RESSALVA

Atendendo solicitação da autora, o texto completo desta tese será disponibilizado somente a partir de 25/10/2025.

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (MICROBIOLOGIA APLICADA)

PRODUCTION OF XYLO-OLIGOSACCHARIDES BY DIFFERENT TREATMENTS OF BANANA PSEUDOSTEM AND THEIR PREBIOTIC EVALUATION VIA PROBIOTIC BACTERIA GROWTH AND THEIR METABOLITES

CAROLINE DE FREITAS

Rio Claro – SP
2024

UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (MICROBIOLOGIA APLICADA)

PRODUCTION OF XYLO-OLIGOSACCHARIDES BY DIFFERENT TREATMENTS OF BANANA PSEUDOSTEM AND THEIR PREBIOTIC EVALUATION VIA PROBIOTIC BACTERIA GROWTH AND THEIR METABOLITES

CAROLINE DE FREITAS

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como requisito para obtenção do título de Doutor em Ciências Biológicas (Microbiologia Aplicada).

Orientador: Prof. Dr. Michel Brienzo

Rio Claro - SP
2024

F866p

Freitas, Caroline de
Production of xylo-oligosaccharides by different treatments of banana pseudostem and their prebiotic evaluation via probiotic bacteria growth and their metabolites / Caroline de Freitas. -- Rio Claro, 2024
136 p. : tabs.

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências, Rio Claro
Orientador: Michel Brienzo

1. xylooligosaccharides. 2. enzymatic hydrolysis. 3. probiotics. 4. prebiotics. 5. metabolites. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto de Biociências, Rio Claro. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Rio Claro

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: PRODUCTION OF XYLO-OLIGOSACCHARIDES BY DIFFERENT TREATMENTS OF BANANA PSEUDOSTEM AND THEIR PREBIOTIC EVALUATION VIA PROBIOTIC BACTERIA GROWTH AND THEIR METABOLITES

AUTORA: CAROLINE DE FREITAS

ORIENTADOR: MICHEL BRIENZO

Aprovada como parte das exigências para obtenção do Título de Doutora em Ciências Biológicas (Microbiologia Aplicada), área: Microbiologia Aplicada pela Comissão Examinadora:

gov.br Documento assinado digitalmente
MICHEL BRIENZO
Data: 28/11/2023 09:54:36-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. MICHEL BRIENZO (Participaçao Virtual)
Instituto de Pesquisa em Bioenergia - IPBEN Rio Claro / UNESP

gov.br Documento assinado digitalmente
DERLENE ATTILI DE ANGELIS
Data: 06/11/2023 23:25:50-0300
Verifique em https://validar.iti.gov.br

Profa. Dra. DERLENE ATTILI DE ANGELIS (Participaçao Virtual)
Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas / Universidade Estadual de Campinas

Prof. Dr. PEDRO DE OLIVA NETO (Participaçao Virtual)
Departamento de Biotecnologia / Unesp - Faculdade de Ciencias e Letras

gov.br Documento assinado digitalmente
ADRIANE MARIA FERREIRA MILAGRES
Data: 31/10/2023 16:24:10-0300
Verifique em https://validar.iti.gov.br

Profa. Dra. ADRIANE MARIA FERREIRA MILAGRES (Participaçao Virtual)
Departamento de Biotecnologia / Universidade de São Paulo

Profa. Dra. SANDRA REGINA CECCATO ANTONINI (Participaçao Virtual)
Departamento de Tecnologia Agroindustrial e Sócio-Economia Rural-UFSCar / Universidade Federal de São Carlos

gov.br Documento assinado digitalmente
SANDRA REGINA CECCATO ANTONINI
Data: 01/11/2023 13:44:57-0300
Verifique em https://validar.iti.gov.br

Rio Claro, 25 de outubro de 2023

gov.br Documento assinado digitalmente
PEDRO DE OLIVA NETO
Data: 28/11/2023 07:42:42-0300
Verifique em https://validar.iti.gov.br

AGRADECIMENTOS

Primeiramente agradeço a Deus, por ter guiado e iluminado meu caminho até aqui, pela sabedoria para conduzir os experimentos e fé para superar os obstáculos. “Tu és o meu refúgio e a minha fortaleza, o meu Deus, em quem eu confio”. (Salmos 91:2)

Ao Prof. Dr. Michel Brienzo, pela orientação, paciência, atenção e auxílio no desenvolvimento deste trabalho.

