BIOREFINERY-DERIVED XYLOOLIGOSACCHARIDES: STRUCTURAL INFLUENCE ON PREBIOTIC POTENTIAL IN FECAL FERMENTATION

Wilian F. Marcondes[1], Samira Prado[2], Tatiana M. Marques[2], Robert Brummer[2], Valdeir Arantes[1]

[1] *Lorena School of Engineering, University of São Paulo (EEL/USP). São Paulo Brazil*
[2] *School of Medical Sciences, Faculty of Medicine and Health, Örebro University, Örebro, Sweden.*
** Corresponding author's email address: wilian.fm@usp.br*

ABSTRACT

The technical and economic feasibility of using plant biomass to produce large-scale biofuels relies on improving process steps and obtaining high-value-added products. This study aimed to valorize the hemicellulosic fraction of sugarcane bagasse to obtain Xylooligosaccharides (XOS-SCB), a prebiotic compounds. The process for obtaining XOS was designed within the biorefinery concept, fully utilizing the sugarcane bagasse to generate nanolignin, nanocellulose, and ethanol. The study included purification and assessment of prebiotic properties through in vitro human fecal fermentation tests. Different process conditions can yield XOS with shorter (XOS-short) or longer chains (XOS-long), potentially affecting prebiotic properties. To elucidate this influence, the extracted XOS-SCB was purified and fractionated into long and short chains. These XOS samples, along with the XOS-SCB and commercial XOS, were subjected to in vitro human fecal fermentation. The results indicated that the degree of polymerization of XOS did not significantly influence bacterial metabolism, with individual differences among donors being more relevant. Cytotoxicity analysis revealed that XOS-Long and XOS-SCB fermentation products adversely affected cell viability, while all XOS from sugarcane bagasse exhibited protective action against cell wall damage. These findings demonstrate that it is possible to obtain XOS as efficient as commercial variants within a biorefinery concept, adding significant value to the process.

Keywords: Xylooligosaccharides - XOS. Prebiotic. Human fecal fermentation. Biorefinery.

1 INTRODUCTION

The recalcitrance of lignocellulose poses a significant challenge in the cost-effective processing of cellulosic biomass for biofuels like ethanol. This limitation has spurred interest in the biorefinery concept, where co-products are produced alongside ethanol to enhance process economics and environmental sustainability. Sugarcane bagasse, abundant and rich in xylan, offers a potential source for xylose and xylo-oligosaccharides (XOS). XOS, derived from xylan, find applications in diverse industries including paper, biofilms, emulsifiers, and increasingly as food additives due to their prebiotic properties, supporting gut health by selectively promoting beneficial bacteria growth. The structural differences of XOS, particularly their degree of polymerization (DP), may significantly impact their prebiotic efficacy. Gel permeation chromatography, despite its cost limitations for large-scale production, remains pivotal in fractionating XOS based on DP. This study focuses on assessing the prebiotic potential of XOS obtained from sugarcane bagasse, emphasizing the influence of DP on human fecal fermentation—an approach crucial for understanding their application as functional dietary fibers.

MATERIAL & METHODS

2.1 Obtaining XOS and its purification

The XOS used were from a commercial source (PrecticX 95P) and from sugarcane bagasse (XOS-SCB) as reported in previous work (Pereira et al., 2021). Briefly, the reactor was simultaneously fed with bagasse at 11.7 kg/h and saturated steam at 25-30 kg/h at 15 bar (approximately 190 °C) for 15 minutes, then depressurized and automatically discharged. To extract the XOS produced during steam explosion, the steam-exploded bagasse was added to a tumbling reactor at 20% w/v for 30 minutes at 30°C. The mixture was vacuum filtered using a 25-mesh membrane, and the filtrate, referred to as SCB-XOS hydrolysate, was collected. The solid fraction, mainly containing cellulose and lignin, was collected for further processing to obtain nanolignin and nanocellulose/ethanol, which are beyond the scope of this work.

