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Agave spp. and *Dasylirion* sp. Fructans as a Potential Novel Source of Prebiotics

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ABSTRACT

Prebiotics of the inulin-type fructans have been studied for many years under a wide range of conditions, including concentration, degree of polymerization and variety of probiotics. This work is the first that addresses the potential of *Agave* spp. and *Dasylirion* sp. fructans as prebiotics. Fructans from five different *Agave* species and from *Dasylirion* sp. grown in six different geographic areas were tested with six different bifidobacteria and four lactobacilli strains, with commercial inulin-type fructans used as positive controls. Results indicate that bifidobacteria and lactobacilli grew using species of *Agave* and *Dasylirion* fructans as a carbon source. Most fructans stimulated the growth of both genera more efficiently than commercial inulin, as indicated by the absorbance and pH values. Fructans of *Dasylirion* sp. from Chihuahua and *Agave tequilana* from Guanajuato were the most effective, followed by Raftilose®Synergy1, a commercial inulin. This study supports previous reports that acetic, formic, and lactic acids were the main detected acids in all cases. This work further proves the potential of *Agave* and *Dasylirion* fructans as prebiotics.

Keywords: agave, bifidobacteria, lactobacilli, prebiotic, SCFA
Abbreviations: DP, degree of polymerization; **HPLC**, high performance liquid chromatography; **MALDI-TOF-MS**, matrix assisted laser desorption/ionization time-of-flight-mass spectrometry; **SCFA**, short chain fatty acids; **TLC**, thin layer chromatography

INTRODUCTION

Agave plants were and continue being of great importance in Mexico, supplying food, beverage, fiber and other resources. Mexico is the origin center, evolution, and diversification of this genus. Of the approximately 300 species described, 75% are found within Mexico (García-Mendoza and Galván 1995). The principal photosynthetic products of the CAM metabolism in agaves are fructans (Sánchez-Marroquín and Hope 1953). López *et al*. (2003) reported the molecular structure of fructans from *A. tequilana* Weber var. azul to be a complex mixture containing primarily β(2-1) and some β(2-6) linkages, and are highly branched with terminal or internal glucose moieties, named graminans and agavins, respectively (Mancilla-Margalli and López 2006). Mancilla-Margalli and López (2006) reported structural differences of fructans between and within species of *Agave*, the latter grown in different environmental regions. The observed structural heterogeneity could be attributed to plant adaptation mechanisms allowing the plants to survive in inhospitable areas.

In general, fructans are non-reducing oligosaccharides that are basically formed of linear or branched structures of fructosyl units with a terminal glucose moiety (Wang *et al.* 1999). Because of the β-configuration of the anomeric C2 in their fructose monomers, fructans are resistant to hydrolysis by human digestive enzymes (α-glucosidase, maltase, isomaltase, and sucrase), which are specific for α-glycosidic bonds. Fructans are thus classified as non-digestible oligosaccharides (Vijn and Smeekens 1999), reaching the large intestine essentially intact. They are also considered as a prebiotic (Wang and Gibson 1993; Gibson *et al.* 1995; Crittenden 1999) in that they serve as a substrate for the colonic bacteria. A prebiotic is an ingredient selectively fermented that affects specific changes on composition and/or activity of the gastrointestinal microflora, which confers benefits upon the host's well-being and health (Gibson *et al.* 2004).

The human intestinal microbial flora represents a rich ecosystem composed of a wide range of metabolically active microorganisms that play an important role in influencing the health of the host. In terms of health, of the several hundred species of bacteria that colonize the large intestine, the most significant organisms are the bifidobacteria (Gibson and Roberfroid 1995). Bifidobacteria is the major component of the microbial barrier to infection, producing a range of antimicrobial agents that are active against Gram-positive and -negative organisms (Gibson and Wang 1994). Lactobacilli also contribute to good health in that they produce a number of antimicrobial agents, but are present in much lower levels in the human colon (Rastall 2004). The presence and predominance of bifidobacteria is essential for the prevention of diseases and maintenance of good health (Mitsuoka 1990; Rastall 2004). Accordingly, considerable research is being directed towards promoting bifidobacteria growth in the large intestine. The fermentation of fructans in the colon generates short chain fatty acids (SCFA), lactic and formic acids, and gases including H_2, CO_2, and CH_4 as products of anaerobic metabolism (Roberfroid 1993, 2005). Fructan fermentation is an important process since it favors the maintenance and the development of bacterial flora, as well as the colonic epithelial cells (García-Peris *et al.* 2002; Gibson *et al.* 2005).

