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## Agave fructans as prebiotics

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**Mercedes G. López and Judith E. Urías-Silvas**

Unidad de Biotecnología e Ingeniería Genética de Plantas, CINVESTAV-IPN  
Unidad Irapuato, A.P. 629, Irapuato, Gto., 36500, México

### Abstract

*Mexico is considered the origin center and biodiversity of the Agave genus, since a large number of Agave species are found in its territory. These plants present a crassulacean acid metabolism (CAM) and their principal photosynthetic products are fructans. Fructans are polymers of fructose generally linked to a moiety of a terminal glucose. Due to the  $\beta$ -configuration of the anomeric C2 in their fructose monomers, fructans are resistant to hydrolysis by the human digestive enzymes and can be fermented by colonic microflora producing short chain fatty acids*

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Correspondence/Reprint request: Dr. Mercedes G. López, Unidad de Biotecnología e Ingeniería Genética de Plantas, CINVESTAV-IPN Unidad Irapuato, A.P. 629, Irapuato, Gto., 36500, México  
E-mail: mlopez@ira.cinvestav.mx

(SCFA). Fructans possess numerous nutraceutical properties, being one of them their prebiotic effect. A prebiotic is a selectively fermented ingredient that allows specific changes, both in composition and/or activity in the gastrointestinal microflora, which confers benefits upon host well-being and health. Fructans affect physiological and biochemical processes in humans and animals, resulting in better health and reducing the risk of many diseases, stimulating the immune system of the body, decreasing the levels of pathogenic bacteria in the intestine, relieving constipation, decreasing the risk of osteoporosis by increasing mineral absorption, reducing the risk of atherosclerosis by lowering the synthesis of triglycerides and fatty acids in the liver and decreasing their level in the serum. These fructans also modulate the hormonal level of insulin and glucagon, thereby regulating carbohydrate and lipid metabolism by lowering the blood glucose levels. Inulin and oligofructose also reduce the incidence of colon cancer. All these properties have been attributed principally to commercial fructans which possess mainly  $\beta(2-1)$  linkages. In the case of *Agave* and *Dasyliirion* fructans, these possess a molecular structure composed of a complex mixture of fructans containing principally  $\beta(2-1)$  linkages, but also some  $\beta(2-6)$  and highly branched, and a neo-type feature, these types of fructans have just been denominated agavins. Agavins stimulated the growth of *Bifidobacterium breve* and *Lactobacillus casei* more efficiently than most commercial inulins.

## 1. Introduction

Fructans of the inulin type possess mainly  $\beta(2-1)$  linkage that escape from the action of digestive enzymes, therefore they reach the large bowel unchanged, consequently serving as fermentative substrates mainly to the colonic microflora. Previous reports indicate that fructans stimulate the growth and activity of Bifidobacteria and Lactobacilli in the guts in a selective manner, and consequently inhibiting the growth of pathogenic bacteria [1-3]. In the last years, the genus *Agave* has been of great importance due to the report of the presence of fructans in several *Agave* species, mainly in *Agave tequilana* [4,5]. Based on the well known health benefits of fructans, there is a great interest on the agavins health properties lately.

### 1.1. Carbohydrates

Starch and sucrose are the most common and well known storage carbohydrates in higher plants, however about 12-15% of these plants store fructans as reserve carbohydrates in at least one of their organs during their life cycle [6,7]. The presence of fructans in plants has been also related to plant protecting aspects against cold and drought stress [8,9]. Fructan containing plants are found in a small number of mono- and di-cotyledonous families such

as Liliaceae, Amaryllidaceae, Gramineae, Compositae, Nolinaceae and Agavaceae. Various fructan containing plant species like asparagus, garlic, leak, onion, Jerusalem artichoke and chicory are often eaten as vegetables [5,10,11]. Some important sources of fructans are given in Table 1. Dicotyledonous plants store fructans that consist mainly of linear  $\beta(2-1)$  fructofuranosyl units (inulin), whereas more complex and branched fructan types with mainly  $\beta(2-6)$  linkages are common to monocotyledonous species [6,8,12].

Fructans isolated from plants have a wide variety of applications. Small DP fructans have a sweet taste, whereas large DP form emulsions with a fat-like texture and neutral taste, there is also a great interest by the food industry in using these fructans as low-calories food ingredients and also as nutraceutical ingredients [12].