Aos meus pais, Adriana e Luiz, pelo amor incondicional, carinho, dedicação, apoio, por tudo que vocês fizeram e ainda fazem por mim. Vocês são as pessoas mais importantes da minha vida, amo vocês demais.

Ao meu marido, Fernando, pelo apoio, paciência, carinho e amparo em todos os momentos, bons e ruins. Você é o amor da minha vida, te amo!

O presente trabalho foi realizado com apoio do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

RESUMO

Xilo-oligossacarídeos (XOS) são oligômeros derivados da xilana, uma hemicelulose encontrada em muitos tipos de plantas. Sua estrutura é composta por unidades de xilose ligadas por ligações β-1,4, tornando-os não digeríveis pelo sistema digestivo humano, mas fermentáveis por microrganismos probióticos presentes na flora intestinal. Os XOS podem ser produzidos por hidrólise ácida ou enzimática de biomassa proveniente de resíduos agroindustriais, como pseudocaule de banana, ou de xilana solubilizada desses resíduos. Nesse contexto, este estudo comparou a produção de XOS por esses diferentes métodos, analisou a estrutura da xilana após a hidrólise e a tolerância de microrganismos probióticos aos compostos utilizados e produzidos no processo de hidrólise ácida. Em seguida, o crescimento dos microrganismos foi avaliado na presença de diferentes concentrações de XOS e dos metabólitos produzidos por sua fermentação. Na hidrólise enzimática da biomassa, utilizando estratégias de deslignificação parcial e moagem, o rendimento de XOS aumentou em 47% em comparação com a biomassa que não passou por esses dois processos. Além disso, o peso molecular da xilana analisado após dois ciclos de hidrólise indica que o substrato complexo restante limitou a ação da enzima. A produção de XOS por hidrólise ácida diluída da biomassa do pseudocaule de banana também é uma alternativa, uma vez que a conversão foi boa e houve baixa produção de xilose e subprodutos de degradação do açúcar. O crescimento de bactérias probióticas foi influenciado por ácidos orgânicos, furfural e HMF em diferentes concentrações, também pelo pH resultante do meio. Quanto maior a concentração desses compostos, menor o crescimento de *Lactobacillus*; no entanto, as bifidobactérias utilizadas neste estudo foram mais resistentes. Concentrações de ácido acético, láctico e fórmico aumentaram, em alguns casos, após o crescimento das bifidobactérias, indicando que também produzem esses ácidos orgânicos. Geralmente, quanto maior a concentração de furfural e HMF, menor o crescimento. Para o *Lactobacillus*, concentrações de 10, 7,5 e 5 g/L de HMF e 10 g/L de furfural inibiram o crescimento. Todos os microrganismos testados cresceram quando cultivados usando XOS produzidos por hidrólise ácida como fonte de carbono. Para a maioria dos microrganismos, a concentração ótima de XOS para o crescimento foi de 20 g/L de fermentação. As concentrações de metabólitos produzidos variaram dependendo da fonte de carbono e do microrganismo utilizado; no entanto, o lactato e o acetato foram os mais produzidos.

Palavras-chave: xilooligossacarídeos, endoxilanase, ácidos orgânicos, subprodutos, probióticos, prebióticos

