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

**XYLOOLIGOSACCHARIDES PRODUCTION FROM FOOD AND
AGROINDUSTRIAL WASTE BY CHEMICAL AND ENZYMATIC HYDROLYSIS**

Beatriz Salustiano Pereira

Dissertação apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Mestre em Microbiologia Aplicada.

Rio Claro/SP - 2020

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TÍTULO DA DISSERTAÇÃO: XYLOOLIGOSACCHARIDES PRODUCTION FROM FOOD AND AGROINDUSTRIAL BY CHEMICAL AND ENZYMATIC HYDROLYSIS

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ABSTRACT

Food production is increasing in the world and consequently solid waste is generated. Moreover, a lack of proper food and waste management plan are growing problems in the food industry. This scenario shows that the optimal reuse of waste generated and the possibility of adding value to the use and obtaining products with higher value is essential. Four large production biomasses in Brazil (banana peels, guava and orange bagasse and restaurant residue) were chosen to evaluate the potential for value-added products generation through pre-treatments, being the conversion by autohydrolysis, acid and alkaline treatment, that present environmental and economic advantages. Treatments may be an alternative to produce xylooligosaccharides (XOS) by lignocellulosic biomass, a product of great commercial value in the food and pharmaceutical markets, mainly due to its prebiotic potential and other value-added products. With autohydrolysis, the maximum obtained was 32.28% of XOS and 5.07% of xylose with banana peel residue, 8.21% of XOS and 1.99% of xylose with guava bagasse, 68.53% XOS and 4.02% xylose with orange bagasse and 33.42% XOS and 2.56% xylose with restaurant residue. The results showed that the ideal conditions to obtain XOS are at 160 °C and 15 min for banana peel residue, at 172.43 °C and 35 min for guava bagasse, at 130 °C and 35 min for orange bagasse and at 130 °C and 6.72 min for restaurant waste. Pretreatment with dilute acid resulted in XOS production of 37.69% for banana peel, 59.60% for guava bagasse, 28.70% for orange bagasse and 49.64% for restaurant residue. The results showed that, for this type of pretreatment, the ideal conditions to produce XOS are at 160 °C, 15 min and 3% H₂SO₄, for guava bagasse at 100 °C, 15 min and 3% H₂SO₄, for orange bagasse at 160 °C, 15 min and 3% H₂SO₄ and for restaurant waste at 160 °C, 55 min and 3% H₂SO₄. For alkaline treatment, biomasses were subjected to three types of alkaline agents: hydrogen peroxide in alkaline medium, sodium hydroxide and potassium hydroxide, in order to solubilize xylan. Subsequently, the xylan was enzymatic hydrolyzed with endoglucanase purified from *Aspergillus versicolor* to obtain xylooligosaccharides. The maximum xylan solubilization with hydrogen peroxide was 90.70% with restaurant residue, for sodium hydroxide was 88.01% with guava bagasse and for potassium hydroxide was 74.20% with banana peel. After enzymatic hydrolysis, the maximum values for XOS production were of 54.14% with banana peel residue (peroxide solubilized), 59.86% with guava bagasse (sodium hydroxide solubilized), 50.42% for orange bagasse (peroxyde solubilized) and 50.80% with restaurant residue (potassium hydroxide solubilized). This study determined autohydrolysis, acid hydrolysis and enzymatic hydrolysis conditions that maximized XOS production with low amount of xylose production using agroindustrial and food waste.

Keywords: agroindustrial waste, fruit waste, xylan, alkaline extraction, autohydrolysis, acid hydrolysis, enzymatic hydrolysis, xylooligosaccharides, prebiotic effect.

