Prebiotic effect of *Agave fourcroydes* fructans: An animal model

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Abstract

The use of prebiotics such fructans has increased in human and animal nutrition, basically becasue they benefit health and productive performance. *Agave fourcroydes* has shown high concentration of fructans in their stems, however, there is no information on new products derived from this plant that might enhance its added value. Therefore, we evaluated the prebiotic effect of *Agave fourcroydes* fructans in an animal model. Mice males (C57BL/6J) were fed on parallel form with a standard diet or diets supplemented with 10 % of fructans from *Cichorium intybus* (Raftilose P95) and *Agave fourcroydes* from Cuba for 35 days. The body weight, food intake, blood glucose, triglycerides and cholesterol, gastrointestinal organs weight, fermentation indicators in cecal and colon contents and mineral content in femurs were determined. The body weight and food intake of mice were not significantly modified by any treatment. However, serum glucose, cholesterol and triglycerides decreased ($P<0.01$) in the fructans groups with respect to the standard diet group, this decrement was higher in the *A. fourcroydes* group with respect to the Raftilose P95 group. Mice groups supplemented with fructans increased ($P<0.01$) total and wall cecum and colon weight. The fermentation indicators, short chain fatty acids (SCFAs) and pH, decreased ($P<0.001$) in the groups that consumed fructans in their diets with respect to the standard diet. The diets supplemented with fructans also increased mineral concentrations calcium ($P<0.01$) and magnesium ($P<0.05$) in the right femurs. In conclusion, the inclusion of fructans from *Agave fourcroydes* in the mice diet induced a prebiotic response, similar to or greater than the commercial product (Raftilose P95) and this constitutes a promising alternative with potentialities to be used not only in animal but also in human feeding.

Keywords:

Agave fourcroydes

Fructans

Prebiotic

Mice

Introduction

The ban to use antibiotics as growth promoters in animal feeding has increased the search for new additives providing hygienic-sanitary guarantees and their efficiency in the agricultural and feeding industry. Nowadays, there is a growing trend to the use for more innocuous products such as prebiotics due to their beneficial effects on health and the productive performance of animals.[1]

Prebiotics are food ingredients that selectively stimulate the growth and activities of specific bacteria in the gastrointestinal tract, usually *Lactobacillus* spp and *Bifidobacterium* spp, thus, improving the animals and humans health[2], fructans are among these compounds. Some researches indicate that fructans increase the metabolic activity of the intestinal microbiota[3], stimulate the immune system[4], modulate glucose and lipid metabolism[5], and improve mineral absorption[6], among other effects.

Fructans are naturally found in garlic *(Allium sativu*m), chicory *(Cichorium intybu*s), Jerusalem artichoke *(Helianthus tuberosu*s), asparagus *(Asparagus officinali*s), onion *(Allium cep*a), and yacon *(Smallanthus sonchifoliu*s), among other species.[7] However, it is still unknown the capacity of other plants to synthesize these compounds.

López *et al.*[8] reported the presence of fructans in *Agave tequilana* finding high concentrations of these carbohydrates in the agave stalks. These authors identified fructans with β(2-1) and β(2-6) bonds with abundant ramifications and high degree of polymerization. *Agave fourcroydes* is within the Agave genus, which is used primarily to obtain fibers for industry.[9] García-Curbelo *et al.*[10] reported the structure of fructans in *Agave fourcroydes* as a complex mixture of oligosaccharides, which presents few ramifications, neo-kestose series, inulin (GFn) and inulo-n-ose (Fn). In spite of this, there are very few reports on new products derived from this plant that could enhance its added value. The lack of knowledge restrains, to a great extent, the use of this new carbohydrate source with prebiotic properties.

Due to a considerable interest on the search for new compounds that improve the animals and humans health, the objective of this research was to evaluate the prebiotic effect of *Agave fourcroydes* fructans in mice as an animal model.