**Table 1.** Fructan content percent on fresh weight basis for some important plant sources.

Source	Fructan <sup>a</sup>
Asparagus root	10-15
Garlic	15-20
Jerusalem artichoke	15-20
Dahlia tubers	15-20
Chicory root	15-20
Agave steam <sup>b</sup>	15-22

<sup>a</sup> [6]; <sup>b</sup> This group.

## 1.2. Fructans

Fructans are non-reducing carbohydrates formed of fructosyl units generally presenting in their structure a moiety of terminal glucose. Their structure can be lineal or branched [13]. Vijn and Smeekens [12] classified fructans into five major groups according to the majority structural units that conform their molecule, those are inulin, levan, mixed levan, inulin neoseris and levan neoseris.

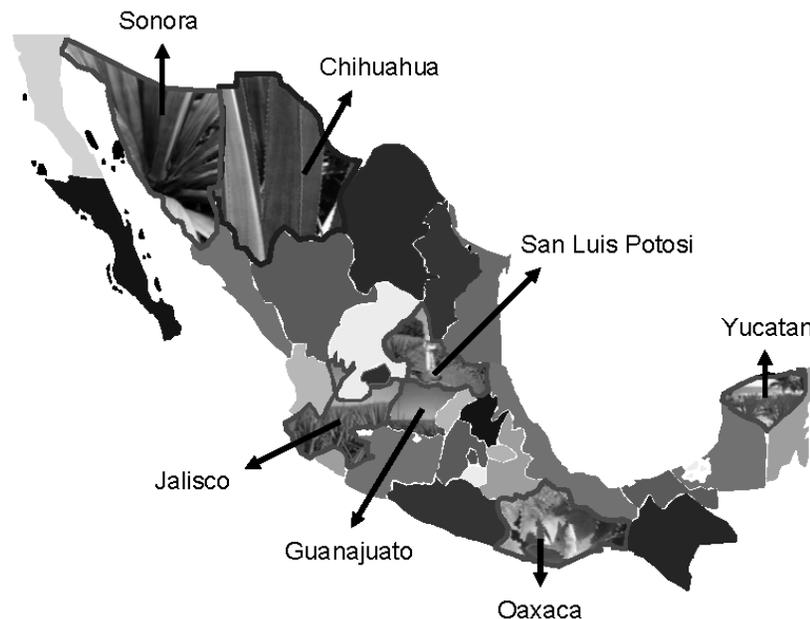
Inulins consist of lineal (2-1)-linked  $\beta$ -D-fructosyl units. The shortest inulin molecule is the trisaccharide 1-kestose, also called isokestose. Levans consist of lineal (2-6)-linked  $\beta$ -D-fructosyl units. Mixed levans are composed of both (2-1)- and (2-6)-linked  $\beta$ -D-fructosyl units. An example of this type of fructan is the molecule known as bifurcose. The inulin neoseris are lineal (2-1)-linked  $\beta$ -D-fructosyl units linked to both C1 and C6 of the glucose moiety of the sucrose molecule. The smallest inulin neoseris molecule is neokestose. The levan neoseris are polymers of predominantly (2-6)-linked  $\beta$ -D-fructosyl residues on either end of the glucose moiety of the sucrose molecule [12].

