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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**

AUTORA: BEATRIZ SALUSTIANO PEREIRA
ORIENTADOR: MICHEL BRIENZO

Aprovada como parte das exigências para obtenção do Título de Mestra em CIÊNCIAS BIOLÓGICAS (MICROBIOLOGIA APLICADA), área: Microbiologia Aplicada pela Comissão Examinadora:

Prof. Dr. MICHEL BRIENZO
Laboratório de Caracterização de Biomassa / Instituto de Pesquisa em Bioenergia do Câmpus de Rio Claro - UNESP

Profa. Dra. SIMONE COELHO NAKANISHI
Laboratório Nacional de Biorrenováveis - LNBR / CNPEM - Centro Nacional de Pesquisa em Energia e Materiais

Prof. Dr. JÚLIO CÉSAR DOS SANTOS
Departamento de Biotecnologia / Universidade de São Paulo - Lorena/SP

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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% H_2SO_4, for guava bagasse at 100 °C,15 min and 3% H_2SO_4, for orange bagasse at 160 °C,15 min and 3% H_2SO_4 and for restaurant waste at 160 °C, 55 min and 3% H_2SO_4. 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 *Aspergiullus 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 (H_2SO_4) 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% H_2SO_4 para resíduo de casca de banana, 100 °C, 15 min e 3% H_2SO_4 para bagaço de goiaba, 160 °C, 15 min e 3% H_2SO_4 para bagaço de laranja e 160 °C, 55 min e 3% H_2SO_4 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 *Aspergiullus 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, xilo-oligossacarídeos, efeito prebiótico.

Sumário

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 endoglucanse 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 Autohydrolisis), 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 2

OBJECTIVES

This study aimed to evaluate the production of xylooligosaccharides by acid pretreatments and autohydrolysis of food and agroindustry residues. The xylan was extracted from biomasses for xylooligosaccharides production by enzymatic hydrolysis.

SPECIFIC OBJECTIVES

• Determine optimum conditions to maximize XOS production with minimum xylose by acid and autohydrolysis of banana peel, guava and orange bagasse residues and restaurant residue;

• Xylan extraction from banana peel, guava and orange bagasse residues and restaurant residue using hydrogen peroxide in alkaline medium, sodium hydroxide and potassium hydroxide;

• Xylooligosaccharides production from enzymatic hydrolysis of xylan extracted from banana peel, guava and orange bagasse residues and restaurant residue.

CHAPTER 3

FOOD AND FRUIT: FROM WASTE GENERATED TO FEEDSTOCK TO NEW PRODUCTS.

Abstract

World fruit production is over 675 million tons per year. Brazil has significant production in the fruit market and in the food sector, responsible for the production of 43.6 million tons of fruits, where 17.5 million tons of production are orange, 6.9 million tons of banana and 349 thousand tons of guava, which makes the country stand out in the generation of fruit and food residues. The country is among the 10 countries that waste the most food in the world and discards about 35% of all agricultural production, and about 42% of this waste is destined for public roads, dumps, rivers and vacant lots, causing environmental, sanitary and social impacts. Among Brazilian production are banana, guava, orange and huge number of restaurants/food industries that discard large volume of waste in their harvest, transportation, storage and end prepare/use. The present review showed the importance of these residues and a scenario of utilization in order to reduce environmental impacts and add value to these residues.

Keywords: food waste, biomass, environmental alternatives, sustainability, fruits.

Introduction

The term 'food waste' defines the waste from the preparation of human food and consists of food leftovers such as peel, pulp, cartilage, leaves or others, and corresponds to 65% of the total organic waste (VIANA et. al., 2005). In addition to household disposal, industrial production also generates a large volume of waste, with a loss of 30 to 40% in the fruit production market (DIAS, 2003). Of all the waste generated in the country, about 50% is composed of organic material, but of all this organic portion, only 1% of this waste is recycled, and this discarded organic matter is fermentable, resulting in negative environmental impacts (BARCELOS, 2009). Industrial productions, such as the manufacture of juices and sweets on a large scale, there is the great use of biomass and, consequently, the generation and disposal of peels and wastes in general. The development of technologies for the utilization of this waste becomes even more important the large industrial scale. Among the environmental impacts are the attraction of vectors, generation of bad odor and visual pollution and soil and groundwater contamination by the leachate.