The nutritional and biological properties of chicory inulin include dietary fiber effects, selective stimulation of bifidobacteria growth in the colon, preventing colon cancer, increasing mineral absorption, stimulation of the immunologic system, and systemic modulation of lipid metabolism (Roberfroid *et al.* 1993, 1998; Roberfroid and Delzenne 1998; Roberfroid 2000).

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Therefore, due to 1) the presence of fructans in Agave species, 2) the DP diversity within the same Agave species, 3) the structural differences within inulin, 4) the importance of fructans on health, and 5) the fact that there have not been studies of agavins as prebiotics, this paper will evaluate the potential of different species of *Agave* and *Dasylirion* fructans as prebiotics.

MATERIALS AND METHODS

Chemicals

Raftilose®Synergy1 and Raftiline®GR were obtained from Orafti, fructans from *Cichorium intybus* and *Dahlia variabilis*, as well as glucose, fructose, and sucrose were purchased from Sigma-Aldrich. Standards for thin layer chromatography (1-kestose (DP3), nystose (DP4), and fructosyl-nystose (DP5)) were obtained from Megazyme International Ltd. and 2-methyl-valeric acid (internal standard for short chain fatty acid quantification) from Sigma-Aldrich.

Plant material

Agave spp. and *Dasylirion* sp. plants were collected: *Agave tequilana* from Guanajuato (ATG) and Jalisco (ATJ), *A. angustifolia* from Sonora (AAS) and Oaxaca (AAO), *A. potatorum* (APO) and *A. cantala* (ACO) also from Oaxaca, *A. fourcroydes* from Yucatán (AFY), and *Dasylirion* sp. from Chihuahua (DSC).

Biological material

Bacterial strains were obtained from the American Type Culture Collections (ATCC). *Bifidobacterium adolescentis* (ATCC 15703), *B. animalis* (ATCC 27536), *B. bifidum* (ATCC 29521), *B. breve* (ATCC 15700), *B. infantis* (ATCC 25962), *B. longum* (ATCC 15707), *Lactobacillus acidophilus* (ATCC 4356), *L. casei* (ATCC 393), *L. paracasei* (ATCC 25302), and *L. rhamnosus* (ATCC 53103).

Extraction of *Agave* spp. and *Dasylirion* sp. fructans

Fructans from each species of *Agave* and *Dasylirion* were extracted as described by López *et al*. (2003). One hundred grams of milled *Agave* spp. and *Dasylirion* sp. stems were extracted twice with 100 ml of 80% v/v ethanol with continuous shaking for 1 h at 55°C. The samples were filtered and the plant material re-extracted with 100 ml of water for 30 min at 55°C. The supernatants were mixed; chloroform was used to eliminate the lipidic fraction. The aqueous phase was concentrated by rotary evaporation under reduced pressure. Samples were freeze dried and stored in a humidity-free container.

Thin layer chromatography (TLC)

The detection and preliminary characterization of *Agave* spp. and *Dasylirion* sp. fructans were carried out by TLC. One microliter of a sample (20 mg/ml) was applied onto the silica gel plates (10 × 10) (Altech). The plates were developed in a saturated TLC-camera using propanol: water: butanol (12: 4: 3, v/v/v) as the mobile phase. Diphenylamine-aniline-phosphoric acid was used as the revealing agent.

Bacterial growth conditions

Bifidobacteria and lactobacilli were used as probiotics strains following the method reported by Gibson and Wang, (1994) with some modifications. Culture broth MRS for bacterial growth was used; in the case of bifidobacteria the culture broth was supplemented with L-cysteine. Bacterial inoculation was done with 1% in 10 ml of the culture broth. The incubation conditions were 20 h at 37°C anaerobically. To evaluate the effect of the different fructans on the bacterial growth, the broth was prepared without glucose and with a fructans concentration of 10 g/l as a carbon source (glucose-free). Bacterial growth was measured by optical density at 630 nm and the uptake of fructans by bacteria (probiotics) was evaluated measuring pH broth changes. The bacterial growth was evaluated by three independent determinations.