Materials and methods

Animals and diets

Eighteen male (C57Bl/6J) mice from CINVESTAV-Mexico (7 weeks old at the beginning of the experiment, with mean body weight (BW) of 21.50 g) were housed in a temperature- and humidity-controlled room with a 12 h light-dark cycle. They were divided into three groups (six mice per group) according to diet. The experiment lasted 5 weeks and the animals had an acclimatization period of 7 days.

The different treatments were: the control mice were fed pelleted 5053 standard diet (STD), prepared by International Center of Nutrition of Laboratory Animals, USA, whereas Raftilose P95 (RSE)- and *Agave fourcroydes* fructans (AF-C)- diet mice received a diet prepared by mixing 90 g 5053 standard diet with 10 g of the corresponding fructans. Mice were given free access to diet and water. All experiments were in accordance with National Research Council guidelines for the care and use of laboratory animals.

Fructans

Cichorium intybus fructans such Raftilose P95 (Orafti, Tienen, Belgium) is a mixture of glucosyl-(fructosyl) n-fructose and (fructosyl) m-fructose with an average DP of 4-8. Fructans from *Agave fourcroydes* present oligosaccharides with a DP < 10 with linkages of the type β(2-1), β(2-6), and branch of the neo type (data not shown).[10]

Body weight, food intake, and organs weight

Body weight was determined at the end of the experimental period and food intake was monitored every day during the experimental period.

On week 5 mice were anaesthetized by intra-peritoneal injection of sodium pentobarbital solution (1 ml 2.5 kg^{-1} body weight, *Anestesal, Pfizer*). A ventral midline incision was made and the gastrointestinal tract organs excised. Immediately after removal, the organs and their contents were weighted to determine total weight. After removal the appropriate samples, the tissues were cleaned with saline solution, blotted to dry, and weighted to determine total wall weights.

They are expressed as weight relative to the body weight in % as:

Organ weight relative (%) = (organ weight/body weight (g)) * 100

Fermentation indicators

Cecum and colon contents were collected, their pH content measured (PHR-146 "*Lazar Research Laboratories, Inc*."), and a 0.4 g aliquot immediately processed for SCFAs analysis.[11]

Blood samples

Blood samples were taken at the end of experiment period, from the mice tails in order to measure serum glucose (digital meter *Prestige smart system, C0 197)*, triglycerides (TAG) (enzymatic kits BioVision 612-100), and cholesterol (enzymatic kits BioVision 603-100).

Mineral content

Quantification of calcium and magnesium in femurs were performed using the protocol by García-Vieyra *et al.* 2014.[12]

Statistical analysis

Results are expressed as mean values with their standard errors of the mean. Statistical differences between groups were evaluated using one-way ANOVA followed by analysis of simple classification and Duncan's[13] multiple range test were used in the necessary cases, using INFOSTAT software.[14] Significant differences were considered at $P<0.05$.

Results

Body weight, food intake, and organs weight

Fructans supplementation did not influence the animal's body weight and daily food intake. However, in both mice groups that consumed fructans from RSE and AF-C and compared with the STD, increases were observed in the relative weight of cecum with content by 19% and wall weight by 21%; the relative weight of the colon with content by 10% and wall weight by 10% (Table 1). Other gastrointestinal organs were unaffected by the fructans administration.

Fermentation indicators

All fructans diets significantly increased the cecum and colon concentrations of total SCFAs compared with the standard diet ($P<0.001$) (Table 2). In addition, the AF-C group had higher butyrate concentration in the cecum than the RSE and STD groups by 24% and 77%, respectively, and in colon by 17% and 47%, respectively.

As shown in Fig. 1, the pH values of cecum and colon were lower ($P<0.001$) in mice fed fructans diets compared to the standard diet.

Serum glucose, triglycerides, and cholesterol

In the postprandial state, serum glucose concentrations were significantly lowered by 27% and 8% in mice fed AF-C diet as compared with STD and RSE, respectively. Only mice fed AF-C diet reduced TAG and cholesterol concentrations by 44.6% and 23.42%, respectively, as compared to the STD diet. In both indicators there were no significant differences between the STD and RSE groups (Table 3).