### 1.3. Agave plants

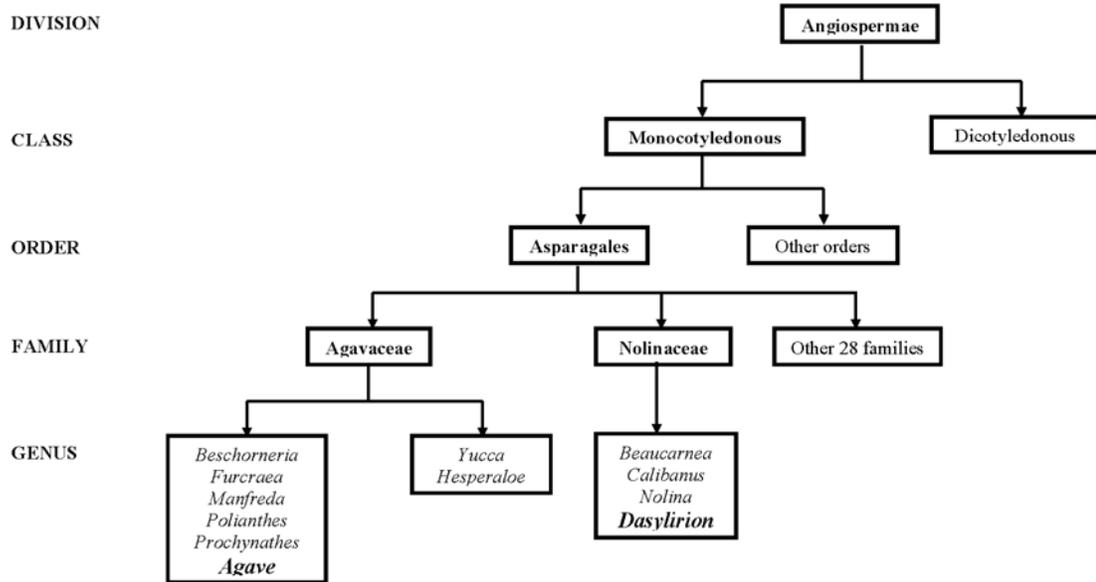
Since immemorial times the Agave or “maguey” plants, as called in Mexico, have represented an important source of long and hard natural fiber, medicines and alcoholic beverage production for prehispanic cultures. Nowadays, Agave plants are of great relevance to Mexico, since it is considered the origin center of evolution and diversification of this genus. Of approximately 300 described species, 75% is found within the Mexican territory [14]. In Mexico, the Agave variability is wide and diverse (Fig. 1), some examples of such variability are *Agave salmiana*, *A. lechuguilla*, *A. atrovirens* and *A. mapisaga* mainly found in San Luis Potosi state; *A. tequilana*, *A. longispala* and *A. subtilis* in Jalisco and Guanajuato; *A. americana*, *A. striata* and *A. lechugilla* in the state of Hidalgo; *A. fourcroydes* and *A. sisalana* in Yucatan; *A. cantala*, *A. angustifolia*, *A. potatorum* and *A. karwinskii* in the state of Oaxaca; *A. angustifolia* in Sonora; *A. salmiana* and *A. mapisaga* in the Valley of Mexico, to mention some [15,16].

Until now, the classification reported by Dahlgren et al. [17] is been the most accepted (Fig. 2). This classification was based on characteristics like chromosome number, chemistry, geographic location and evolutionary analyses, proposing the Asparagales order with 30 families, which include the *Agavaceae* and *Nolinaceae*.

*Dasyliirion* possesses similar characteristics with Agave such morphology, geographical distribution and pollen characteristics, however, they present large genetic differences as the number of chromosomes, principal reason for which *Dasyliirion* was classified in a different family.



**Figure 1.** Biodiversity of the Agave genus in Mexico.



**Figure 2.** Agave and Dasyliirion taxonomic classification.

## 1.4. Agave metabolism

The most important physiologic adaptation of Agave and Dasyliirion plants is their photosynthetic metabolism known as Crassulacean Acid Metabolism (CAM). CAM is based on the opening of stomata at night and their closure during the day, this metabolism involves reduction of transpirational water loss [18].

In Agave plants, the principal photosynthetic products have been reported to be fructans [5,19]. Fructans are synthesized and stored in the stems of Agave plants, and their main function in the steam of such CAM plants is the same as for  $C_3$  and  $C_4$  plants, reserve carbohydrates. They may also act as osmoregulators during drought, constituting this aspect another possible physiological adaptation to arid environments [9]. Because fructans are water soluble, are osmotically active, by changing the degree of polymerization (DP) of the molecule in the plant vacuole, the plant can readily change the osmotic potential of its cells without altering the total amount of carbohydrates. The internal hydrolysis of fructans by endoinulinases generates different DP fructans (F<sub>m</sub> and GF<sub>n</sub>; where m and n could be different). These molecules allow plants to selfsmoregulate, surviving winter periods in cold to moderately cold and drought-stricken regions [20].