The SCB-XOS was subjected to purification and fractionation. Initially, the hydrolysate was filtered and concentrated. The concentrated solution was then applied to a chromatographic column permeated with Bio-Gel P-2. This process yielded two distinct fractions: one enriched with shorter XOS chains (XOS-short) and another with longer chains (XOS-long). These fractions were collected separately and analyzed for their prebiotic properties.

2.2 Fecal fermentation

Three biological triplicates were performed using fecal samples from three healthy participants, collected with ethics committee approval (Dnr 2022-01696-01). Participants had not used antibiotics or fiber supplements in the previous six months. Fresh fecal samples were collected and kept anaerobically using an anaerobic punch until homogenized in sterile 50 mM PBS at 200 g/L. The slurry was prepared just before inoculation in the fermenter.

The substrates (1% w/w) and 100 mL of the basal medium, pre-bubbled with sterile nitrogen, were mixed in an anaerobic chamber (37°C; 10% H_2, 80% N_2, 10% CO_2) and inoculated with 10% v/v fecal slurry, reaching a final fecal concentration of 2% w/v. Fermentation was conducted in a Gas Endeavor system using 500 mL bottles for 24 hours at 100 rpm. Samples were collected at 0, 4, 8, and 24 hours, centrifuged, and both supernatant and pellet were stored at -80°C until further use.

2.3 Metabolite and Cytotoxicity Analysis

Ammonia was analyzed using a Megazyme Ammonia Assay Kit, following the manufacturer's protocol. Caco-2 cells were cultured according to ATCC guidelines and exposed to filtered fermentation supernatants for cytotoxicity assays using resazurin and LDH assays. Cell viability was assessed through fluorescence and absorbance measurements, comparing treated samples to controls.

2 RESULTS & DISCUSSION

Even though the applied hydrothermal treatment has not been optimized for selective obtain XOS from the SCB, but rather to benefit cellulosic ethanol production (Rocha et al., 2012), it was possible to obtain a XOS-rich hydrolysate with a ratio of oligomers to xylose (selectivity) of ~ 2:1 (Table 1). In general, this SCB-XOS has a composition similar to that of the commercial XOS, however, for SCB-XOS it is worth highlighting the higher concentration of xylobiose and xylotriose and practically no oligomers with size greater than six xylose units (Table 1). Unlike commercial. In addition, the concentration of glucose oligomers and monomers derived from cellulose hydrolysis were very low.

Xylan's components	**XOS (% w/w)**			
	XOS commercial	XOS-SCB	XOS-Long	XOS-Short
Xylose	0,05	39,10	0,02	6,74
Total XOS	99,05	60,90	99,98	93,26
Xylobiose	31,47	30,37	9,08	72,63
Xylotriose	25,16	24,03	32,61	24,94
Xylotriose	19,47	20,58	32,28	2,42
Xylopentose	10,63	16,14	18,02	0,00
Xyloexose	13,26	8,88	8,01	0,00

Table 1. Degree of polymerization of XOS extracted from SCB before and after fractionation process by gel permeation chromatography. Xylobiose, Xylotriose, Xylotetraose, Xylopentaose and Xylohexaose are expressed in relation to Total XOS

The fractionation technique by gel permeation chromatography exploits the difference among the sizes of the molecules of the components to be fractionated. To obtain purified XOS, both short and long chain fractions, a Bio-Gel P-2 chromatographic column was employed. The eluted fractions containing short and long XOS showed much higher purity than XOS-SCB relative to XOS than xylose (Table 1). This is because the fraction containing monomers was easily discarded and only fractions containing XOS was grouped. XOS-short fraction was mostly composed of xylobiose and xylotriose while the XOS-long fraction was poor in xylobiose and had more XOS with DP up to 6 xylose units. This higher purity allows to better study the influence of the XOS DP as a prebiotic, since there is no interference of monomers in the probiotic fermentation.