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 food offered exceeds the need for consumption, resulting in the occurrence of leftovers and waste, contributing to an increase 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. Reuse of waste generated could add value producing molecules of industrial interest.

Residual biomasses resulting from industrial food processes are mostly lignocellulosic biomasses, which are manly composed of cellulose, hemicellulose and lignin, as well as smaller amounts of other components such as extractives and ashes. Cellulose is the main constituent, appearing as a major fraction of lignocellulosic material. In the plant cell wall, hemicelluloses are tightly bound to lignin and cellulose that makes it difficult to separate these components without significant change in their structures (MELATI, 2019). Lignocellulose, composed of cellulose, hemicellulose and lignin, appears as a promising alternative raw material in the production of biofuels and chemicals, capable of promoting sustainable development from the use of alternative biomass in biorefineries to produce high value products (Rinaldi et al., 2016).

Brazil has expressive fruit production and among the main fruits and of greater production are the banana nanica, guava and orange pear, besides the large number of restaurants. Along with the large volume of production is the large volume of disposal at various stages of production, as well as the final disposal of waste from products generated from these materials. These biomasses have, in addition to many added nutrients being wasted, great potential for reuse. This review highlighted the qualities and potential of these waste biomasses, in order to stimulate alternatives uses and a better destination of these wastes, adding value to them and avoiding environmental damage.

Brazilian panorama

Urban solid waste (USW) is characterized as waste generated by human settlements in urban areas and its main types are residential, commercial, industrial, and from public cleaning, health services and construction. The growing concern about these wastes is due to the large amount generated and ways of disposal (dos SANTOS, 2019). About 50% of USW is composed of organic material, which is composed by animal or vegetable remains from human activities, that can naturally be decomposed in the environment. However, there is a problem related to the large volume generated that improperly disposal results of emissions of methane, proliferates disease vectors, among many other problems (MMA, 2017).

According to the 2017 Solid Waste Panorama in Brazil, made by the Brazilian Association of Public Cleaning and Special Waste Companies (ABRELPE), solid waste generation in Brazil grew 1% between 2016 and 2017, reaching a total of 214,868 tons of urban solids waste generated per day. These values reveal a total of 78.4 million tons of municipal solid waste generated annually. Of this amount generated, a total of 91.2% is covered by collection in the country, which highlights the problem of 6.9 million tons of waste not being collected annually, which results in improper disposal. In 2017, 59.1% of the collected waste was disposed of in landfills, while the remaining 40.9% was disposed of in inappropriate places, such as dumps and controlled landfills, which are not prepared to receive this waste, not containing protection systems against damage to the environment, degradation and health of the surrounding population.

According to the National Solid Waste Policy, all dumps in Brazil should have been closed by 2014. However, according to ABRELPE, in 2017 Brazil had about 3,000 dumps operating in 1,600 cities, showing a 3% increase in inadequate waste disposal compared to 2016. This portion of discarded organic waste generates a huge volume of waste. According to the United Nations Food and Agriculture Organization (FAO ONU, 2017), about 1.3 billion tons of food goes to trash every year, representing one third of all food production in the world. More than enough food is produced in the world for the demand, despite that in many regions hunger affects thousands of people and remains one of the most pressing development challenges. In addition to food waste, this loss also implies a waste of resources and inputs used in production, such as land, water and energy as well as an increase in greenhouse gas emissions. Around

25% of habitable land is used for food production, accounting 70% of drinking water consumption, 80% of deforestation and 30% of greenhouse gas emissions (FILHO, 2017).

Environmental impacts

The generation of waste from human activities is one of the major environmental concerns, given that much of this waste is discarded without planning. At the same time, several studies propose the correct management of waste, which includes selective collection, proper disposal and recycling, which are activities linked to the concept of sustainable development (BOCHNIA et al,2013).

According to Institute of Applied Economic Research (IPEA, 2009), Brazil is among the 10 countries that most waste food in the world. The country discards about 35% of all agricultural production. In addition, the IPEA points out that this waste amounts to more than 10 million tons of food, which could supply 54 million Brazilians living in the poverty line.