Analysis of short chain fatty acids (SCFA) by HPLC

After the incubation period (20 h), one milliliter of culture broth was analyzed according to Al-Tamimi *et al*. (2006) with some modifications. In brief, samples were centrifuged at 13,000 × *g* for 5 min to remove bacterial cells and any particulate material. Acetic, formic and lactic acids concentrations were determined by HPLC on an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad). Degassed 5 mM H_2SO_4 was used as eluent at a flow rate of 0.6 ml min^{-1} and an operating temperature of 50°C. Organic acids were detected by UV at a wavelength of 220 nm, and calibrated against standards of corresponding organic acids at concentrations between 1 and 100 mM. The injection volume was 20 µl.

Statistical analysis

Results are expressed as mean values +/- standard error of the mean (SEM). Statistical differences between groups were evaluated using one-way ANOVA followed by Tukey's *post hoc* test using SPSS 14.0 for Windows (SPSS, Chicago IL, US). Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Fructans samples were analyzed by TLC prior to bifidobacteria and lactobacilli growth in pure culture, supplemented with *Agave* spp., DSC or commercial fructans. Fructans DP profile differences are depicted in **Fig. 1**. Fructans from *Dasylirion* sp. (DSC) showed the largest amounts of low DP compared with other species, followed by *A. tequilana* from Guanajuato (ATG). Based on the weak spot left at the application origin, DSC contained almost no fructans with a DP > than 20. For commercial fructans, Raftilose®Synergy1 (RSE) presented a large amount of low DP compared to Raftiline®GR. No marked differences were observed among fructans from *A. fourcroydes* from Yucatan (AFY) and *A. angustifolia* Sonora (AAS). It is important to note that the presence of a spot between DP2 and DP3 in all agavins corresponds to the neo-type fructans reported by Mancilla-Margalli and López (2006), which is indicative of the presence of a neo-type fructans. Different effects were observed on the stimulation growth of both bacteria genera

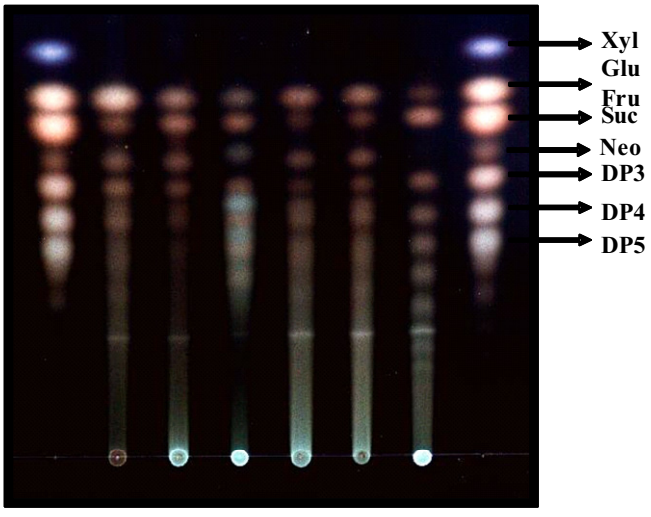

Fig. 1 Thin layer chromatography of fructans from *Dasylirion* sp. (DSC), *Agave tequilana* Gto (ATG), Raftilose®Synergy1 (RSE), *A. fourcroydes* Yuc (AFY), *A. angustifolia* Son (AAS), and Raftiline®GR (RNE). STD, Standard. Xyl, Xylose; Glu, glucose; Fru, Fructose; Suc, sucrose; DP3, 1-kestose; DP4, nystose; DP5, fructosyl-nystose.

