fermentation. Lactic acid was the metabolite produced in the greatest amount after the administration of either agave fructan fraction. Following the fructan IPD fraction administration, significant decreases in lactic acid concentrations were observed on day 4 in the ascending and transverse colon ($p < 0.05$) compared to day 0. Regarding the administration of the fructan HPD fraction, increases in lactic acid concentrations were observed on day 4 in all colon sections ($p < 0.05$).

Acetic acid decreased after the fructan IPD fraction was administered for nine days. Furthermore, while the acetic acid concentration was higher in the proximal colon during the control period, after the IPD fraction was administered, significantly higher acetic acid concentrations ($p < 0.05$) appeared in the distal colon section on days 4 and 9 of administration. When the fructan HPD fraction was administered, a decrease in acetic acid concentrations was demonstrated on day 4 of administration.

After the IPD fraction was administered, propionic acid concentrations changed over time in a similar manner within the three colon sections. The propionic acid concentrations had increased on day 4 of administration; however, it decreased by day 9 of administration ($p < 0.05$). In the case of the HPD fraction administration, there was a decrease in propionic acid on days 4 and 9 of administration in the three colon sections compared to the control day ($p < 0.05$). In addition, the propionic acid concentration during the control period was greater in the proximal colon section, while on day 9 of administration of both fructan fractions, a greater propionic acid concentration was observed in the distal colon ($p < 0.05$). Fallowing the IPD fraction administration, butyric acid concentrations showed a decrease on days 4 and 9 of administration in the three colon sections with respect to the control period (day 0) ($p < 0.05$). When the fructan HPD fraction was administered, different effects were observed in the different regions; for example, butyric acid had increased in the ascending colon on day 4 of administration and then showed a decrease by day 9 of administration. There were no significant changes in the traverse colon section, whereas the distal colon showed a decrease on day 4 ($p < 0.05$).

Isobutyric acid production showed a tendency to decrease by day 9 of IPD fructan administration. However, after HPD fructan administration, no changes in isobutryic acid production were observed in any of the colon sections ($p < 0.05$).

Lactic acid, which is not considered to be an SCFA, was the compound that was generated at highest concentrations during the fermentation of both fructans. This organic acid may decrease the pH of the culture and as consequence the growth of the growth may decrease of pathogens as a reported in other studies (García-Gamboa *et al*., 2018; Tejero-Sariñena *et al*., 2012). It is important to mention that physiologically, this metabolite does not generally accumulate in the colon because it can serve as a substrate for other bacteria, such as propionic acid- and butyric acid-producing bacteria, which can convert lactic acid into other SCFAs (Ríos-Covián *et al*., 2016). Therefore, since lactic acid is produced in large quantities, it is likely that these two fructan fractions are related to both propionic and butyric acid production. Similar to lactic acid, acetic acid production is important because this acid is a precursor of butyric acid (Belzer *et al*., 2017). Because of the increase in acetic acid presented during IPD fermentation, it is possible to infer that this fraction can generate higher butyric acid production. Likewise, the IPD versus the HPD fraction exhibited a greater effect on the production of propionic acid. This is beneficial because propionic acid contributes to serum cholesterol reduction (Louis & Flint, 2017).

In addition of SCFAs production, there are other substances capable of suppressing the growth of pathogenic bacteria; inhibitory peptides are an example of these substances (bacteriocins). *Lactobacillus* (Ge *et al*., 2016) and *Bifidobacterium* (Cheikhyoussef *et al*., 2009) species may produce bacteriocins. In this study, the growth of this probiotics was achieved through the metabolization of agave fructans and it can be assumed that the lack of production of SCFAs and the pathogen inhibition presented in some colon sections after HPD fructans metabolization was due to the production of bacteriocins.

Table 2 illustrates the amount of sucrose, fructose, and glucose after the metabolism of the carbohydrates. When the fructan IPD fraction was administered, a significant decrease in sucrose concentration was observed in the different colon sections. The ascending colon demonstrated an increase on day 4 and a decrease by day 9 after administration. There were no changes over time in the transverse colon section, while in the descending colon, there was a decrease on day 4 ($p < 0.05$). After administration of the fructan HPD fraction, similar effects were found in the three colon sections, revealing a decrease on day 4 of administration ($p < 0.05$).