Poor management of waste generated implies health problems such as pollution, flooding and the creation of disease vectors. About 42% of this waste produced in Brazil is destined to public roads, dumps, rivers and vacant lots (BRAZILIAN ASSOCIATION OF PUBLIC ENTERPRISES AND SPECIAL WASTE, 2011). However, until the destination currently considered more appropriate and used, landfills have environmental risks and losses. If improperly handled, landfills can lead to soil and water contamination, methane generation, waste of resources and landscaping and social impacts. There are also operational and logistical factors that cause environmental impacts, such as the large amount of trucks for waste collection and transport to disposal. This waste collection/transport generate pollutants and involves high costs, as well as the need of available areas for the implementation of waste landfills, which are becoming smaller (ABRELPE, 2011).

Food and industrial waste

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 food offered exceeds 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). Another factor that generates huge amount of waste is the worldwide product standard. The US market, for example, fails to harvest more than 20 billion pounds of fruit and vegetables due to the selective nature of consumer buying habits, even though they are still perfectly fit for consumption (SOBOL, 2018).

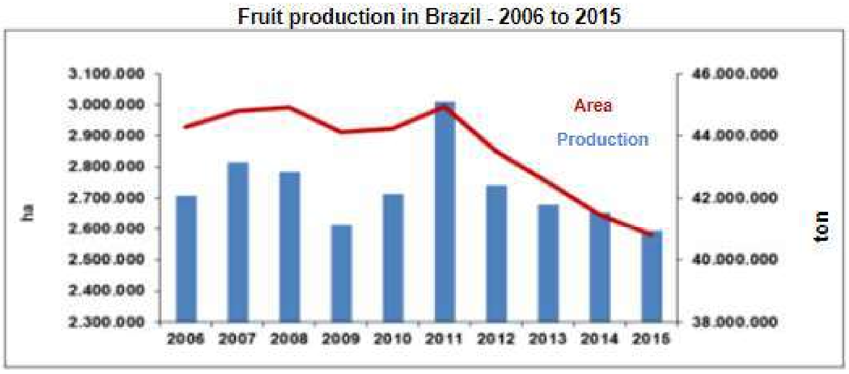
To minimize this type of waste, it is necessary investment and awareness of both those involved in the production process and the consumers of the products. However, the benefits of these actions are diverse, as highlighted by Crittenden and Kolaczkowski (1995), among them, the reduction of costs with handling, transportation and final disposal, reduction of waste storage space, improvement of company image, reduction of environmental impacts, reduction of input consumption.

Food waste is produced in the various activities of preparation, presentation, consumption and cleaning of utensils. Each of the activities should be observed with a view to reducing waste generation. Already in preparation should seek the intelligent use of food, avoiding waste. Hard leaves and stalks for use in raw salads, for example, can be used in creams or soups. Even if all generation reduction measures are implemented, there will always be waste to be managed. Each restaurant should design an action plan aimed at the implementation (or improvement, where it already exists) of a selective waste collection system and possible reuse.

In addition to domestic waste and other activities, there is also agro-industrial waste. In Brazil, about 35% of agricultural production is wasted, which places the country among the 10 most wasted in the world (FILHO, 2017). Fruit production in the country places it as the third largest fruit producer in the world, surpassing 40 million tons per year, production that covers 2.6 million hectares in the country. According to the survey Municipal Agricultural Production (PAM 2016), in 2016 the national fruit production recorded a record production of R$ 33.3 billion, value released by the Brazilian Institute of Geography and Statistics (IBGE) and that was not so high since

1974 (DCI, 2017). A variation in the Brazilian production of fruits over 9 years, from 2006 to 2015, showed the area in hectares used for such production (Figure 1).

Figure 1 – Representative chart of the Brazilian fruit production and area used in 9 years.



Source: IBGE; Elaboration: SEAB/DERAL

In the 2015 survey, in Brazil, the indices had a fruitful production of 40.9 million tons. Among the main fruits produced in Brazil, orange leads in volume, totaling 16.7 million tons from orchards in 2015, which corresponded to 40.9% of the total fruit crops. Among the Brazilian states, São Paulo leads the production, with 12.3 million tons, representing 73.3% of the volume produced (ANDRADE, 2017).

The second most produced fruit in Brazil, in volume, is banana, which had a volume of about 6.7 million tons in 2017, with the main producers being the states of São Paulo and Bahia (ABF, 2018). Another fruit of growing national production is guava, which grew 11% in its planted area in 2018, while in sugarcane and orange, the increase was, respectively, 0.5% and 2.5% (O Globo, 2018). In addition to this growth, the greatest diversity of guava species is found in the country, which occupies a prominent position among the world's guava producers (POMMER, 2006).