ABSTRACT

Xylooligosaccharides (XOS) are oligomers from xylan, a hemicellulose found in many types of plants. Their structure is composed of xylose units linked through β-1,4 bonds, which makes them non-digestible by the human digestive system but fermentable by probiotic microorganisms present in the gut flora. XOS can be produced by acid or enzymatic hydrolysis of biomass from agro-industrial residues, such as banana pseudostem, or from solubilized xylan from these residues. In this context, this study compared the production of XOS by these different methods, analyzed the xylan structure after hydrolysis, and the tolerance of probiotic microorganisms by compounds used and produced in the acid hydrolysis process. After that, the growth of the microorganisms was evaluated in the presence of different concentrations of XOS and the metabolites produced by its fermentation. In the enzymatic hydrolysis of biomass, using both strategies of partial delignification and milling, the XOS yield went up by 47% compared to the biomass that has not gone through these two processes. In addition, the xylan molecular weight analyzed after two hydrolysis cycles indicate that the remaining complex substrate limited enzyme action. The production of XOS by diluted acid hydrolysis of banana pseudostem biomass is also an alternative since the conversion was good and there was low production of xylose and sugar degradation by-products. Probiotic bacteria growth was influenced by organic acids, furfural, and HMF in different concentrations, also by the consequent pH of the medium. The higher the concentration of these compounds, the lower the *Lactobacillus* growth, however, the bifidobacteria used in this study were more resistant. Concentrations of acetic, lactic, and formic acid increase, in some cases, after bifidobacteria growth, indicating that they also produce these organic acids. Generally, the higher the concentration of furfural and HMF, lower the growth. For *Lactobacillus*, concentrations of 10, 7.5 and 5 g/L HMF and 10 g/L furfural inhibited growth. All tested microorganisms grew when cultured using XOS produced via acid hydrolysis as their carbon source. For most microorganisms, the optimal XOS concentration for growth was 20 g/L fermentation. Concentrations of metabolites produced varied depending on the carbon source and microorganism used, however, lactate and acetate were the most produced.

Keywords: xylooligosaccharides, endoxylanase, organic acids, by-products, probiotics, prebiotics

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

Xylooligosaccharides (XOS), oligomers composed of xylose units linked by β-1,4-glycosidic bonds, are reported to have several biological activities. They can present antioxidant activity, anti-inflammatory and immunomodulatory effects, among other properties, depending on their degree of polymerization that can vary from 2 to 10 (YAN et al., 2022c). XOS present properties of interest for the food industry, such as low-calorie sweetness, no off-taste, non-cariogenic, and stability over a wide range of pH and temperature (FREITAS; CARMONA; BRIENZO, 2019). Prebiotics are defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” (GIBSON et al., 2017a). XOS can promote the proliferation of beneficial bacteria, such as *Lactobacilli* and *Bifidobacteria*, which are able to ferment this oligomer and produce beneficial metabolites that can be absorbed by the host and confer a health effect.

Prebiotics are not absorbed by the human digestive system, they remain intact until reaching the colon, where probiotic bacteria will ferment them (FERNÁNDEZ et al., 2016). The major metabolic products produced after prebiotic fermentation by probiotic bacteria are short-chain fatty acids (SCFA), such as acetate, propionate, and butyrate. These compounds are essential for colonic mucosal health and are unavailable from the diet. They are related to bowel function, calcium absorption, and lipid metabolism (YAN et al., 2022a). The ratio of SCFAs produced can vary with the fermenter microorganism and the fiber source. Acetate suppresses appetite and enhances lipogenesis, while propionate suppresses cholesterol synthesis, and both of them share anti-inflammatory action. Butyrate has a unique role as the chief energy source for the colonic mucosa. The consumption of prebiotics is also related to decreased protein fermentation, which could lead to the formation of potentially harmful metabolites (O’KEEFE, 2016).

XOS production can contribute to a sustainable generation of products with value-added, since this process occurs mainly using inexpensive lignocellulosic residue. It can be carried out by several methods, diluted acid and enzymatic hydrolysis are examples. Diluted acid hydrolysis has the advantage of using the biomass, without the previous need for xylan solubilization. However, it can release monosaccharides and sugar degradation by-products, which can interfere in the XOS yield. Enzymatic hydrolysis has to be carried out after xylan solubilization and with a purified enzyme (1,4-β-endoxylanase) to obtain a better XOS concentration, however, it’s a much cleaner process with no by-products and xylose release.

Because of the current high demand for functional foods, the interest in prebiotics is growing. XOS show great promise, as they can be produced from agricultural industry residues. The Global Info Research, in 2018, projected a growth of 94 million USD in 2017 to 130 million USD in 2025 of the international market of XOS (PALANIAPPAN; ANTONY; EMMAMBUX, 2021a). However, XOS are still more expensive to produce compared to other prebiotics. Therefore, studies on the most feasible way to produce them still need to be developed. Furthermore, since the metabolites produced can vary depending on the microorganism and XOS source, studies in this area are important to better evaluate this process.