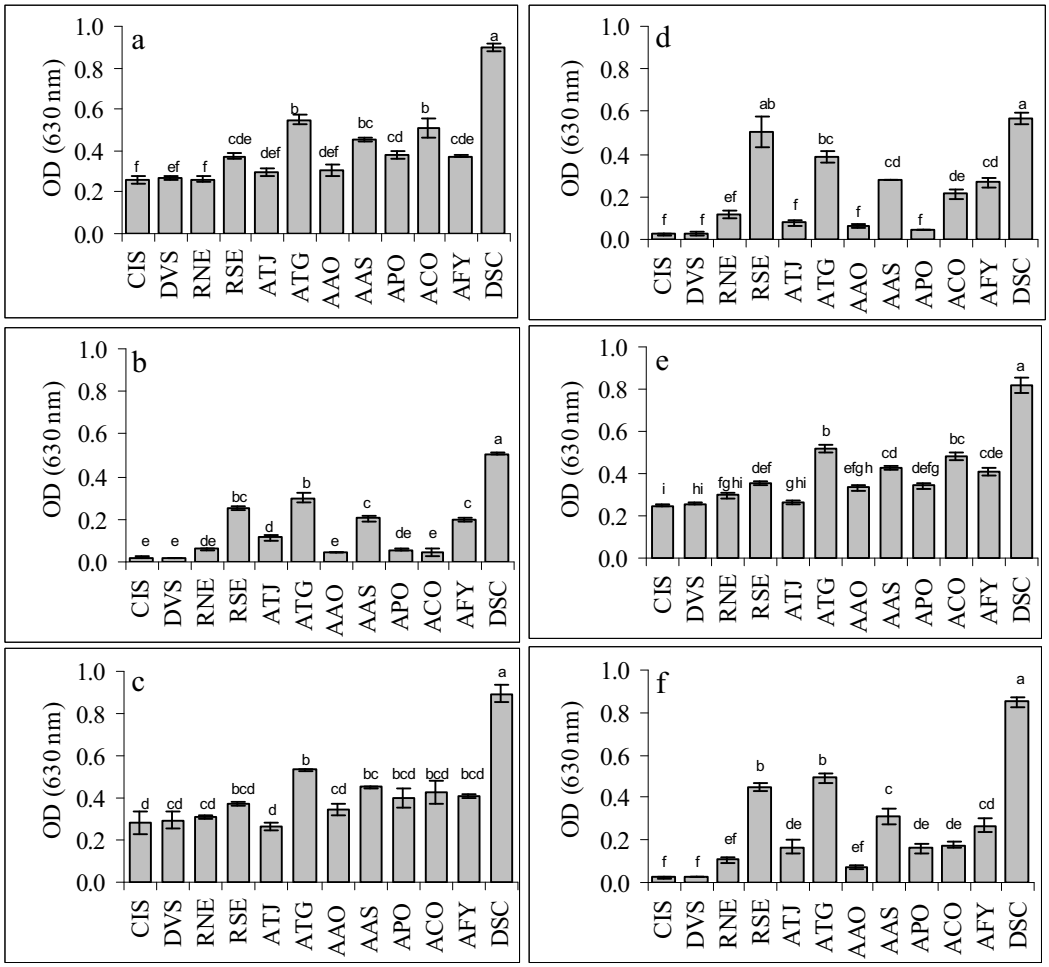

Fig. 2 Effect of different fructans on the growth of *Bifidobacterium adolescentis* (a), *B. animalis* (b), *B. bifidum* (c), *B. breve* (d), *B. infantis* (e), and *B. longum* (f) incubated anaerobically at 37°C in the presence of 10 g of fructan/L. Results are means of 3 independent determinations ± SEM. Differences were considered significant at $P \leq 0.05$. OD, Optical density; CIS, *Cichorium intybus* Sigma; DVS, *Dahlia variabilis* Sigma; RNE, Raftiline®GR; RSE, Raftilose®Synergy1; ATJ, *A. tequilana* Jal; ATG, *A. tequilana* Gto; AAO, *A. angustifolia* Oax; AAS, *A. angustifolia* Son; APO, *A. potatorum* Oax; ACO, *A. cantala* Oax; AFY, *A. fourcroydes* Yuc; DSC, *Dasylirion* sp. Chih.