Main fruits produced in São Paulo State

The state of São Paulo is responsible for much of the Brazilian fruit Production, producing, in 2018, 10.6 billion reais in the fruit market, corresponding to 32% of the national collection. In addition, it is the second state that exports the most fruits in the country, exporting 135.6 thousand tons in 2017, the largest volume in the southeast and second largest in the country, second only to Bahia (ABF, 2018).

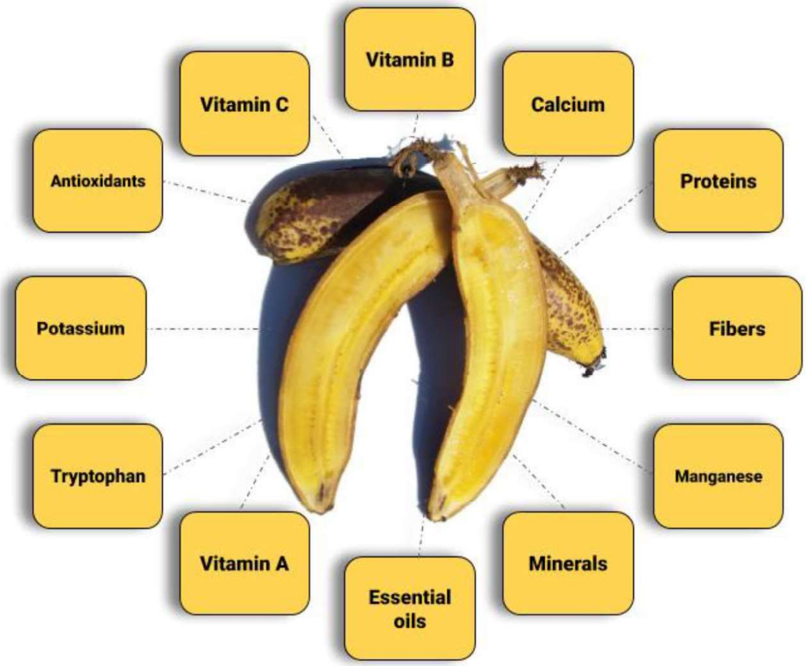
Orange is one of the most produced fruits in the country, and corresponds to the largest production in the state, accounting for 59.2% of São Paulo production, followed by banana production, which corresponded to 13.8% of fruticulture in 2016. In addition to the expressive orange and banana production, the state is the largest producer of guava in the country, with the production area expanded by 24% between 2013 and 2017 (FREITAS and FREDO, 2019).

Banana

Banana is a fruit originated in Asia and only came to be known from the 17th century. It is a typically tropical fruit and its production currently occurs mostly by the American, Asian and African countries. Both in production volume and in commercial value, it is one of the most important fruits in the world, besides allowing its production and trade to occur throughout the year (Toda Fruta, 2016).

Bananas are an important source in human food due to their calorific value, energy and, especially, for their mineral and vitamin content (LIMA, 2000). The fruit is rich in carbohydrates and energy (calories), important nutrients such as vitamins and minerals, leave that appear in greater quantity in the still green fruit, and composed mainly of potassium, phosphorus, calcium, sodium and magnesium, also featuring iron, manganese, iodine, copper, aluminum and zinc. In addition to the numerous minerals, bananas still have as main vitamins A, C and B complex (B1, B2 and niacin), proteins such as albumin and globulin in small amounts, and amino acids such as asparagine, glutamine and histidine (BIANCHI, 2005), among other properties as shown in the figure 2.

Figure 2 - Scheme with representative properties of the banana.



Source: author.

In Brazil, banana cultivation has spread and today the fruit stands out as the most consumed in the country, being in its raw form or processed, such as in jams, liqueurs, brandy, fried, roasted, flour, raisins or puree. This wide range and appreciation of the fruit is combined with the fact of its ease cultivation and handling, which explains its growing consumption (SEBRAE, 2008). Banana cultivation stands out internationally as one of the main agricultural businesses, considering that bananas are the most consumed fresh fruit in the world. Brazil has significant production of this fruit, being the fourth largest producing country, estimated at 7.1 million tons of banana per year, mostly destined for domestic consumption, as only 1.5% of its production is exported, which makes the country the world's first consumer of fruit. The type of banana most consumed in Brazil varies by region. In the North and Northeast regions the predominant consumption is of the Silver type banana, in the South and Southeast regions, Nanica and Nanicão are more consumed (LIMA, 2012).