RESUMO

A produção de alimentos no mundo é cada vez maior e, junto a isso, o desperdício, geração de resíduos sólidos e a falta de um plano adequado de gerenciamento causam crescentes problemas ambientais, sociais e econômicos, cenário que mostra que a reutilização ideal e a possibilidade de agregar valor à esses resíduos está se tornando cada vez mais essencial. Quatro biomassas de grande produção no Brasil (banana, goiaba, laranja e preparo de restaurante) tiveram seu resíduo escolhido para avaliar o potencial de geração de xilooligosacarídeos através de pré-tratamentos, sendo eles a conversão por auto-hidrólise, tratamento ácido e alcalino. A partir da auto-hidrólise dessas biomassas, o teor máximo de XOS foi de 32,28% com o resíduo de casca de banana, 8,21% com bagaço de goiaba, 68,53% com bagaço de laranja e 33,42% com resíduo de restaurante, mostrando que as condições ideais para obter XOS foram em 160 °C e 15 min para resíduos de casca de banana, em 172,43 °C e 35 min para bagaço de goiaba, em 130 °C e 35 min para o bagaço de laranja e para resíduos de restaurantes em 130 °C e 6,72 min. O pré-tratamento com ácido sulfúrico (H₂SO₄) diluído resultou na produção de XOS de 37,69% para casca de banana, 59,60% para bagaço de goiaba, 28,70% para bagaço de laranja e 49,64% para resíduos de restaurantes, que mostraram que, para este tipo de pré-tratamento, as condições ideais para produzir XOS são: 160 °C, 15 min e 3% H₂SO₄ para resíduo de casca de banana, 100 °C, 15 min e 3% H₂SO₄ para bagaço de goiaba, 160 °C, 15 min e 3% H₂SO₄ para bagaço de laranja e 160 °C, 55 min e 3% H₂SO₄ para resíduos de restaurantes. No tratamento alcalino, as biomassas foram submetidas a três tipos de agentes alcalinos: peróxido de hidrogênio em meio alcalino, hidróxido de sódio e hidróxido de potássio, a fim de solubilizar a xilana. Posteriormente, a xilana foi hidrolisada enzimaticamente com endoglucanase purificada de *Aspergillus versicolor* para obter XOS. A solubilização máxima de xilana com peróxido de hidrogênio foi de 90,70% com resíduo de restaurante, para hidróxido de sódio foi de 88,01% com bagaço de goiaba e para hidróxido de potássio foi de 74,20% com casca de banana. Após a hidrólise enzimática, os valores máximos para produção de XOS foram 54,14% com resíduo de casca de banana (solubilizado com peróxido), 59,86% com bagaço de goiaba (solubilizado com hidróxido de sódio), 50,42% com bagaço de laranja (peróxido solubilizado) e 50,80% com resíduo de restaurante (hidróxido de potássio solubilizado). Este estudo determinou condições de auto hidrólise, hidrólise ácida e hidrólise enzimática que maximizavam a produção de XOS com baixa quantidade de produção de xilose, utilizando resíduos agroindustriais e de alimentos.

Palavras-chave: resíduo agroindustrial, resíduo de frutas, xilana, extração alcalina, auto-hidrólise, hidrólise ácida, hidrólise enzimática, xilooligosacarídeos, efeito prebiótico.

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CHAPTER 1

Introduction

According to the 2017 Solid Waste Panorama in Brazil, prepared by the Brazilian Association of Public Cleaning and Special Waste Companies (ABRELPE), total waste generation in 2017 reached the equivalent of 214,868 t/day, a number 1% higher than 2016. Of this total, approximately 52% is composed of organic material (fruit, vegetables, legumes and food in general), and 6.9 million tons of waste were not collected and thus disposed incorrectly. Food waste, solid waste generation and lack of proper food management plan have become an increasing problem in the food industry. In restaurants in general, the variety and quantity of foods offered exceed the need for consumption, resulting in the occurrence of leftovers and waste, contributing to an increase in solid waste generation (WRAP, 2013; NASPOLINI et al., 2009). This scenario shows that the proper planning in food preparation and conscious use of resources is increasingly essential, implementing preventive measures for solid waste generation or an ideal reuse of waste generated, and can add value to the waste obtaining of products with interest to the society.

In industrial productions, such as the manufacture of juices and sweets on a large scale, there is a high processing of fruit and, consequently, a large disposal of peels and waste. The elaboration of technologies for the utilization of this waste becomes even more important on an industrial scale. Residual biomasses resulting from industrial food processes are mostly lignocellulosic biomasses, which are basically composed of cellulose, hemicellulose and lignin. Lignocellulosic materials currently represent a potential source of raw material for biotechnological processes. This material can be used for energy generation through burning and for animal feed. However, this material has great potential for new applications, providing better use of this material generating new products. Among the products with the highest added value and commercial interest are highlight the xylooligosaccharides (XOS), which are prebiotic compounds.

XOS can be obtained industrially from xylan-rich lignocellulosic materials such as agroindustrial by-products (abundant sources of lignocellulosic biomass). In order for solubilization and/or hydrolysis of xylan, present in xylose oligomers of the

hemicellulosic fraction, different processes can be performed, among which are autohydrolysis in liquid water or steam explosion, chemical pre-treatments in dilute or oxidative acid solutions, dilute alkali extraction or organosolve, and direct enzymatic hydrolysis of biomass (QING et al., 2013; VÁZQUEZ et al., 2000).