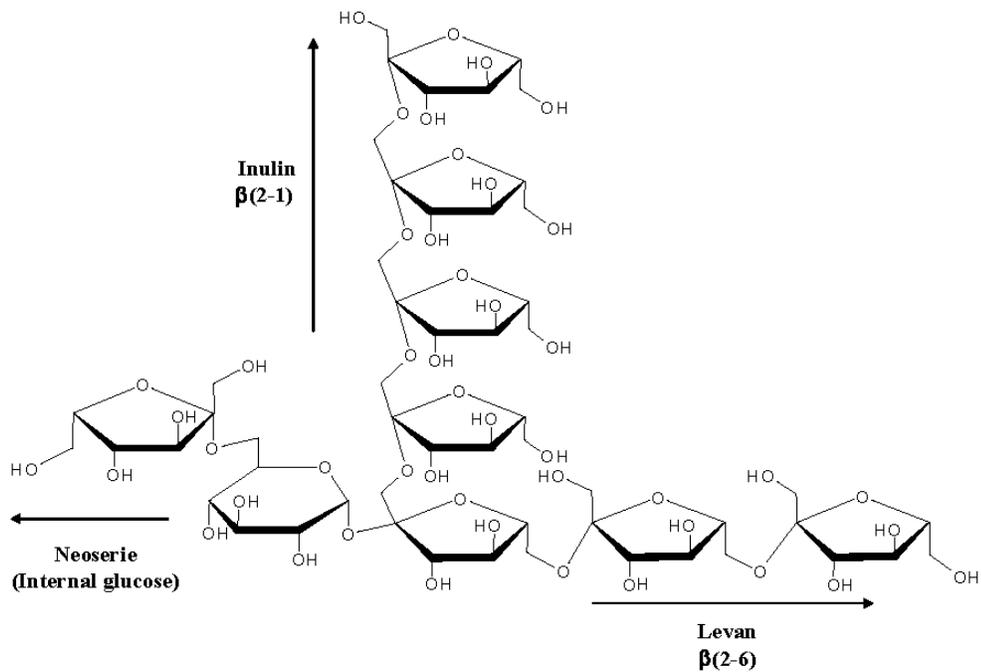
## 1.5. Agave fructans

Fructan structures seem to be species dependent; in fact, Bonnett et al. [21] proposed the elucidation of fructan structures as a potential taxonomic marker

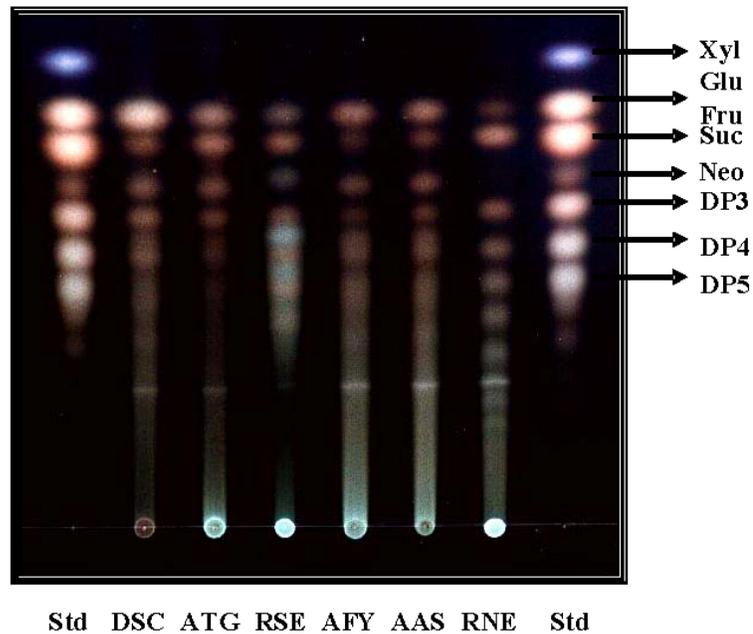
for Poaceae. However, in *Agave* species more than one fructan structure has been reported, Sánchez-Marroquín and Hope [19] and Bathia and Nandra [22] reported inulin as the principal storage carbohydrate in *Agave tequilana* and *A. americana*, respectively. On the other hand, Aspinall and Das Gupta [23] and Satyanarayana [24,25] reported the presence of a complex mixture of fructans highly branched with internal glucose and linkages of the type  $\beta(2-1)$  and  $\beta(2-6)$ . Some years later, Wang and Nobel [9] working with *A. deserti* found the presence of 1-kestose (DP3), nystose (DP4), neokestose (DP3, with an internal glucose moiety) and fructans with DP5, named by them as pentofructan. More recently, Mancilla-Margalli and López [4] and López et al. [5] reported the molecular structure of fructans from *A. tequilana* Weber var. azul and many other species. They proposed structures based on derivatization upon linkage analysis coupled to gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance and matrix-assisted laser desorption time-of flight mass spectrometry (MALDI-TOF-MS). The structures they proposed have been named agavins, which consist of a complex mixture of fructans containing principally  $\beta(2-1)$  linkages, but also some  $\beta(2-6)$ , highly branched and a neo-type (Fig. 3). Mancilla-Margalli and López [4] reported structural differences not only among *Agave* but also within *Agave* species grown in different environmental regions. The observed structural heterogeneity could be attributed to the plant adaptation mechanisms to survive in very inhospitable areas. On the other hand, Urías-Silvas and López [26] analyzed the DP profile of some *Agave* fructans by thin layer chromatography (TLC), the observed differences on fructan DP profiles can be seen in Figure 4. Fructans from *Dasyilirion* spp. (DSC) showed the largest amount of low DP compared with other species, followed by *A. tequilana* from Guanajuato (ATG) and based on the weak spot left at the application origin. Among commercial fructans, Raftilose®Synergy1 (RSE) presented also a large amount of low DP compared to Raftiline®GR. No marked differences were observed between fructans from *A. fourcroydes* from Yucatan (AFY) and *A. angustifolia* Sonora (AAS). An important point to mention on the case of these agavins, is the presence of a spot between sucrose and DP3, which corresponds to the neo-type fructans reported by Mancilla-Margalli and López [4], where is mentioned that the spot is an indicative of the presence of a neo-type fructan.