Table 2. Sugar production by fermentation of agave fructans in a gastrointestinal tract simulator.

SUGAR/ COLON SECTION	FRUCTAN FRACTION					
	IPD			HPD		
	CONTROL PERIOD (MMOL L^{-1})	DAY 4 OF ADMINISTRATION (MMOL L^{-1})	DAY 9 OF ADMINISTRATION (MMOL L^{-1})	CONTROL PERIOD (MMOL L^{-1})	DAY 4 OF ADMINISTRATION (MMOL L^{-1})	DAY 9 OF ADMINISTRATION (MMOL L^{-1})
Sucrose						
AC	0.00 ± 0.00bB	4.63 ± 0.62aA	0.73 ± 0.02bA	0.40 ± 0.00aA	1.28 ± 0.00bA	0.84 ± 0.11cA
TC	0.00 ± 0.00B	0.00 ± 0.00B	0.02 ± 0.00B	0.16 ± 0.00aB	0.00 ± 0.00bB	0.00 ± 0.00bB
DC	2.89 ± 0.0.1aA	0.06 ± 0.00bB	0.00 ± 0.00cB	0.02 ± 0.00aC	0.00 ± 0.00bB	0.00 ± 0.00bB
Fructose						
AC	0.75 ± 0.03bB	3.74 ± 0.26aA	0.40 ± 0.00bA	0.52 ± 0.00cC	1.06 ± 0.00bA	1.31 ± 0.01aA
TC	0.74 ± 0.02aB	0.18 ± 0.07bB	0.25 ± 0.00bB	0.68 ± 0.00aA	0.67 ± 0.01aB	0.54 ± 0.02bB
DC	0.90 ±0.02aA	0.27 ± 0.03bB	0.21 ± 0.03bB	0.57 ± 0.01aB	0.30 ± 0.09bC	0.26 ± 0.01bC
Glucose						
AC	1.91 ± 0.03cB	6.29 ± 0.51aA	4.29 ± 0.63bA	1.74 ± 0.32bB	5.51 ±0.48aA	1.99 ± 0.07bA
TC	2.33 ± 0.02aA	0.42 ± 0.31bB	0.44 ± 0.00bB	2.20 ± 0.33aA	2.21 ± 0.02aB	0.82 ± 0.00bB
DC	2.00 ± 0.02aA	0.00 ± 0.00bB	0.00 ± 0.00bB	0.38 ± 0.01aC	0.19 ± 0.01bC	0.12 ± 0.01cC

IPD: intermediate polymerization degree fructans; HPD high polymerization degree fructans; AC; Ascending Colon; TC: Transverse Colon; DC: Descending Colon. Different lowercase letters indicate significant differences in rows among administration for a specific colon section. Different capital letters in columns indicate significant differences between colon sections for a time ($P < 0.05$).

Monomers, fructose, and glucose, had results similar to those with fermentation of both fructan fractions. Their concentrations increased in the ascending colon on day 4 of administration and then decreased by day 9 of administration. These compounds showed a decrease after day 4 in the transverse and descending colon sections ($p < 0.05$).

Agave fructans are non-digestible substrates for colonic hydrolytic enzymes and are generally of the β-fructofuranosidase type (EC 3.2.1.26). Simpler fructans are generated with a PD between two and eight monomers; in addition, fructose and glucose are products of this hydrolysis (Falony *et al*., 2009). Some strains of bacteria, mainly bacteria of the genus *Lactobacillus* spp. and *Bifidobacterium* spp., are capable of metabolizing fructose and glucose through the fructose 6-phosphate phosphoketolase pathway (Rivière *et al*., 2016) and the Embden-Meyerhof-Parnas pathway (Moens *et al*., 2016). Furthermore, it has also been well documented that *Bifidobacterium* spp. is able to metabolize fructooligosaccharides (Payne *et al*., 2012). Intestinal microbiota can undergo a period of adaptation to new substrates; on the other hand, it can also face excessive amounts of family substrate (Hollie *et al*., 2009). Therefore, it is possible that the bacteria belonging to the ascending colon underwent this adaptation period due to the increase in these types of carbohydrates. Consequently, after nine days of administration of both fructan fractions, it is possible to speculate that the ARIS-related intestinal microbiota underwent the adaptation period due to the reduction of sucrose, fructose, and glucose. Consequently, metabolic activities are subject to modifications during the adaptation period (Payne *et al*., 2012).

