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Prebiotic Activity of Xylooligosaccharides from Corncob

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Abstract: Prebiotics are substances that promote the growth of beneficial microorganisms (probiotics) inthe gut. Probiotics are microorganisms such as bacteria or yeast that is believe to improve health.The xylooligosaccharides (XOS) are a type of prebiotic substances, these compounds are oligomers formed by xylose units and can be obtained by enzymatic hydrolysis of xylan. In this work the xylan was obtained through thermochemical pretreatment of the corncob, which is a by-product from corn processing. To analyse the prebiotic activity of XOS obtained from corncob and reagent grade xylan, they were tested in *L. brevis, L. plantarum, L. acidophilus, L. rhamnosus* cultures, and in a co-culture with *Escherichia coli* as a challenge microorganism to prove the bacteriostatic activity of lactobacilli strains.Xylooligosaccharides stimulated *L. brevis* and *L. plantarum* growth: these microorganisms grew faster than the other lactobacilli strains. *L. acidophilus* grew better in the presence of XOS and maintained the absorbance of the culture.

In the co-culture in presence of both XOS the challenge microorganism did not grow; lactobacilli colonies appeared in MRS agar. No colonies of *E. coli* grew in EMB plaques.

Key Words: *Prebiotic, Xylan, Xylooligosaccharides, Corncob, Lactobacilli*

INTRODUCTION

The concept of functional food is defined as any food or ingredient that may provide a health benefit beyond the traditional functions. Number of products can be defined as healthier and have functional and health properties, and these days have gained prominence in research, for the development of new products [(1)].

The biological and clinical importance of resident gastrointestinal microbiota drawn attention to health workers, because many disease states involve bacterial metabolism and human gut microbiota may also be considered as a host health. Bifidobacteria and lactobacilli (probiotcs) may help to prevent infections, reduce cholesterol levels and even can help to response and syntheses vitamins [(2)].Prebiotics can be defined as non-

digestible food ingredients, but they stimulate the growth and activity of a limited number of non-pathogenic bacteria in the microbiota with health promoting potential [1].

Some examples of prebiotics include fructooligosaccharides, galactooligosaccharides, arabinose, galactose, inulin, raffinose, mannose, lactulose, stachyose, mannanoligosaccharides, xylooligosaccharides, palatinose, lactosucrose, etc, [3, 4, 5].Xylooligosaccharides (XOS) are sugar oligomers made up of xylose units that can appear naturally in vegetables, milk and honey. In the literature there is a vast number of researches about xylooligosaccharides in feedstock like hardwoods, softwoods, barley hulls and barley spent grains, brewery spent grains, almond shells, corn fiber and rice hulls [6]. XOS are obtained by acid or enzymatic hydrolysis of xylan.In this work xylan is obtained from the corncob, which is a by-product from corn processing it has potential applications in food and pharmaceutical industries, because they have immunomodulatory, anti-cancerous and anti-microbial activities, also they stimulate the intestinal mineral absorption and are mildly laxative [7].Xylan is hydrolysed to xylose that is a carbon source for the ethanol and xylitol fermentations [8], enzymatic hydrolysis is controlled in order to produce xylooligosaccharides, which contained a few units of xylose. The combination of XOS and *Lactobacilli* might formulate strain-specific symbiotic product with selective properties on desired probiotics. The objective of this research is to analyse the prebiotic activity of XOS obtained from corncob in four strains from *Lactobacillus* and bacteriostatic activity against *Escherichia coli.*

METHODS

The XOS used in this work where obtained from the corncob, this was submitted to thermochemical treatments for the obtention of xylan. In first place the corncob was grinded to obtain particles no bigger than 2 mm of diameter, then is thermochemically treated to degrade around 27 % of the material, the fraction that was degraded is compounded of hemicellulose and acid soluble lignin. The other 73 % was used to obtained lignin and xylan; delignification was carried out using hydrogen peroxide. After the reaction the soluble xylan was precipitated to split the lignin, the leftover of the liquor is reduced in order to recover the xylan precipitating with methanol. At last the xylan was centrifuged from the methanol solution and then dryed at a temperature superior to the boiling point of methanol to eliminate the solvent completely.