## 2. Prebiotics

Because of the  $\beta$ -configuration of the anomeric C2 of fructose monomers, all fructans are resistant to hydrolysis by human digestive enzymes ( $\alpha$ -glucosidase, maltase, isomaltase and sucrase) which are specific for  $\alpha$ -glycosidic bonds, and are thus classified as non-digestible oligosaccharides on the basis of both *in vitro* and *in vivo* data. Since the stomach hydrolysis of



**Figure 3.** Proposed Agave fructan core structure.



**Figure 4.** Thin layer chromatography of fructans from *Dasyliirion* spp. (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.

fructans is of a limited physiological significance, these products pass undigested through the upper part of the gastrointestinal tract into the colon. This has been confirmed by *in vivo* studies on humans [27]. The most convincing studies were done with the use of an ileostomy model which provides a valuable alternative to the study of digestive physiology in man and which has often been used to quantify the small intestinal excretion of nutrients, carbohydrates in particular. It was found that 86-88% of a dose of inulin and oligofructose was recovered in the ileostomy effluent which supported the hypothesis that inulin and oligofructose are practically indigestible in the small intestine of man [28]. Further proof of their indigestibility rests on the fact that there is no observed increase in serum glucose after they were ingested [6,27,29,30]. Therefore, the criteria used for the classification of food components as prebiotics are as follows: resistance to digestion, hydrolysis and fermentation by colonic microflora, and most importantly, selective stimulation of growth of one or a limited number of bacteria in the colon [31, 32].