Mineral content

Calcium and magnesium concentrations in the mice femurs fed a STD or a diet supplemented with fructans from RSE and AF-C are shown in Fig. 2 and Fig. 3, respectively. Calcium concentrations were significantly increased by 27 % and 15 % as compared with the STD in mice fed AF-C and RSE diets, respectively. Magnesium concentrations were significantly increased by 33 % and 28 % when compared to the STD in mice fed AF-C and RSE diets, respectively.

Discussion

Plants of the genus Agave are of great importance as they stored high concentration of fructans as reserve carbohydrates in their stems.[15] These authors showed that the agave species type had an impact not only on the degree of polymerization but also on their complexity. They have proposed a classification for agave fructans, e.i. *Agave tequilana* belonging to group I and *A. fourcroydes* within group II with an estimated DP of 18.12 and 6.66, respectively. Recent research on non-digestible carbohydrates has increased their relevant importance due to the proved action of fructans as prebiotics by stimulating the growth and/or the activity of one or a limited number of beneficial intestinal bacteria thus, improving the health of animals and men.[16]

The purpose of this work was to study the prebiotic effect of fructans from *Agave fourcroydes* in mice as an animal model. The present study demonstrated that the intake of fructans increased the total and wall weight of the cecum and colon, being more significant in the cecum. Fructans with β links are not degraded by the digestive enzymes of the host in the upper gastrointestinal tract, reaching the large intestine unchanged, a site with high microbial populations (microbiota), where beneficial bacteria uses these compounds as energy source, and consequently increasing SCFAs, which may increase crypt depth, cell density by providing energy source, and normalizing cell proliferation.[17] The difference in the site of action of these compounds may result from the fact that mice are cecal fermenters.

In addition, the pH of cecum and colon were significantly lowered in the mice groups that ingested fructans. The large levels of total SCFAs production during fermentation might cause the pH drop.

SCFAs, particularly acetate, propionate, and butyrate, are the dominating end-products of bacteria fermentation in the large bowel.[18] In this study, it was observed that fructans intake improved concentrations of cecal SCFAs. On the other hand, butyrate concentrations were higher in the AF-C group than in the RSE and STD groups. The difference in the chemical composition of these fructans migth influence different fermentation by the microbiota. In previous studies, it was reported that when adding inulin-type oligosaccharides in mice diets short chain fatty acids (acetic, propionic, and butyric) concentrations increased.[19] However, Campbell *et al.*[20] found only increases in the concentration of acetic and butyric acids in rats that consumed fructans with a high degree of polymerization (DP>10), suggesting that the stoichiometry of the carbohydrates reactions during fermentation depends on several factors, among which are their structure, chemical composition, chain length, and microbiota in different animal species.

Different experiments have shown that the inclusion of fructans as prebiotics have positive effects on the physiology and productive performance of animals. Halas *et al.*[21] found changes on the bacterial metabolic activity in pigs. Other investigations have reported diminishing cecal pH and increase short chain fatty acids concentrations, mainly butyric acid in ducks[22] and also, diminishing mortality and increase of short chain fatty acids with lower cecal pH in rabbits.[23]

Short chain fatty acids play essential roles in the growth and physiology of intestinal tissue as well as in systemic metabolism.[24] Acetate is an important energy source for the body and is metabolized by the skeletal muscle, the heart, and the brain.[25] Propionate stimulates proliferation of normal crypt cells[26] and acts as a regulation of cholesterol metabolism (reducing hepatic cholesterol).[27] Finally, butyrate is the main source of energy for the intestinal epithelium and regulates cell growth and differentiation.[28]