Conclusions

As a conclusion, the favourable growth of *Lactobacillus* spp. and *Bifidobacterium* spp. by means of the colonic fermentation of the IPD and HPD fractions suggest that they possess prebiotic activity when they are administrated long-term. In addition, these fructan fractions may inhibit of the growth of *Clostridium* spp. and *Salmonella* spp., which are pathogens associated with GIT diseases. Fructan HPD fractions produced major inhibitory effects that may be related to bacteriocins production. SCFA production was higher during the IPD fraction's fermentation, particularly with respect to acetic and propionic acids. These effects appear to specifically depend on the length of the administration period into the GIT simulator. These results suggest that these fructan fractions could potentially play a role in health and could be of interest for new product development.

Acknowledgements

The authors thank CONACYT (granted number 402440) and AGARED for the financial support granted to carry out this project. We thank MS Pamela Aldrete Herrera for the kind donation of fructan

fractions needed to carry out this project. We also thank Jorge Valente for the support provided in the generation of the ARIS system illustration.

References

Acosta-Domínguez, L., Alamilla-Beltrán, L., Calderón-Domínguez, G., Jiménez-Aparacio, A. R., Gutiérrez-López, G. F., & Azuara-Nieto, E. (2018). Determination of total and incipient solubilization point of fructans extracted of *A. Tequilana* weber var. azul. *Revista Mexicana de Ingeniería Química 17*, 379-388.

Arrizon, J., Hernandez-moedano, A., Toksoy-Oner, E., & Gonzalez-Avila, M. (2014). *In vitro* prebiotic activity of fructans with different fructosyl linkage. *International Journal of Probiotics and Prebiotics 9*, 69-76.

Atlas, R. M., & Snyder, J. W. (2006). *Handbook of Media for Clinical Microbiology*. CRC Press, Boca Raton.

Belzer, C., Chia, L. W., Aalvink, S., Chamlagain, B., Piironen, V., Knol, J., & de Vos, W. M. (2017). Microbial metabolic networks at the mucus layer lead to diet-independent butyrate and vitamin b12 production by intestinal symbionts. *America Society for Microbiology 8*, e00770-17. https://doi.org/10.1128/mBio.00770-17.

Cheikhyoussef, A., Pogori, N., Chen, H., Tian, F., Chen, W., Tang, J., & Zhang, H. (2009). Antimicrobial activity and partial characterization of bacteriocin-like inhibitory substances (BLIS) produced by *Bifidobacterium infantis* BCRC 14602. *Food Control 20*, 553-559.

Falony, G., Lazidou, K., Verschaeren, A., Weckx, S., Maes, D., & De Vuyst, L. (2009). *In vitro* kinetic analysis of fermentation of prebiotic inulin-type fructans by *Bifidobacterium* species reveals four different phenotypes. *Applied and Environmental Microbiology 75*, 454-461. Retrieved from http://aem.asm.org/content/75/2/454.abstract.

Fedewa, A., & Rao, S. S. C. (2013). Dietary fructose intolerance, fructan intolerance and FODMAPs. *Current Gastroenterology Reports 16*, 370. https://doi.org/10.1007/s11894-013-0370-0.

Fernandes, J., Eaton, P., Franco, I., Ramos, Ó. S., Sousa, S., Nascimento, H., ...Pintado, M. E. (2012). Evaluation of chitoligosaccharides effect upon probiotic bacteria. *International Journal of Biological Macromolecules 50*, 148-152. https://doi.org/10.1016/j.ijbiomac.2011.10.011.