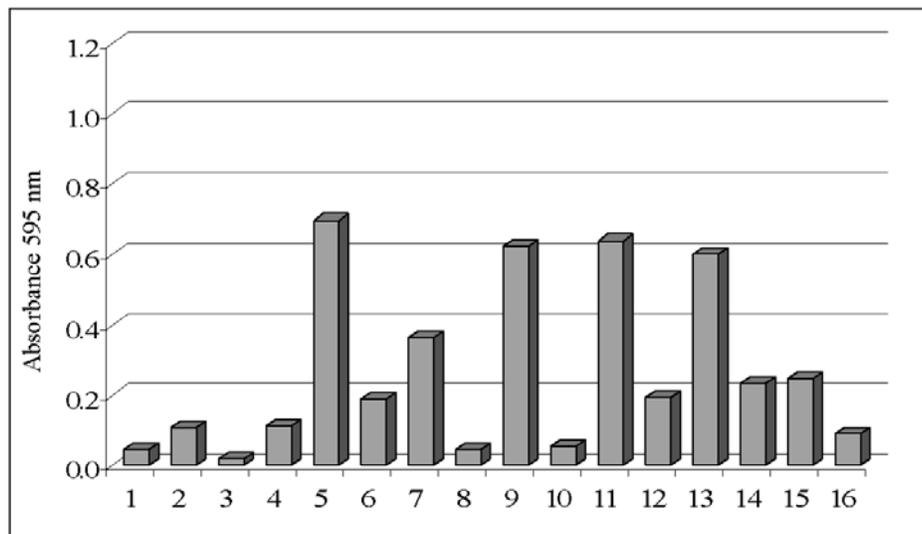
A prebiotic is a selectively fermented ingredient that allows specific changes, both in composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health [1]. Inulin-type fructans are an example of such carbohydrates [33]. They reach the colon unabsorbed and are utilized selectively as a substrate for the endogenous bacteria [34-37] and by fermentation producing SCFA's, mainly acetate, propionate and butyrate as well as lactate [2,31, 38]. Collectively, there is convincing evidence to indicate that inulin-type fructans selectively stimulate the growth of Bifidobacteria both *in vitro* and *in vivo* [2,27,35,39-44].

All current prebiotics are nondigestible oligosaccharides (NDO). Examples of NDO prebiotic beside fructans include galacto-oligosaccharides and lactulose all of which have bifidogenic effect [45]. However, for xylo-, manno- and galacto-oligosaccharides and lactulose, more studies are required on their prebiotic effect [46]. Despite the variety of new potential candidates as prebiotics becoming available for human use, the consensus of scientists as a result of the European Project ENDO (DGXII AIRII-CT94-1095) indicated that  $\beta(2-1)$ -fructans exclusively possess well-documented prebiotic abilities in humans [47,48].

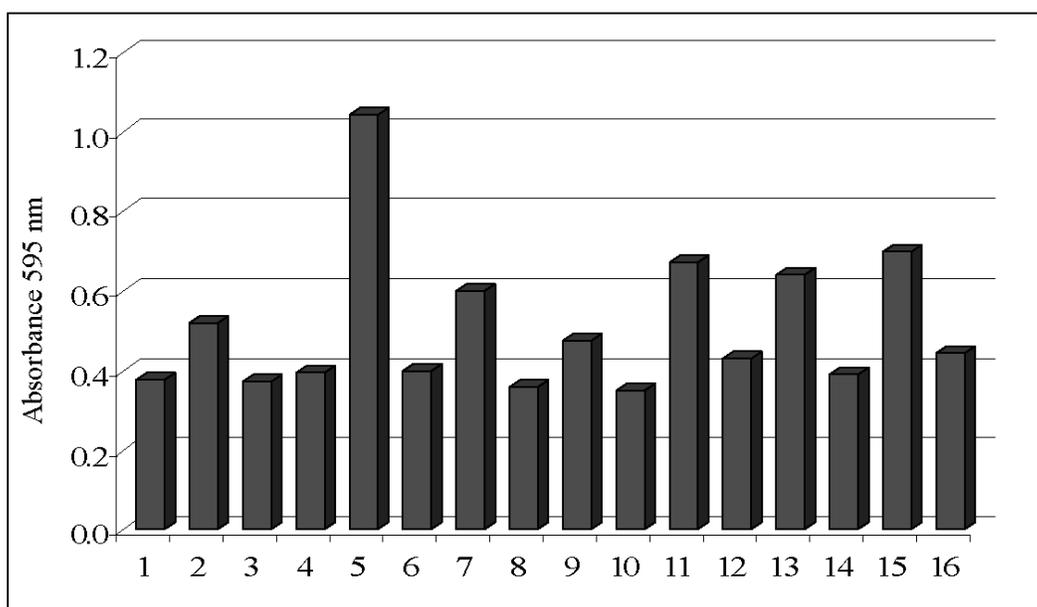
Similarly, bifidobacterial  $\alpha$ -galactosidase allows a prebiotic effect of oligosaccharides from soybean. Galacto-oligosaccharides are manufactured from lactose by transglycosylation reactions and consist of galactosyl derivatives of lactose with  $\beta(1-3)$ - and  $\beta(1-6)$ -linkages. The prebiotic nature of galacto-oligosaccharides may be due to the linkage-specificity of the *Bifidobacterium*  $\beta$ -galactosidase. Isomalto-oligosaccharides  $\alpha(1-6)$ -linked and gluco-oligosaccharides  $\beta(1-6)$ -linked are potential candidates as prebiotics, as are xylo-oligosaccharides. However, specific enzymes for the degradation of those