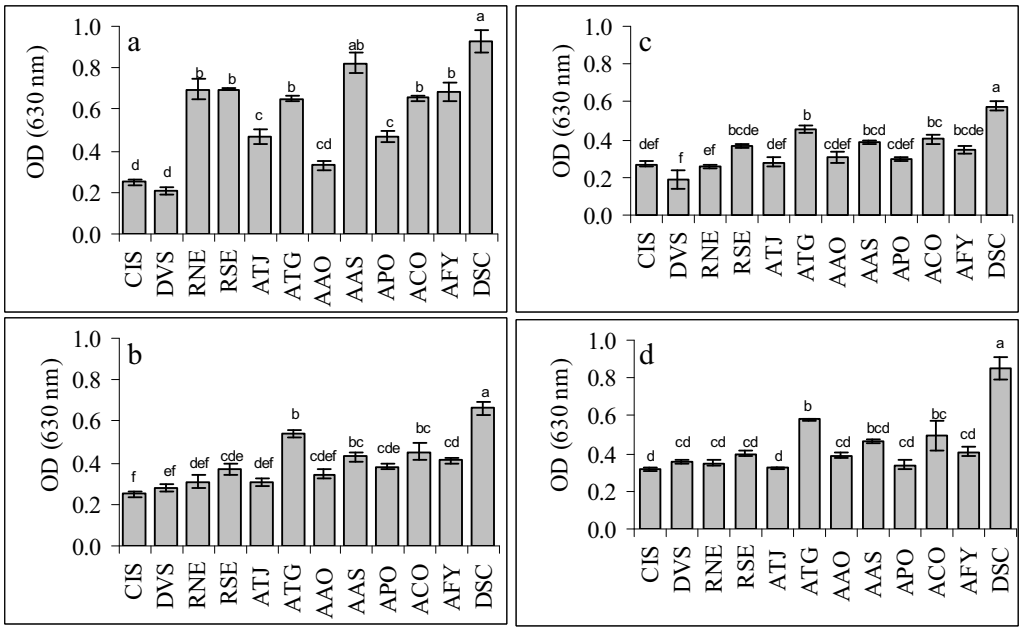

Fig. 3 Effect of different fructans on the growth of *Lactobacillus acidophilus* (a), *L. casei* (b), *L. paracasei* (c), and *L. rhamnosus* (d) incubated anaerobically at 37°C in the presence of 10 g of fructan/L. Results are means of 3 independent determinations ± SEM. Differences were considered significant at $P \leq 0.05$. OD, Optical density; CIS, *Cichorium intybus* Sigma; DVS, *Dahlia variabilis* Sigma; RNE, Raftiline®GR; RSE, Raftilose®Synergy1; ATJ, *A. tequilana* Jal; ATG, *A. tequilana* Gto; AAO, *A. angustifolia* Oax; AAS, *A. angustifolia* Son; APO, *A. potatorum* Oax; ACO, *A. cantala* Oax; AFY, *A. fourcroydes* Yuc; DSC, *Dasylirion* sp. Chih.

(**Figs. 2, 3**). In general, the medium supplemented with DSC fructans showed the best growth for bifidobacteria (**Fig. 2**). Only in the case of *B. breve* (**Fig. 2d**), DSC was not significantly different to RSE. As second better source of prebiotics, ATG showed good results, followed by ACO on *B. adolescentis* (**Fig. 2a**), by RSE on *B. animalis* (**Fig. 2b**), *B. breve* (**Fig. 2d**), and *B. longum* (**Fig. 2f**), on *B. bifidum* (**Fig. 2c**), ATG was not significantly different to RSE, AAS, APO, ACO, and AFY, and finally, on *B. infantis* (**Fig. 2e**) was similar to ACO. For Lactobacilli (**Fig. 3**), DSC was again best, stimulating better the bacterial growth of all species compared with other fructans, only with *L. acidophilus* (**Fig. 3a**), DSC was not significantly different to AAS; also it was followed by ATG and this did not show any difference compared to ACO, and AAS on *L. casei* (**Fig. 3b**) and *L. rhamnosus* (**Fig. 3d**). With *L. acidophilus* (**Fig. 3a**), ATG, AAS, and ACO were similar to RNE, RSE, and AFY, and with *L. paracasei* (**Fig. 3c**), were similar to RSE, and AFY. These results are according to McKellar and Modler (1989), where they mentioned that the inulinases of *Bifidobacterium* spp. were most active against neosugars, and least active against inulins.