García-Gamboa, R., Basurto, R. I. O., Santoyo, M. C., Madrigal, J. B., Álvarez, B. E. R., & Ávila, M. G. (2018). *In vitro* evaluation of prebiotic activity, pathogen inhibition and enzymatic metabolism of intestinal bacteria in the presence of fructans extracted from agave: A comparison based on polymerization degree. *LWT Food Science and Technology 92*, 380-387. https://doi.org/10.1016/j.lwt.2018.02.051.

Garcia-Curbelo, Y., Bocourt, R., Savon, L. L., Garcia-Vieyra, M. I., & Lopez, M. G. (2015). Prebiotic effect of *Agave fourcroydes* fructans: an animal model. *Food & Function 6*, 3177-3182. https://doi.org/10.1039/C5FO00653H.

Ge, J., Sun, Y., Xin, X., Wang, Y., & Ping, W. (2016). Purification and partial characterization of a novel bacteriocin synthesized by *Lactobacillus paracasei* HD1-7 isolated from Chinese sauerkraut juice. *Scientific Reports 6*, 19366. https://doi.org/10.1038/srep19366.

Gómez-Angulo, J. H., Amaya-Delgado, L., Gschaedler-Mathis, A., & Arrizon-Gaviño, J. (2018). Production of fructooligosaccharides by two new recombinant β-fructofuranosidase enzymes from mezcal fermenting yeasts. *Revista Mexicana de Ingeniería Química 17*, 1039-1049.

Gomez, E., Tuohy, K. M., Gibson, G. R., Klinder, a., & Costabile, A. (2010). *In vitro* evaluation of the fermentation properties and potential prebiotic activity of Agave fructans. *Journal of Applied Microbiology 108*, 2114-2121. https://doi.org/10.1111/j.1365-2672.2009.04617.x.

González-Avila, M., Prado-Ramírez, Rogelio Flores-Montaño, J. L., Perez-Martinez, F. J., Gaspar,

O. R., Alonso-Segura, D., Vol, P., Leon, G. N. (2014). Evaluation of prebiotic potential of *Agave fructans* from different regions of Colima and Zacatecas, México. *International Journal of Probiotics and Prebiotics 9*, 93-100.

Gutiérrez-Zamorano, C., González-Ávila, M., Díaz-Blas, G., Smith, C. T., González-Correa, C., & García-Cancino, A. (2019). Increased anti-*Helicobacter pylori* effect of the probiotic *Lactobacillus fermentum* UCO-979C strain encapsulated in carrageenan evaluated in gastric simulations under fasting conditions. *Food Research International 121*, 812-816.

Hernández-Moedano, A., Moreno-Ramos, E. F., Herrera-Rodríguez, E. S., & González-Avila, M. (2014). changes in intestinal microorganisms influenced by Agave fructans MakeLowercasein A Digestive Tract Simulator. *Journal of Chemical , Biological and Physical Sciences 4*, 19-25.

Hollie A. Grabitske MS, R. D., & Joanne L. Slavin PhD, R. D. (2009). Gastrointestinal effects of low-digestible carbohydrates. *Critical Reviews in Food Science and Nutrition 49*, 327-360. https://doi.org/10.1080/10408390802067126.

Krumbeck, J. A., Maldonado-Gomez, M. X., Ramer-Tait, A. E., & Hutkins, R. W. (2016). Prebiotics and synbiotics: dietary strategies for improving gut health. *Current Opinion in Gastroenterology 32*, 110-119.

Lopez, M. G., Mancilla-Margalli, N. a., & Mendoza-Diaz, G. (2003). Molecular Structures of Fructans from *Agave tequilana*º Weber var. azul. *Journal of Agricultural and Food Chemistry 51*, 7835-7840. https://doi.org/10.1021/jf030383v.

Louis, P., & Flint, H. J. (2017). Formation of propionate and butyrate by the human colonic microbiota. *Environmental Microbiology 19*, 29-41. https://doi.org/10.1111/1462-2920.13589.

Macfarlane, G. T., Macfarlane, S., & Gibson, G. R. (1998). Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colon. *Microbial Ecology 35*, 180-187. https://doi.org/10.1007/s002489900072.

Macfarlane, S., & Macfarlane, G. T. (2003). Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society 62*, 67-72. https://doi.org/10.1079/PNS2002207.