molecules have not yet been evaluated; thus the explanatory mechanism for any prebiotic effect is not yet sufficiently evidence [49].

For agavins, the growth of *Bifidobacterium breve* and *Lactobacillus casei* in pure culture was evaluated (Fig. 5 and 6) [26]. In general, the media supplemented with fructans from *Dasyilirion* spp. (DSC) showed the best growth for both bacteria. For *B. breve* (Fig. 5) fructans from *A. tequilana* from Guanajuato (ATG) was ranked in second and Raftilose®Synergy1 (RSE, commercial fructan) was third. But in the case of *L. casei* (Fig. 6) fructans from *A. cantala* from Oaxaca (ACO) was ranked in second and ATG was third. Agavins from the same *Agave* species, but from plants grow in different geographic zones showed differences on their prebiotic effect. Fructans from *A. tequilana* from Jalisco (ATJ) seem as a very poor substrate, when compared to the same *Agave* species but grown in Guanajuato (ATG). The same behavior was observed for *A. angustifolia* from Sonora (AAS) and from Oaxaca (AAO), being AAS a better substrate. These results can be explained based on the structural diversity within *Agave* species [4]. The preferential stimulation of bacterial growth of certain fructans, especially those with a low DP was observed. Commercial fructans presented the larger DP's (*Cichorium intybus* (CIS), *Helianthus tuberosus* (HTS) and *Dahlia variabilis* (DVS)), showed the least effect as prebiotics (Figs. 5 and 6).



**Figure 5.** Effect of different fructans on the growth of *Bifidobacterium breve* incubated anaerobically at 37 °C in the presence of 10 g of fructan/L. Results are means of 3 independent determinations. **1**, *Helianthus tuberosus* Sigma; **2**, *A. potatorum* Oax; **3**, Inulin Merk; **4**, *A. tequilana* Jal; **5**, *Dasyilirion* spp. Chih; **6**, Inulin Helm; **7**, *A. angustifolia* Son; **8**, *Cichorium intybus* Sigma; **9**, Raftilose®Synergy1; **10**, *Dahlia variabilis* Sigma; **11**, *A. tequilana* Gto; **12**, *Opuntia* spp.; **13**, *A. fourcroydes* Yuc; **14**, Raftiline®GR; **15**, *A. cantala* Oax; **16**, *A. angustifolia* Oax.



**Figure 6.** Effect of different fructans on the growth of *Lactobacillus casei* incubated anaerobically at 37 °C in the presence of 10 g of fructan/L. Results are means of 3 independent determinations. **1**, *Helianthus tuberosus* Sigma; **2**, *A. potatorum* Oax; **3**, Inulin Merk; **4**, *A. tequilana* Jal; **5**, *Dasyilirion* spp. Chih; **6**, Inulin Helm; **7**, *A. angustifolia* Son; **8**, *Cichorium intybus* Sigma; **9**, Raftilose®Synergy1; **10**, *Dahlia variabilis* Sigma; **11**, *A. tequilana* Gto; **12**, *Opuntia* spp.; **13**, *A. fourcroydes* Yuc; **14**, Raftiline®GR; **15**, *A. cantala* Oax; **16**, *A. angustifolia* Oax.

Another important parameter during fermentation is the pH drop, due to the production of SCFA's. Urías-Silvas and López [26] observed the larger pH drop in the culture broth containing *Dasyilirion* spp., a direct relationship between bacterial growth and pH drop is shown in Table 2. It can be said, that the larger the bacterial growth the larger pH drop, which has been reported to have an increasing beneficial effect, inhibiting the growth of pathogenic bacteria [40].