Enzymatic hydrolysis to obtain XOS offers the advantage of enabling work with milder conditions, acting specifically on xylan chains. However, enzymatic methods require longer reaction times than acid hydrolysis or autohydrolysis, as well as producing different hydrolysis end products depending on the xylanase substrate specificity and having a difficult control of XOS production with a range desired degree of polymerization (QING et al., 2013). The process conditions influence the yield of hemicellulose extraction. With the isolation of hemicellulose, enzymatic hydrolysis acts efficiently releasing the XOS. This extraction process is divided into two stages, the first being the alkaline extraction of hemicelluloses and the second the enzymatic hydrolysis (BRIENZO, 2016). As an advantage of this extraction in the form of xylooligosaccharides we have the fact that fermentation to obtain a final product is not necessary, having XOS itself as a high added value end product (MARCONDES, 2018).

Xylooligosaccharides are prominent compounds and are considered with prebiotic properties, which act to improve the composition of the intestinal microbiota and are considered emerging prebiotics in the functional dietary fiber scenario (GOBINATH et al., 2010; MÄKELÄINEN et al., 2010). In addition to prebiotics, xylan extraction in its oligomeric form is an alternative to the use of this polysaccharide because it has great application as adhesives, reinforcements in cellulosic pulps and emulsifiers (MARCONDES, 2018).

In the scenario of waste utilization, this project aims to study the extraction of hemicellulose from waste from the food industry and agroindustry. Food preparation and fruit residues are rich in carbohydrates and can be a source for hemicellulose production. The biomasses were submitted to acid hydrolysis and autohydrolysis treatments, hemicellulose was solubilized with different chemicals and submitted to enzymatic hydrolysis with purified endoglucanase from *Aspergillus versicolor* to produce xylooligosaccharides.

The present work was structured in the form of chapters, where, from the introduction chapter, the objective chapters (chapter 2), bibliographic review about the material studied (chapter 3: Food and Fruit: From Waste Generated to Feedstock to New Products), obtaining xylooligosaccharides from the autohydrolysis of materials (Chapter 4: Xylooligosaccharides from Agroindustrial and Restaurant Waste Produced by Autohydrolysis), obtaining xylooligosaccharides from acidic hydrolysis of materials (Chapter 5: Xylooligosaccharides Production from Fruit Waste by Acid Hydrolysis), obtaining xylan and enzymatic hydrolysis of materials (Chapter 6: Xylan Solubilization from Food and Agroindustrial Waste and XOS production by Enzymatic Hydrolysis) and, finally, the work conclusion chapter (Chapter 7).

CHAPTER 7

CONCLUSION

Banana, guava and orange fruits are produced in large quantities and, consequently, generate a large volume of waste, as is the case with restaurant waste, due to the large quantity and large production volume. The National Policy on Solid Waste (NBR 12.305/10) proposes that for optimal management there should be no waste generation in the biomass processing chain. Considering that with the chosen biomass this scenario is not yet possible, it is emphasized that the policy also proposes the reuse of waste when its non-generation is not possible, evidencing that, through the project results, this factor proves to be possible and potential. This scenario showed that the ideal reuse of the generated residues and the possibility of adding value to these biomasses are increasingly essential, a possibility evidenced by the study, which presented alternatives for this reuse with good results.

Compared to other biomasses, the materials (banana peels, guava and orange bagasse and restaurant residue) demonstrated superior potential as feedstock for generation of value-added products. Moreover, they are generated in abundance in Brazil, what is an environmental concern, that can be diminished.

The treatments chosen were effective. Enzymatic hydrolysis showed the highest levels of XOS production, in addition to presenting operational advantages and the non-formation of degradation products. Acid hydrolysis was the second method with the greatest XOS production, presenting almost zero degradation products. Autohydrolysis was the least effective, but it has operational and economic advantages, in addition to almost zero formation of degradation products.

Xylan extraction was high and evidenced another potential of the chosen residues and reagents. In addition, with xylan extracted from enzymatic hydrolysis, there was a high production of XOS, mainly with guava and restaurant residues, showing the potential of the material and the enzyme used.

The study biomasses showed great potential in the production of xylan and xylooligosaccharides, therefore, residues of potential interest and use in the production of prebiotics compounds.

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