Whether the nature of the carbohydrate determines its fermenting characteristics is a question that has barely been addressed (Cummings and Macfarlane 2002). van Laere *et al.* (1997) tested the breakdown of a range of different fructooligosaccharides with wide variety of sugar compositions and molecular sizes by several bacteria strains including bifidobacteria and lactobacilli. They observed that fructooligosaccharides were extensively fermented by most bacteria, except by Clostridia. Besides, low DP carbohydrates were utilized better or faster by bifidobacteria. Therefore, the structure of the carbohydrates and the bacteria strains present in an ecosystem are definitely determining factors controlling the fermentation of prebiotics. In this work, DSC with a DP range from 3 to 20 and ATG from 3 to 22 (data not shown, obtained by MALDI-TOF-MS) stimulated better the growth of both bacteria genera. Similar behavior was observed with RSE and RNE, being RSE a mixture of larger amounts of low DP compared to RNE, as it was also reported by Al-Tamimi *et al*. (2006), bifidobacteria were stimulated to different extents depending on arabinan and arabinooligosaccharide molecular weight, i.e. a maximum increase in bifidobacteria was seen after 48 h with the lower molecular weight fractions. Palframan *et al*. (2003) mentioned that bifidobacteria differed in fermentation profiles when tested on different carbohydrates. Similarly, in other work, van der Meulen *et al*. (2004) evaluated the growth of *B. animalis* on different energy sources through small- and large-scale fermentations, in general, the fermentation of inulin-type fructans resulted in changes on the growth and metabolic production, due to the preferential metabolism of certain fructans, especially the fructooligosaccharides.

In this work, fructans with the larger DP's were commercial inulins from *Cichorium intybus* (CIS) and *Dahlia variabilis* (DVS), both inulins showed the least effect on bacterial growth for all the different evaluated strains (**Figs. 2, 3**).

According to the results reported by Mancilla-Margalli and López (2006), where Agave fructans were classified into three groups depending on branched points and presence of terminal and internal α-D-glucopyranose moieties, DSC belongs to group II, ATG is classified into group III, and ATJ into group I. The best prebiotic effect was found in agave fructans that belong to groups II and III, which are characterized by having less branched structures and low DP. In contrast, fructans in group I possess highly-branched structures with a higher DP.

On the other hand, fructans from individuals within the same species, but grown in different geographic zones, showed a greater number of differences when were evaluated with the same bacteria (**Figs. 2, 3**). Fructans from *A. tequilana* from Jalisco (ATJ) seem as a very poor substrate for all bacteria, when compared with ATG. The same behavior was observed for *A. angustifolia* from Sonora (AAS) and from Oaxaca (AAO), being AAS a better substrate for all tested bacteria (**Figs. 2, 3**). These results can also be explained based on the structural diversity within *Agave* species (Mancilla-Margalli and López 2006).

Another important consideration during fructans fermentation is the drop in pH level as a consequence of SCFA production, in this work, the larger pH drop was observed in the culture broth containing *Dasylirion* sp., illustrating a direct relationship between bacterial growth and decline in pH as shown in **Tables 1** and **2**. The larger amount of bacterial growth results in the larger pH drop, a relationship reported as being beneficial as it inhibits the growth of pathogenic bacteria (Gibson and Wang 1994).

The short chain fatty acids proportions from four different fructans are listed in **Table 3**. Acetic, formic, and lactic acids were the major SCFAs detected. The established proportions of acids varied depending on the substrate used by the different bacteria (Shene *et al.* 2005).

The production of acetic and formic acids is of high interest for the inhibition of intestinal pathogens such as *Escherichia coli* and *Salmonella* spp. It is indeed well known that the inhibitory property of bifidobacteria depends on the production of organic acids (Ibrahim and Bezkorovainy 1993; Fooks and Gibson 2002; van der Meulen *et al.* 2004). Moreover, it has been reported that bifidobacteria and lactobacilli do not produce butyric acid, which plays a particularly important role as the preferred energy source for the colonic epithelium and a proposed role providing protection against colon cancer and colitis (Archer *et al.* 1998; Christl *et al.* 1996; Csordas 1996; Pool-Zobel 2005), however, acetic and lactic acids generated by these bacteria

Table 1 Culture broth pH drop due to fermentation of fructans by *Bifidobacterium adolescentis*, *B. animalis*, *B. bifidum*, *B. breve*, *B. infantis*, and *B. longum*.