Márquez-Aguirre, A. L., Camacho-Ruiz, R. M., Arriaga-Alba, M., Padilla-Camberos, E., Kirchmayr, M. R., Blasco, J. L., & González-Avila, M. (2013). Effects of *Agave tequilana* fructans with different degree of polymerization profiles on the body weight, blood lipids and count of fecal *Lactobacilli/Bifidobacteria* in obese mice. *Food & Function 4*, 1237-1244. https://doi.org/10.1039/c3fo60083a.

Márquez-Aguirre, A. L., Camacho-Ruíz, R. M., Gutiérrez-Mercado, Y. K., Padilla-Camberos, E., González-Ávila, M., Gálvez-Gastélum, F. J., Ortuño-Sahagún, D. (2016). Fructans from *Agave tequilana* with a lower degree of polymerization prevent weight gain, hyperglycemia and liver steatosis in high-fat diet-induced obese mice. *Plant Foods for Human Nutrition 71*, 416-421. https://doi.org/10.1007/s11130-016-0578-x.

Marzorati, M., Qin, B., Hildebrand, F., Klosterbuer, A., Roughead, Z., Roessle, C., . . . Possemiers, S. (2015). Addition of acacia gum to a FOS/inulin blend improves its fermentation profile in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). *Journal of Functional Foods 16*, 211-222. https://doi.org/10.1016/j.jff.2015.04.039.

Melgar-Lalanne, G., Ley-Martinez, J., Azuara-Nieto, E., Tellez-Medina, D. I., Meza, T., González-González, C. R., & Gutierrez-Lopez, G. F. (2019). Insight over Lactobacillus plantarum 299V physicohemical characteristics of aggregation kinetics under starvation and different pH conditions. *Revista Mexicana de Ingeniería Química 18*, 151-164.

Moens, F., Weckx, S., & De Vuyst, L. (2016). Bifidobacterial inulin-type fructan degradation capacity determines cross-feeding interactions between bifidobacteria and *Faecalibacterium prausnitzii*. *International Journal of Food Microbiology 231*, 76-85. https://doi.org/10.1016/j.ijfoodmicro.2016.05.015.

Molly, K., Vande Woestyne, M., & Verstraete, W. (1993). Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. *Applied Microbiology and Biotechnology 39*, 254-258. https://doi.org/10.1007/BF00228615.

Moreno-Vilet, L., Garcia-Hernandez, M. H., Delgado-Portales, R. E., Corral-Fernandez, N. E., Cortez-Espinosa, N., Ruiz-Cabrera, M. a., & Portales-Perez, D. P. (2014). *In vitro* assessment of agave fructans (*Agave salmiana*) as prebiotics and immune system activators. *International Journal of Biological Macromolecules 63*, 181-187. https://doi.org/10.1016/j.ijbiomac.2013.10.039.

Morrison, D. J., & Preston, T. (2016). Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes 7*, 189-200. https://doi.org/10.1080/19490976.2015.1134082.

Nagao-Kitamoto, H., Kitamoto, S., Kuffa, P., & Kamada, N. (2016). Pathogenic role of the gut microbiota in gastrointestinal diseases. *Intestinal Research 14*, 127-138. http://synapse.koreamed.org/DOIx.php?id=10.5217%2Fir.2016.14.2.127.

Olivarez-Romero, R., Faustino-Vega, A., Miranda-Calderon, J. E., González-Vázquez, R., & Azaola-Espinosa, A. (2018). Microencapsulation of Lactobacillus acidophilus la-5 increases relative survival under simulated gastrointestinal tract stress. *Revista Mexicana de Ingeniería Química 17*, 641-650. https://doi.org/10.24275/10.24275/uam/izt/dcbi/revmexingquim/2018v17n2/Olivarez.

Payne, A. N., Chassard, C., & Lacroix, C. (2012). Gut microbial adaptation to dietary consumption of fructose, artificial sweeteners and sugar alcohols: implications for host-microbe interactions contributing to obesity. *Obesity Reviews 13*, 799-809. https://doi.org/10.1111/j.1467-789X.2012.01009.x.