Together, these results show the potential of XOS fractionation using Bio-gel P-2 as a chromatographic column. Although it is known that the fractionation process could be improved, this would require a detailed study of the experimental conditions (i.e., flow rate, temperature, fraction collect time and volume of samples). Other compounds present in XOS-SCB, such as degradation compounds (i.e., furfural and HMF) or lignin (polyphenols) were not quantified in this work but may still be present in these XOS fractions.

Ammonia is a metabolite released mainly by the fermentation of proteins and their derivatives in the fecal microbiota and the process of deamination (Diether & Willing, 2019; Tomé, 2021). From **Figure** 1, it is possible to notice that the highest values of ammonia accumulation occur in the control, a culture medium that does not contain any carbohydrate. This is due the fermentation of the protein itself that makes up the culture medium. In culture media containing XOS, the accumulation of ammonia is lower because there is a greater microbial growth due to the use of XOS as a carbon source, evidenced by the decrease in pH (figure 2. This results in a greater need for nitrogen sources for the growth of bacteria, being the ammonia an important source of nitrogen for microorganisms (Tomé, 2021). As the pH values, the release of ammonia in the medium does not present a significant difference between the different carbon sources but it is more influenced by the individuality of the donors.

A greater diversity in the DP of XOS may provide better growth of bifidobacteria in relation to the XOS with a lower variety of DP, which indicates that a mixture of XOS with different DP can be more effective than a mixture of defined DP (Mäkeläinen et al., 2010). Other work indicates that XOS with DP_{2-3} have better prebiotic properties (Moura et al., 2007). However, our study showed that there was no significant difference in prebiotic property between short-chain XOS (Xlobiose-xylotriose) and Xos-Long (xylotriose-xylohexaose).

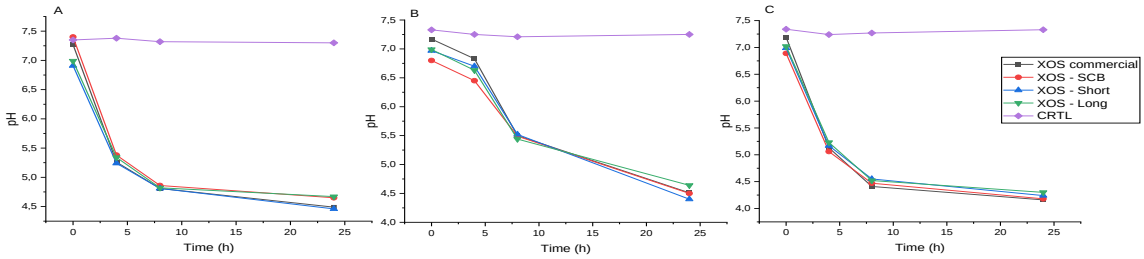

Figure 1 Ammonia concentration profile during fecal fermentation with different donors (A, B and C).

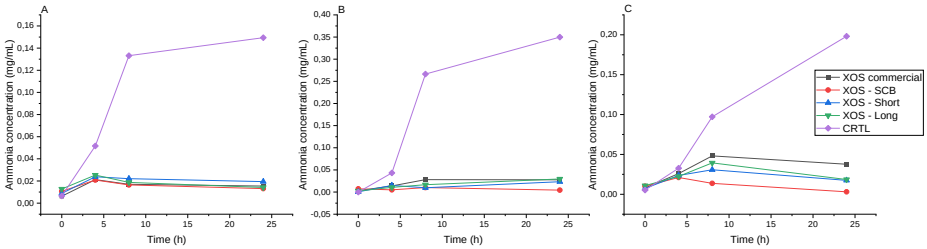

Figure 2 pH profile during fecal fermentation with different donors (A, B and C)