Our results suggest that butyric acid is highly correlated with glucose serum. In the present work, the group of mice that consumed agave fructans (AF-C) was the most efficient to decrease glucose serum and increase butyric acid concentration. Bacterial fermentation yielding short chain fatty acids, mainly butyrate, are believed to be responsible for increases in the glucose-regulating and satiety-inducing hormone glucagon-like peptide-1 (GLP-1) observed in prebiotic-fed animals.[29] Oligofructose given to rats at a dose of 10% for 30 d reduces postprandial glycemia and insulinemia by 7% and 26%, respectively.[30] The supplementation with agave fructans (TEQ and DAS) in mice induced a higher excretion of GLP-1 and its precursor, proglucagon mRNA, in the different colonic segments, thus suggesting that fructans are able to promote the production of satietogenic/incretin peptides in the lower part of the gut, with promising effects on glucose metabolism.[31]

The hypotriglyceridemic and hypocholesterolemic effects of prebiotics have been described both in humans[5] and in animals.[32,33] The mechanism of these carbohydrates on the serum-lipid lowering effect remains to be elucidated. However, in the present study it was demonstrated that only the mice group fed with fructans (AF-C) decreased serum triglyceride and cholesterol, these responses may result from the structural difference between agave fructans with respect to chicory inulin. The mechanism by which these non-digestible nutrients modify the lipid metabolism remains also to be clarified. However, our results coincide with those of Urías-Silvas *et al.*[31], who also reported a significant decrease in serum cholesterol level with a significant decrease in liver cholesterol for *Agave tequilana* fructans of mice. Feeding rats a diet supplemented with 10% oligofructose, significantly lowered triacylglycerol and phospholipid serum concentrations.[7] Janssens and Van Loo[6] found in laying hens a hardness increment of the eggshell and a decrement of the yolk cholesterol.

Researchers have demonstrated the hypothesis that a decreased in *de novo* lipogenesis in the liver, through a corresponding reduction of the activity of all lipogenic enzymes, is a key event in the reduction of VLDL-triglyceride secretion in fructan-fed rats.[34] In a previous report, it was considered that propionate, which is largely produced through the fermentation of all tested fructans, has been shown to decrease cholesterol synthesis in different models[2], evidences suggest it may result from propionate-induced inhibition of hepatic cholesterol synthesis.[35]

Calcium and magnesium concentrations in the mice femurs were increased in both groups that were fed with diets supplemented with fructans. The increase in the bioavailability of minerals, due to the administration of prebiotics is mainly attributed to the high production of SCFAs resulting in a decrease in luminal pH and in an increase on the concentration of ionized minerals in the large intestine. Consequently, increasing the solubility, as well as active and passive diffusion of minerals through the intestinal cells.[36,37]

Studies of García-Vieyra *et al.*[12] showed that mice fed agave fructans absorbed more calcium from food, excreted less calcium in their feces, and showed a 50% increase in levels of a protein (osteocalcin) associated with the build-up of new bone tissue. These results suggest that the supplementation of the standard diet with agave fructans prevented/restored bone loss and improved bone formation, indicating the important role of agave fructans on the maintenance of healthy bone.

Conclusions

In conclusion, the inclusion of fructans from *Agave fourcroydes* in the diet of mice produced a prebiotic response similar to or greater than the commercial product (Raftilose P95), related to fermentation and morphometric indicators in the cecum and colon of mice, as well as reduction of serum glucose, triglycerides and cholesterol and increased mineral concentrations of mice femurs. Fructans of *Agave fourcroydes* constitutes a promising alternative as prebiotic with potentialities to be used in animals and humans feeding.

Abbreviations

SCFAs	Short chain fatty acids
STD	Standard diet
RSE	Raftilose P95
AF-C	*Agave fourcroydes* fructans from Cuba

TAG	Triglycerides
GLP-1	Glucagon-like peptide-1
TEQ	*Agave tequilana* fructans
DAS	*Dasylirion* fructans
VLDL	Very-low-density lipoprotein

Conflict of interest

The authors declare no conflict of interest.