Fructan	pH drop by					
	B. adolescentis	***B. animalis***	***B. bifidum***	***B. breve***	***B. infantis***	***B. longum***
CIS	0.25 ± 0.07 d	0.16 ± 0.04 e	0.19 ± 0.06 c	0.18 ± 0.05 d	0.31 ± 0.04 e	0.49 ± 0.13 e
DVS	0.26 ± 0.03 d	0.19 ± 0.01 e	0.14 ± 0.10 c	0.15 ± 0.07 d	0.30 ± 0.04 e	0.43 ± 0.09 e
RNE	0.31 ± 0.05 d	0.53 ± 0.01 d	0.17 ± 007 c	0.40 ± 0.10 cd	0.36 ± 0.04 e	0.82 ± 0.05 de
RSE	0.45 ± 0.08 cd	1.54 ± 0.04 ab	0.43 ± 0.07 bc	1.54 ± 0.05 a	0.43 ± 0.09 de	1.83 ± 0.02 ab
ATJ	0.22 ± 0.06 d	0.39 ± 0.01 de	0.21 ± 0.06 c	0.24 ± 0.06 d	0.27 ± 0.07 e	0.64 ± 0.14 e
ATG	0.76 ± 0.07 bc	1.27 ± 0.04 bc	0.74 ± 0.07 b	0.83 ± 0.08 b	0.85 ± 0.09 b	1.29 ± 0.09 c
AAO	0.44 ± 0.05 cd	0.32 ± 0.02 de	0.33 ± 0.12 bc	0.17 ± 0.06 d	0.46 ± 0.07 cde	0.60 ± 0.02 e
AAS	0.82 ± 0.07 b	1.07 ± 0.07 c	0.70 ± 0.15 b	0.76 ± 0.04 b	0.88 ± 0.04 b	1.41 ± 0.08 bc
APO	0.48 ± 0.03 bcd	0.30 ± 0.05 de	0.39 ± 0.11 bc	0.11 ± 0.03 d	0.44 ± 0.04 cde	0.65 ± 0.10 e
ACO	0.80 ± 0.14 b	1.08 ± 0.11 c	0.49 ± 0.04 bc	0.67 ± 0.10 bc	0.74 ± 0.07 bc	1.21 ± 0.07 cd
AFY	0.67 ± 0.05 bc	1.30 ± 0.06 bc	0.55 ± 0.12 bc	0.92 ± 0.05 b	0.73 ± 0.07 bcd	1.33 ± 0.11 c
DSC	1.76 ± 0.04 a	1.71 ± 0.09 a	1.83 ± 0.10 a	1.56 ± 0.02 a	1.79 ± 0.02 a	2.17 ± 0.05 a

The drop in pH induced by fructans fermentation is expressed as pH (averaged value ± SEM) at the end of fermentation in the presence of test carbohydrate minus pH at the beginning of the fermentation (adapted from Roberfroid *et al.* 1998). Differences were considered significant according to Tukey's *post hoc* test at $P \leq 0.05$. The drop in pH of the cultures was measured directly in culture tube. CIS, *Cichorium intybus* Sigma; DVS, *Dahlia variabilis* Sigma; RNE, Raftiline®GR; RSE, Raftilose®Synergy1; ATJ, *A. tequilana* Jal; ATG, *A. tequilana* Gto; AAO, *A. angustifolia* Oax; AAS, *A. angustifolia* Son; APO, *A. potatorum* Oax; ACO, *A. cantala* Oax; AFY, *A. fourcroydes* Yuc; DSC, *Dasylirion* sp. Chih.

Table 2 Culture broth pH drop due to fermentation of fructans by *Lactobacillus acidophilus*, *L. casei*, *L. paracasei*, and *L. rhamnosus*.