Reagent grade xylan (Sigma Aldrich) was used as a control. Both type of xylan were hydrolysed with xylanase (DT enzyme, Dechema), at 40ºC, with Citrate 0.05M buffer for 72 hours at 150 rpm.

At the end of the hydrolysis the XOS are submitted to a process of semi-purification, by a spin-dry to separate the insoluble solids.

The hydrolysis solution was analysed by high pressure liquid chromatography, using a HPX-87H column, at 65 C with sulphuric acid 50 mM as eluent at 0.6 mL/min, with a HPLC Agilent (model 45) in the IR detector. The XOS solution was also analysed by infrared spectrometry.

The molecular weight was determined with a HPLC Waters 2695 equipped with two modules, the first one is a refracted index Waters 2414 and the other module of warming Waters that heats from 30 to 80ºC. using a PL aquagel-OH mixed column, 8 m, 7.5 x 300 mm, at 40ºC and water grade HPLC as mobile phase at 0.7 mL/min flow. The standards used as a calibration curve were ghati rubber (P.M. 12000 Da), raffinose (P.M. 504.5 Da), cellobiose (P.M. 342.3 Da), glucose (P.M. 180.16) and xylose (P.M. 150.13 Da).

Microorganisms used in this work were: *L. brevis, L. rhamnosus, L. plantarum and L. acidophilus.* The prebiotic activity was determined culturing the lactobacilli with and without XOS in MRS broth (Difco) at

37ºC, for 48 hrs and measuring the optical density at 24 and 48 hrs at 620 nm in a Spectrophotometer Spectronic Genesys [10].

To prove the bacteriostatic activity, *Lactobacilli* were co-culture with *Escherichia coli,* at 37ºC for of 24 h. The colony forming units (CFU) were determined in Eosin-Methylene-Blue Agar (Bioxon) and MRS agar (Difco). All the microbiological tests were made with corn cob and reagent grade xylan.

RESULTS

Table 1 shows characteristics of hydrolysed xylan; the molecular weight decreases from 1077-1080 Da to 508-539 Da, according with this results the oligomers contains between two and three xylose units. Polydispersity also diminishes from an interval of 13.34-11.04 to 7.05-7.71, then molecular weight distribution is narrower than for the non-hydrolysed xylan. MP value indicates that most of oligomers are formed by two xylose units.

Table 1: Molecular weight and polidispersity of whole and hydrolysed xylan

Sample	Mn	Mw	MP	Mz	Mz + 1	Polydispersity
Xylan	81 - 98	1077 - 1080	273	6705 - 6647	9755 - 9747	13.34 - 11.04
Hydrolysed xylan	72 - 70	508 - 539	271 - 223	4693 - 5545	9345 - 9652	7.05 - 7.71

When the microorganisms were culture with and without XOS, different behaviors was observe for each strain. **Fig.1** depicts the lactobacilli growth at 24 and 48 h, with and without XOS as in *L. brevis* grew in both media but at h 48 optical density had diminished; apparently XOS maintain the culture alive, since the difference between optical density at 24 and 48 h is more significant without XOS. For *L. plantarum* there is no apparent effect in microbial growth with XOS addition to MRS broth, neither 24 nor 48 h. *L. rhamnosus* apparently conserve their metabolic activity at 48 hours of cultivation, is the only microorganism that continues growing in the presence of prebiotic. *L. acidophilus* with XOS grew better than the culture with MRS only, this microorganism shows a different behaviour, with a significate difference in optical density at 24 h between both media culture.

To observe the effect of the lactobacilli and the prebiotics against *E. coli*, *L. brevis, L. plantarum, L. acidophilus,* and *L. rhamnosus* were co-cultured with and the challenge microorganism. Fig. 2 depicts the effect in the co-culture growth of XOS, in this case there is a slight difference between the co-cultures which were supplemented with XOS from the reagent grade xylan and from the corncob, however the current price of the reagent grade xylan is $306.46 for 100 g dollars while the price of xylan from corncobs is $7.60 dollars for 100 g.