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Table 1 Body weight, food intake, total and wall relative weights of cecum and colon of mice fed a diet fructans supplementation (Raftilose P95 (RSE) and *Agave fourcroydes* (AF-C)) at the end of the experimental period

Diets	Total weight cecum	Wall weight cecum	Total weight colon	Wall weight colon
STD	2.26[a]	0.50[a]	2.14[a]	1.03[a]
RSE	2.71[b]	0.60[b]	2.37[b]	1.13[b]
AF-C	2.70[b]	0.60[b]	2.36[b]	1.13[b]
Pooled SEM	0.21	0.05	0.28	0.02
P-value	**	**	**	**

Probability of significance: **P<0.01. Values followed by a different superscript in each row are significantly different (P<0.05).

433 **Table 2** Cecum and colon concentrations of short chain fatty acids (SCFAs) of mice fed a diet fructans supplementation (Raftilose P95 (RSE) and *Agave fourcroydes* (AF-C)) at the end of the experimental period

Treatment	SCFAs (mmol/l)	Acetic (mmol/l)	Propionic (mmol/l)	Butyric (mmol/l)
		CECUM		
STD	21.67[a]	17.05[a]	2.17[a]	1.05[a]
RSE	26.43[b]	20.50 [b]	3.03[b]	1.50[b]
AF-C	26.76[b]	20.36 [b]	3.14[b]	1.86[c]
Pooled SEM	0.30	0.21	0.13	0.07
P-value	***	***	***	***
		COLON		
STD	12.30[a]	9.51[a]	1.10[a]	0.59[a]
RSE	14.74[b]	12.12[b]	1.48[b]	0.74[b]
AF-C	14.81[b]	12.05[b]	1.56[b]	0.87[c]
Pooled SEM	0.20	0.15	0.09	0.02
P-value	***	***	***	***

Probability of significance: ***P<0.001. Values followed by a different superscript in each row are significantly different (*P*<0.05).

Table 3 Effect of diet fructans supplementation (Raftilose P95 (RSE) and *Agave fourcroydes* (AF-C)) on serum glucose, triglycerides, and cholesterol from mice tails

Diets	Glucose (mmol/l)	Triglycerides (nmol/µl)	Cholesterol (µg/µl)
STD	7.77[a]	1.21[b]	1.11[a]
RSE	6.15[b]	1.08[b]	0.94[ab]
AF-C	5.68[c]	0.67[a]	0.85[b]
Pooled SEM	0.11	0.06	0.07
P-value	**	**	**

Probability of significance: **P<0.01. Values followed by a different superscript in each row are significantly different (*P<0.05*)

Figure legends

Fig. 1 pH values of cecum and colon contents of mice fed a diet fructans supplementation (Raftilose P95 (RSE) and *Agave fourcroydes* (AF-C)) at the end of the experimental period. Probability of significance: ***P<0.001. Bars represent the mean values, error bars represents standard deviation. Bars with different letters within the same bread type are significantly different (*P*<0.05).

Fig. 2 Calcium concentrations of mice femurs fed a diet fructans supplementation (Raftilose P95 (RSE) and *Agave fourcroydes* (AF-C)) at the end of the experimental period. Probability of significance: **P<0.01.Bars represent the mean values, error bars represents standard deviation. Bars with different letters are significantly different (*P*<0.05).

Fig. 3 Magnesium concentrations of mice femurs fed a diet fructans supplementation (Raftilose P95 (RSE) and *Agave fourcroydes* (AF-C)) at the end of the experimental period. Probability of significance: *P<0.05. Bars represent the mean values, error bars represents standard deviation. Bars with different letters are significantly different (*P*<0.05).