### 3. Physiological effect of fructans on the health

Fructans are considered as functional food ingredients since they affect physiological and biochemical processes on humans and animal, resulting in better health and reduction on the risk of many diseases. Experimental studies have shown their use as bifidogenic agents, stimulating the immune system of the body, decreasing the levels of pathogenic bacteria in the intestine, relieving constipation, decreasing the risk of osteoporosis by increasing mineral absorption [30,50-53], especially of calcium (Ca), reducing the risk of atherosclerosis by lowering the synthesis of triglycerides (TAG) and fatty acids in the liver and also decreasing their level in serum [32,54]; decreasing fast and

**Table 2.** Culture broth pH drop due to fermentation of fructans by *Bifidobacterium breve* and *Lactobacillus casei*.

Number <sup>1</sup>	Substrate	pH drop <sup>2</sup> by	
		<i>Bifidobacterium breve</i>	<i>Lactobacillus casei</i>
1	<i>Helianthus tuberosus</i> Sigma	0.13	0.59
2	<i>Agave potatorum</i> Oax.	0.30	0.88
3	Inulin Merk	0.21	0.65
4	<i>A. tequilana</i> Jal.	0.40	0.65
5	<i>Dasylirion</i> spp. Chih.	1.66	2.21
6	Inulin Helm	0.70	0.93
7	<i>A. angustifolia</i> Son.	1.08	1.09
8	<i>Cichorium intybus</i> Sigma	0.19	0.72
9	Raftilose®Synergy1	1.71	1.15
10	<i>Dahlia variabilis</i> Sigma	0.22	0.73
11	<i>A. tequilana</i> Gto.	1.55	1.29
12	<i>Opuntia</i> spp.	0.70	1.22
13	<i>A. fourcroydes</i> Yuc.	1.61	1.19
14	Raftiline®GR	0.75	0.93
15	<i>A. cantala</i> Oax.	0.83	1.33
16	<i>A. angustifolia</i> Oax.	0.25	0.75

<sup>1</sup> The numbers in the first column correspond to the order of the prebiotics in the graphs. <sup>2</sup> The drop in pH induced by fructans fermentation is expressed as pH (means of 3 independent determinations) at the end of the substrate fermentation minus the pH at the beginning of the fermentation (adapted from [33]). The drop in pH of the cultures was measured directly in culture tube.

postprandial plasma glucose concentrations and improving of glycaemic control [55-57]. They are also effective in lowering blood urea [58,59] and uric acid levels, thereby maintaining the nitrogen balance. These fructans modulate the hormonal level of insulin and glucagon [60,61], thereby regulating carbohydrate and lipid metabolism [54] by lowering the blood glucose levels. Inulin and oligofructose also reduce the incidence of colon cancer [62-66]. Oligofructose are non-cariogenic as they are not used by *Streptococcus mutans* to form acids and insoluble glucans that are the main culprits in dental caries [6]. These fructans are low energy food ingredient; their energy content is only 40-50% of that of digestible carbohydrates, giving them a caloric value of 1.0-2.0 Kcal/g. Both inulin and oligofructose are used in the diet of obese people [6].

#### 4. Fructans and the food industry

The utilization of *Cichorium intybus* (chicory) for the production of fructans with the finality to be used as food ingredients has been authorized in all European countries as well as in the United States, Canada and Japan. On the other hand, fructans obtained by an enzymatic process via a transfer of fructosyl units from sucrose molecules is widely used in the Japanese food

industry [33,64]. Different functional attributes of inulin and oligofructose are due to the difference in their DP's as it was mentioned before.

Inulin and oligofructose can be used either for their nutritional advantages or for their multi-functional properties, but they are frequently used for their dual benefits: an improved organoleptic quality and a better-balanced nutritional composition [68].

## 5. Remarks

This is a brief communication concerning the implication of agavins as prebiotics. The prebiotic concept with the overall of its implications on health, is generating a great interest on the search for new sources of fructans to be used in a wide range of food products, agavins could be one of them.

However, the mechanisms implicated on the positive effects of agavins on health, must be done under well-controlled studies, rigorously and carefully performed.

## Note

Most of the data presented in this chapter is a part of a Mexican patent number PA/A/2004/011739.

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