The present study compared three types of XOS production: enzymatic hydrolysis using solubilized xylan and biomass, and acid hydrolysis using biomass. In addition, the molecular mass and structure of xylan after hydrolysis were evaluated, along with the effect of a second hydrolysis cycle with the addition of a new enzyme load. Subsequently, the XOS generated through two distinct processes (enzymatic hydrolysis of xylan and acid hydrolysis of biomass) were subjected to evaluation as a carbon source for probiotic bacteria. The study further examined the growth of probiotics in the presence of potential inhibitors produced during acid hydrolysis and analyzed the resulting metabolites produced after fermentation of enzymatically produced XOS.

References

BHATIA, R. et al. Pilot-scale production of xylo-oligosaccharides and fermentable sugars from Miscanthus using steam explosion pretreatment. **Bioresource Technology**, v. 296, n. October 2019, p. 122285, 2020.

BYRNE, C. S. et al. The role of short chain fatty acids in appetite regulation and energy homeostasis. **International Journal of Obesity**, v. 39, n. 9, p. 1331–1338, 10 set. 2015.

CANO CARMONA, E. et al. **Purification and biochemical characterization of an endoxylanase from Aspergillus versicolorFEMS Microbiology Letters**. [s.l: s.n.].

CORIM MARIM, A. V.; GABARDO, S. **Xylooligosaccharides: prebiotic potential from agro-industrial residue, production strategies and prospects**. **Biocatalysis and Agricultural Biotechnology**Elsevier Ltd, , 1 out. 2021.

CUNNINGHAM, M. et al. **Shaping the Future of Probiotics and Prebiotics**. **Trends in Microbiology**Elsevier Ltd, , 1 ago. 2021.

DEN BESTEN, G. et al. **The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism**. **Journal of Lipid Research**, set. 2013.

DONG, C. DI et al. Bioprocess development for the production of xylooligosaccharide prebiotics from agro-industrial lignocellulosic waste. **Heliyon**, v. 9, n. 7, 1 jul. 2023.

GIBSON, G. R. et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. **Nature Reviews Gastroenterology and Hepatology.** Nature Publishing Group, , 1 ago. 2017.

GUPTA, M. et al. Bioprocess development for production of xylooligosaccharides prebiotics from sugarcane bagasse with high bioactivity potential. **Industrial Crops and Products**, v. 178, 1 abr. 2022.

HAMER, H. M. et al. Review article: The role of butyrate on colonic function. **Alimentary Pharmacology and Therapeutics**, jan. 2008.

ILIEV, I. et al. Metabolic profiling of xylooligosaccharides by lactobacilli. **Polymers**, v. 12, n. 10, p. 1–18, 1 out. 2020.

KOH, A. et al. From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. **Cell.** Cell Press, , 2 jun. 2016.

MACFARLANE, G. T.; MACFARLANE, S. Bacteria, colonic fermentation, and gastrointestinal health. **Journal of AOAC International**, v. 95, n. 1, p. 50–60, 2012.

MÄKELÄINEN, H. et al. Xylo-oligosaccharides enhance the growth of bifidobacteria and Bifidobacterium lactis in a simulated colon model. **Beneficial Microbes**, v. 1, n. 1, p. 81–91, 2010.

MAO, B. et al. Effects of different doses of fructooligosaccharides (FOS) on the composition of mice fecal microbiota, especially the bifidobacterium composition. **Nutrients**, v. 10, n. 8, 16 ago. 2018.

MARKOWIAK, P.; ŚLIZEWSKA, K. Effects of probiotics, prebiotics, and synbiotics on human health. **Nutrients.** MDPI AG, , 15 set. 2017.

MARKOWIAK-KOPEĆ, P.; ŚLIŻEWSKA, K. The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. **Nutrients** MDPI AG, , 1 abr. 2020.

MENSAH, R. Q. et al. Application of green produced xylooligosaccharides from sugarcane residues and their properties – Recent progress towards sustainability. **Bioresource Technology Reports.** Elsevier Ltd, , 1 set. 2023.

MOURA, P. et al. In vitro fermentation of xylo-oligosaccharides from corn cobs autohydrolysis by Bifidobacterium and Lactobacillus strains. **LWT**, v. 40, n. 6, p. 963–972, 2007.