The growth of *Escherichia coli* was compared in two different media cultures in order to analyze the bacteriostatic activity of *Lactobacilli* and the effect of XOS. The **Fig. 2** shows the increase of optical density at 24 h of incubation. It can be noted that, regardless of the precedence of XOS, the growth of co-culture is similar in MRS broth with and without prebiotic. But an important aspect to consider is that *E. Coli* did not

grow neither EMB or MRS agar, when serial dilutions of culture broth were plated in these media in contrast to control conditions, without lactobacilli.

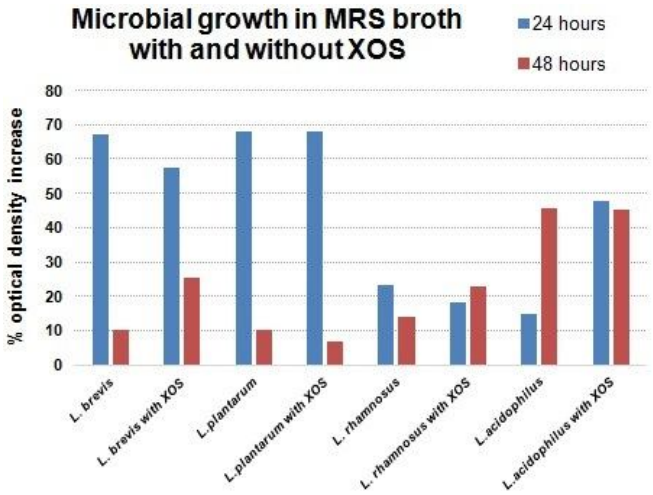

Fig.1: Comparison of Microbial growth in MRS broth with and without XOS.

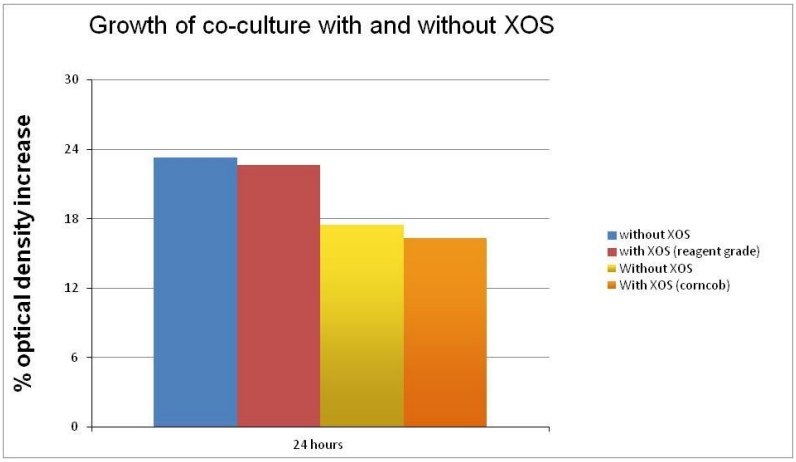

Fig. 2: Comparison of co-culture growth, with and without reagent grade and corncob XOS.

CONCLUSION

Enzymatic hydrolysis conditions of xylan allow obtaining a mixture of low molecular weight oligomers, with dimer predominance. The solution of xylooligosaccharides was analyzed by HPLC and IR.Among the tested species of lactobacilli the growth of *L. acidophillus* in the presence of xylooligosaccharides was the most

notable effect. For *L. rhamnosus* the population remains active at 24 and 48 h when XOS are added to media culture. The other strains do not modify their behaviour with supplementation of prebiotics.

The co-culture of *L. brevis, L. plantarum, L. acidophilus, L. rhamnosus* and *Escherichia coli,* shows that the growth of the challenge microorganism was inhibited, the same result was found with the XOS obtained from the reagent grade xylan or from the corncob. The XOS can be important in the food industry either as an ingredient for probiotic nutrition or, in the bio-refinery process, as a value added product using corncob which is a high volume residue from the corn, the main food in the Mexican diet.

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