The XOS fractions before fermentation did not show significant differences in relation to cell viability, except for XOS-long that had a negative impact. When there was fermentation, only the experiment with XOS-SCB had a slight negative impact on cell viability, while the other sources of XOS from sugarcane bagasse had a slightly positive impact. In the LDH assay, all hydrolysates containing XOS from sugarcane bagasse, before and after fermentation, had a positive impact on the test. That is, they had lower relative amounts of LDH released in the medium. Together, the results of both tests indicate that XOS from sugarcane bagasse, either by its DP or by the presence of other compounds in the hydrolysate, has a cell wall protection action. However, the cell viability assay points to a lower cell viability when it comes from XOS-Long or the product of fermentation using XOS-SCB, which may represent that the decrease in this cell viability is due to factors other than cell wall damage

3 CONCLUSION

Gel permeation chromatography effectively separated xylan derivatives, facilitating the isolation of two distinct groups of XOS: short-chain (XOS-short) and long-chain (XOS-long). When tested in human fecal fermentation, both XOS-SCB and these fractions exhibited similar fermentation profiles in terms of pH and ammonia levels, indicating comparable metabolic responses. However, XOS fractions derived from sugarcane bagasse demonstrated notable benefits in protecting cell wall integrity. They highlight that XOS from sugarcane bagasse, irrespective of chain length, offer significant protective effects on cell viability, positioning them as valuable components in prebiotic applications. This research suggests that integrating XOS obtain within biorefinery processes not only enhances sustainability by utilizing waste biomass but also contributes to the development of functional food ingredients with health-promoting properties.

REFERENCES

Diether, N. E., & Willing, B. P. (2019). Microbial fermentation of dietary protein: An important factor in diet–microbe–host interaction. *Microorganisms*, *7*(1). https://doi.org/10.3390/MICROORGANISMS7010019

Mäkeläinen, H., Forssten, S., Saarinen, M., Stowell, J., Rautonen, N., & Ouwehand, A. (2010). Xylo-oligosaccharides enhance the growth of bifidobacteria and Bifidobacterium lactis in a simulated colon model. *Beneficial Microbes*, *1*(1), 81–91. https://doi.org/10.3920/BM2009.0025

Moura, P., Barata, R., Carvalheiro, F., Gírio, F., Loureiro-Dias, M. C., & Esteves, M. P. (2007). In vitro fermentation of xylo-oligosaccharides from corn cobs autohydrolysis by Bifidobacterium and Lactobacillus strains. *LWT - Food Science and Technology*, *40*(6), 963–972. https://doi.org/10.1016/j.lwt.2006.07.013

Pereira, B., Marcondes, W. F., Carvalho, W., & Arantes, V. (2021). High yield biorefinery products from sugarcane bagasse: Prebiotic xylooligosaccharides, cellulosic ethanol, cellulose nanofibrils and lignin nanoparticles. *Bioresource Technology*, *342*. https://doi.org/10.1016/j.biortech.2021.125970

Rocha, G. J. M., Gonçalves, A. R., Oliveira, B. R., Olivares, E. G., & Rossell, C. E. v. (2012). Steam explosion pretreatment reproduction and alkaline delignification reactions performed on a pilot scale with sugarcane bagasse for bioethanol production. *Industrial Crops and Products*, *35*(1), 274–279. https://doi.org/10.1016/j.indcrop.2011.07.010

Tomé, D. (2021). Protein digestion and bioavailability. In *Reference Module in Food Science*. Elsevier. https://doi.org/10.1016/B978-0-12-821848-8.00034-2

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. This research used facilities of the National Bio-renewables Laboratory (LNBR), at the National Center for Research in Energy and Materials (CNPEM), a Social Organization overseen by the Ministry of Science, Technology and Innovation (MCTI). The authors would like to thank Barbara Pereira for helping during XOS extraction, Giulia Bevilacqua for her help during the laboratory work, Pauline Seeburger for her help during fermentation process and Dr Ignacio Rangel for kindly providing Caco-2 cells for the cytotoxicity assay.