Fructan	pH drop by			
	L. acidophilus	***L. casei***	***L. paracasei***	***L. rhamnosus***
CIS	0.65 ± 0.04 f	0.33 ± 0.05 e	0.35 ± 0.05 d	0.39 ± 0.04 e
DVS	0.52 ± 0.01 f	0.30 ± 0.04 e	0.31 ± 0.04 d	0.29 ± 0.02 e
RNE	2.10 ± 0.12 b	0.45 ± 0.01 de	0.48 ± 0.02 cd	0.40 ± 0.02 e
RSE	2.40 ± 0.03 a	0.61 ± 0.01 cd	0.52 ± 0.08 bcd	0.65 ± 0.04 bcd
ATJ	1.27 ± 0.02 de	0.34 ± 0.02 e	0.32 ± 0.03 d	0.31 ± 0.04 e
ATG	1.77 ± 0.03 c	0.76 ± 0.03 bc	0.81 ± 0.04 b	0.68 ± 0.07 bc
AAO	1.10 ± 0.03 e	0.46 ± 0.01 de	0.48 ± 0.05 bcd	0.47 ± 0.04 cde
AAS	2.30 ± 0.03 ab	0.91 ± 0.04 b	0.73 ± 0.05 bc	0.78 ± 0.05 b
APO	1.46 ± 0.05 d	0.47 ± 0.03 de	0.46 ± 0.05 cd	0.45 ± 0.01 de
ACO	2.08 ± 0.04 b	0.77 ± 0.04 bc	0.78 ± 0.09 bc	0.78 ± 0.07 b
AFY	2.11 ± 0.11 b	0.82 ± 0.03 b	0.76 ± 0.12 bc	0.77 ± 0.03 b
DSC	2.27 ± 0.04 ab	1.81 ± 0.06 a	1.92 ± 0.09 a	1.82 ± 0.04 a

CIS, *Cichorium intybus* Sigma; DVS, *Dahlia variabilis* Sigma; RNE, Raftiline®GR; RSE, Raftilose®Synergy1; ATJ, *A. tequilana* Jal; ATG, *A. tequilana* Gto; AAO, *A. angustifolia* Oax; AAS, *A. angustifolia* Son; APO, *A. potatorum* Oax; ACO, *A. cantala* Oax; AFY, *A. fourcroydes* Yuc; DSC, *Dasylirion* sp. Chih.

Table 3 Short chain fatty acid (mM) generated by bifidobacteria and lactobacilli from the fermentation of *Agave* spp., *Dasylirion* sp. and commercial fructans.

Fructan	ACID	BA	BAN	BBF	BI	BBR	BLON	LA	LCC	LPC	LR
DSC	Lactic	38.66	8.63	43.23	45.17	5.24	6.99	63.78	33.41	54.70	37.45
	Formic	13.18	18.28	14.14	12.78	17.75	14.09	40.95	13.11	6.56	3.70
	Acetic	10.16	17.50	12.72	11.68	23.42	19.93	14.93	12.30	12.50	10.35
RSE	Lactic	4.39	3.66	7.07	7.17	4.11	0.00	62.25	21.03	9.41	0.00
	Formic	3.62	7.99	2.73	4.35	13.57	5.56	14.98	10.12	0.00	0.89
	Acetic	7.07	6.06	10.09	9.35	17.55	7.95	10.01	10.73	9.87	7.31
ATG	Lactic	10.64	0.00	6.64	11.15	0.00	0.00	45.33	6.75	21.32	15.13
	Formic	17.38	10.47	7.97	14.67	9.79	10.57	76.78	7.16	2.76	5.89
	Acetic	9.79	7.72	8.02	8.58	12.15	10.26	18.43	9.04	14.24	9.40
RNE	Lactic	1.59	0.00	0.31	4.87	0.89	0.00	51.26	0.00	0.00	0.00
	Formic	3.22	1.55	0.08	4.06	6.70	1.33	18.26	2.66	0.00	1.57
	Acetic	7.36	0.00	7.04	9.31	8.26	0.00	14.24	5.95	5.46	8.33

BA, *Bifidobacterium adolescentis*; BAN, *B. animalis*; BBF, *B. bifidum*; BI, *B. infantis*; BLO, *B. longum*; LA, *Lactobacillus acidophilus*; LR, *L. rhamnosus*. DSC, *Dasylirion* sp.; ATG, *Agave tequilana Gto*; RSE, Raftilose®Synergy1; RNE, Raftiline®GR.

have been reported as butyrogenic precursors (Duncan *et al.* 2002, 2004a, 2004b). To conclude, it has been shown that fructans from *Agave* spp. and *Dasylirion* sp. were able to stimulate the growth of probiotics on MRS medium; this might be due to the linkage type, degree of polymerization, and the highly branched structural features of these fructans. Bifidobacteria and lactobacilli growth better with shorter fructans. HPLC was very useful on the determination of the SCFA's proportions generated by the fermented fructans. Acetic, formic, and lactic acids were the main fermentation products. The final remark of this work is that *Agave* spp. and *Dasylirion* sp. fructans offer a possible prebiotic potential, opening new and excited alternatives as food ingredients and/or health promoting ingredients.

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