NARISETTY, V. et al. Valorization of renewable resources to functional oligosaccharides: Recent trends and future prospective. **Bioresource Technology**Elsevier Ltd, , 1 fev. 2022.

PALFRAMAN, R. J.; GIBSON, G. R.; RASTALL, R. A. Carbohydrate Preferences of Bifidobacterium Species Isolated from the Human Gut. **Issues Intest. Microbiol**. [s.l: s.n.]. Disponível em: <www.caister.com/bacteria-plant>.

POKUSAEVA, K.; FITZGERALD, G. F.; VAN SINDEREN, D. **Carbohydrate metabolism in Bifidobacteria**. **Genes and Nutrition**, ago. 2011.

RATAJCZAK, W. et al. Immunomodulatory potential of gut microbiome-derived shortchain fatty acids (SCFAs). **Acta Biochimica Polonica**Acta Biochimica Polonica, , 2019.

RATNADEWI, A. A. I. et al. Lactobacillus casei fermentation towards xylooligosaccharide (XOS) obtained from coffee peel enzymatic hydrolysate. **Biocatalysis and Agricultural Biotechnology**, v. 23, 1 jan. 2020.

RENYE, J. A.; WHITE, A. K.; HOTCHKISS, A. T. Identification of lactobacillus strains capable of fermenting fructo-oligosaccharides and inulin†. **Microorganisms**, v. 9, n. 10, 1 out. 2021.

YAN, F. et al. Advances in xylooligosaccharides from grain byproducts: Extraction and prebiotic effects. **Grain and Oil Science and Technology.** KeAi Communications Co., , 1 jun. 2022.

CHAPTER 7: CONCLUSION

In order for the production of xylooligosaccharides to meet the high and growing demand in the prebiotic market, more studies need to be conducted on different production techniques. Furthermore, the use of these prebiotics, produced by different techniques, by probiotic microorganisms should also be studied in order to evaluate the best way for them to be introduced for human consumption.

XOS production by enzymatic hydrolysis of banana pseudostem biomass is improved by partial delignification, milling and using only endoxylanase. However, the concentration of XOS produced is higher if the hydrolysis is performed in previous solubilized xylan. The increase in xylan concentration led to a decrease in yield but an increase in XOS concentration. Interestingly, a higher reaction volume improved the conversion of xylan into XOS. A cycle of a second hydrolysis adding a new enzyme load to the remaining substrate has a low increment in XOS yield. The molecular weight and polydispersity of remaining xylan increased after the second hydrolysis compared to the first one, making enzyme action more difficult.

The production of XOS through diluted acid hydrolysis of banana pseudostem biomass can be an alternative since there is a good conversion and low production of xylose for optimized conditions. Furthermore, the probiotic microorganisms used were able to grow in the medium containing different concentrations of the by-products generated in this reaction (furfural and HMF) but for the *Lactobacillus*, the presence of the organic acids was inhibitory. However, the concentration of XOS produced via acid hydrolysis is also lower compared to the enzymatic hydrolysis of xylan. The study demonstrated that all evaluated microorganisms could thrive when XOS produced through acid hydrolysis was used as the carbon source in the culture medium. While most microorganisms were unaffected by the purification of XOS, the removal of extractives from biomass significantly enhanced their growth. This suggests that XOS produced through acid hydrolysis has the potential to be utilized as prebiotics to stimulate the growth of probiotic microorganisms.

Additionally, the growth and metabolic activities of microorganisms were significantly influenced by the type and concentration of carbon sources present in their environment. Glucose consistently emerged as the preferred and easily assimilated carbon source among all the microorganisms studied. However, their responses to other carbon sources such as xylose, XOS (xylooligosaccharides), and FOS (fructooligosaccharides) varied, suggesting specific preferences or efficiencies in metabolizing these alternative sources. Notably, some carbon sources resulted in delayed growth phases, while others exhibited potential inhibitory effects, particularly at higher concentrations.

For most of the microorganisms examined, the fermentation of XOS and xylose led to a higher production of short-chain fatty acids (including acetate, propionate, and butyrate) compared to glucose fermentation, which primarily resulted in higher production of lactate. This observation suggests that the XOS produced in this study possesses favorable prebiotic properties, as it promotes the production of beneficial short-chain fatty acids by the microorganisms.