Procura, F., Bueno, D. J., Bruno, S. B., & Rogé, A. D. (2019). Prevalence, antimicrobial resistance profile and comparison of methods for the isolation of *Salmonella* in chicken liver from Argentina. *Food Research International 119*, 541-546. https://doi.org/https://doi.org/10.1016/j.foodres.2017.08.008.

Ramnani, P., Costabile, A., Bustillo, A. G. R., & Gibson, G. R. (2015). A randomised, double- blind, cross-over study investigating the prebiotic effect of agave fructans in healthy human subjects. *Journal of Nutritional Science 4*. https://doi.org/10.1017/jns.2014.68.

Rastall, R. A., & Gibson, G. R. (2015). Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. *Current Opinion in Biotechnology 32*, 42-46. https://doi.org/10.1016/j.copbio.2014.11.002

Ringel-Kulka, T., Benson, A. K., Carroll, I. M., Kim, J., Legge, R. M., & Ringel, Y. (2016). Molecular characterization of the intestinal microbiota in patients with and without abdominal bloating. *American Journal of Physiology - Gastrointestinal and Liver Physiology 310*, G417-G426. https://doi.org/10.1152/ajpgi.00044.2015.

Ríos-Covián, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., de los Reyes-Gavilán, C. G., & Salazar, N. (2016). Intestinal short chain fatty acids and their link with diet and human health. *Frontiers in Microbiology 7*, 185. https://doi.org/10.3389/fmicb.2016.00185.

Rivière, A., Selak, M., Lantin, D., Leroy, F., & De Vuyst, L. (2016). Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Frontiers in Microbiology 7*, 979. https://doi.org/10.3389/fmicb.2016.00979.

Ruiz-Alvarez, B. E., Galvez-Alvarez, N. S., Hernandez-Moedano, A., Carmona-Vargas, G. A., & González-Avila, M. (2014). A supplement enriched with agave fructans for childhood obesity evaluated in *Ex vivo* system. *Sustainable and Integral Exploitation of Agave*, 104-107.

Santiaguín-Padilla, A. J., Peña-Ramos, E. A., Pérez-Gallardo, A., Rascón-Chu, A., González-Ávila, M., González-Ríos, H., Islava-Lagarda, T. (2019). *In vitro* digestibility and quality of an emulsified meat product formulated with animal

fat encapsulated with pectin. *Journal of Food Science 7*, 1331-1339.

Santos, A., Mauro, M. S., & Díaz, D. M. (2006). Prebiotics and their long-term influence on the microbial populations of the mouse bowel. *Food Microbiology 23*, 498-503. https://doi.org/10.1016/j.fm.2005.07.004.

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-Y, M., ... Garrett, W. S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic treg cell homeostasis. *Science 341*, 569-573. https://doi.org/10.1126/science.1241165.

Tejero-Sariñena, S., Barlow, J., Costabile, A., Gibson, G. R., & Rowland, I. (2012). *In vitro* evaluation of the antimicrobial activity of a range of probiotics against pathogens: Evidence for the effects of organic acids. *Anaerobe 18*, 530-538. https://doi.org/10.1016/j.anaerobe.2012.08.004.

Terpend, K., Possemiers, S., Daguet, D., & Marzorati, M. (2013). Arabinogalactan and fructo-oligosaccharides have a different fermentation profile in the simulator of the human intestinal microbial ecosystem (SHIME). *Environmental Microbiology Reports 5*, 595-603. https://doi.org/10.1111/1758-2229.12056.

Wilson, B., & Whelan, K. (2017). Prebiotic inulin-type fructans and galacto-oligosaccharides: definition, specificity, function, and application in gastrointestinal disorders. *Journal of Gastroenterology and Hepatology 32*, 64-68. https://doi.org/10.1111/jgh.13700.

Zhang, Y.-J., Li, S., Gan, R.-Y., Zhou, T., Xu, D.-P., & Li, H.-B. (2015). Impacts of gut bacteria on human health and diseases. *International Journal of Molecular Sciences 16*, 7493-7519. https://doi.org/10.3390/ijms16047493.