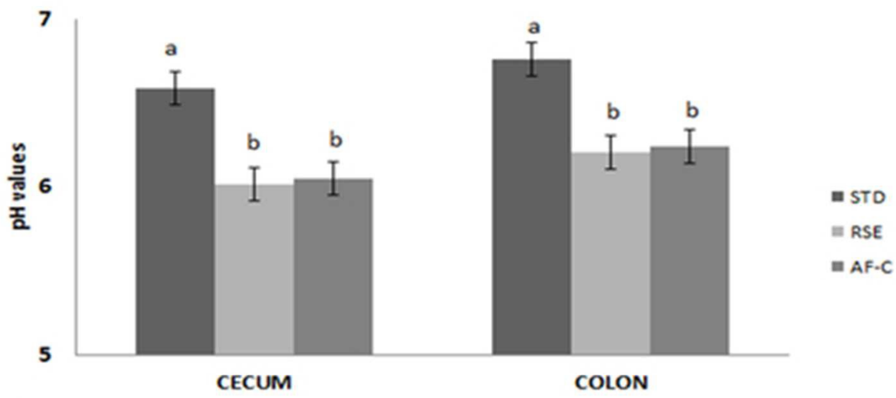


Fig. 1

39x19mm (300 x 300 DPI)

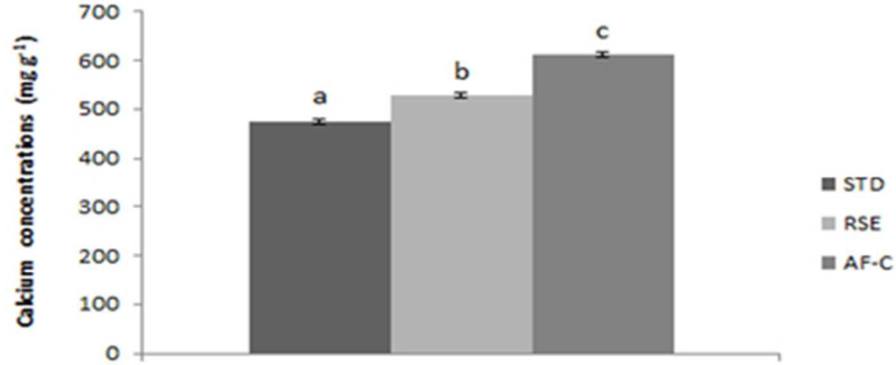


Fig. 2

39x19mm (300 x 300 DPI)

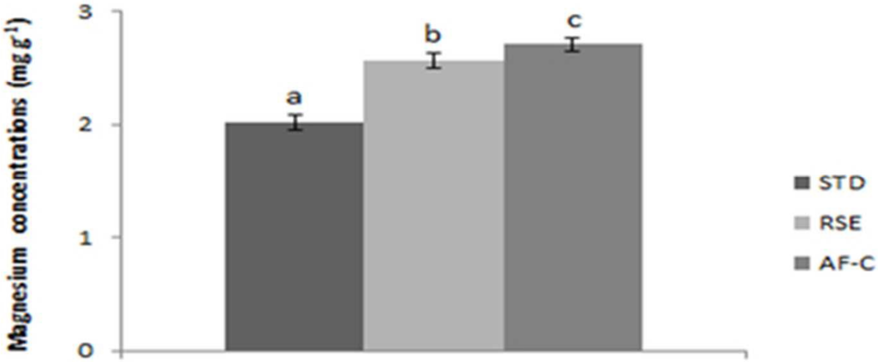
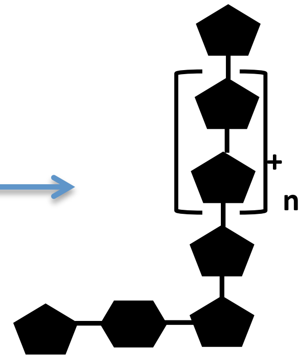


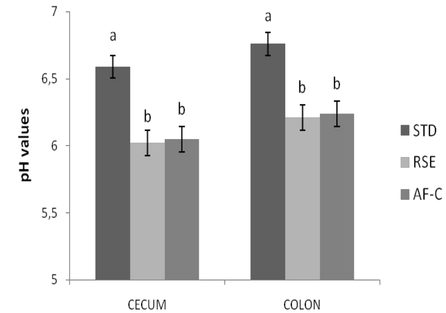
Fig. 3

39x19mm (300 x 300 DPI)

***Agave fourcroydes* from Cuba**



DP < 10



Prebiotic Effect in Mice