In terms of volume of fruit produced, bananas rank second in Brazil, reaching 6.8 million tons harvested, which corresponds to 16.7% of the total volume. Regarding the most representative states, Bahia appears as the main producer, with 1.1 million tons harvested, followed by the state of São Paulo, with 998 thousand tons, and Minas Gerais, with 795.9 thousand tons produced. The participation of the three states in the banana volume produced in 2015 was 15.6%, 14.6% and 11.6%, respectively. The planted area in the country reached 469.5 thousand hectares in 2017 and the amount produced reached 6.7 million tons, generating a total production value of R$ 8.1 billion (IBGE, 2019).

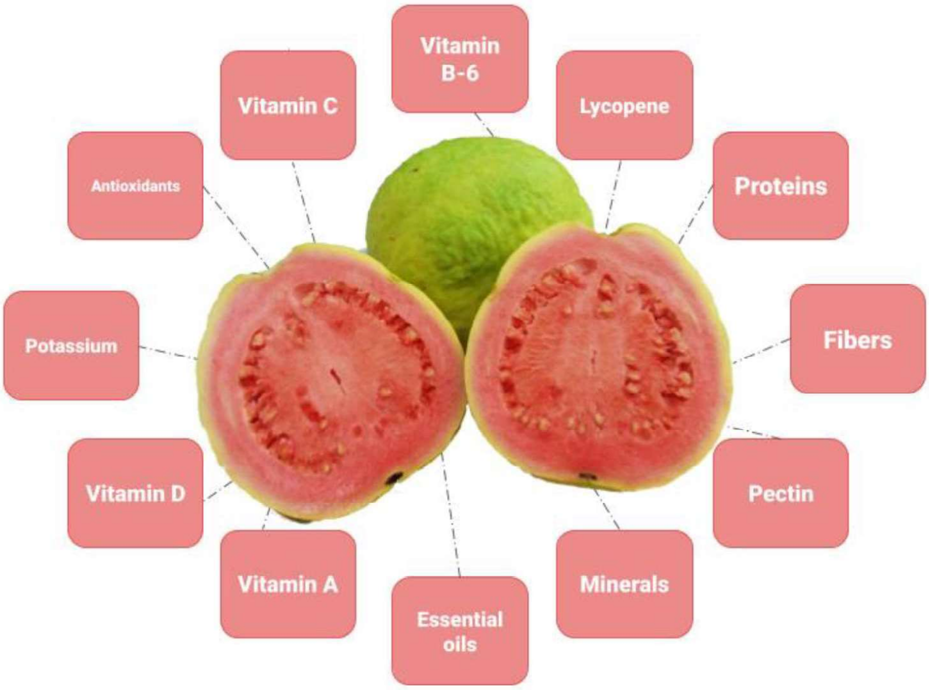
Despite having a high consumption potential, among tropical and subtropical fruits the only one that has significant presence in international trade is banana, being the most produced fruit in the world in 2014, totaling 144.8 million tons produced (SEAB, 2017). In the Brazilian intern market, due to the great dedication to banana growing in addition to its low participation in the export market, the dimensions of its domestic consumption are intensely reflected. The fruit represents 30.7% of the volume of fruit sold, leading the domestic market.

As a result, the need for the development of new products and/or the utilization of fruit by-products becomes even greater and may represent new sales and profitability opportunities for untapped banana growers (SEBRAE, 2008).

Guava

Guava stands out for its high nutritional value, being one of the best sources of vitamin C, lycopene, potassium, copper and fiber, besides being able to supply 100% of the recommended daily intake of vitamin C and lycopene with only one fruit, approximate weight 150 g, in addition to other beneficial components for human health as antioxidants, vitamin B-6 and essential oils, as can be seen in the figure 3. Its fresh consumption has great acceptance and the fruit still presents great yield in pulp for industrial application (QUEIROZ, 2008).

Figure 3 – Representation of guava elements and properties.



Source: author.

Brazil is the world's largest producer of guava, producing approximately 424.305 tons of guava annually, from an area of approximately 17.688 thousand hectares of planted guava. The guava production is concentrated in the Southeast and Northeast regions of the country, with São Paulo and Pernambuco are the states with the largest commercial production (IBGE, 2015).

Guava is important among Brazilian fruits, not only for its gastronomic characteristics, but also for its nutritional values, making Brazil the world's largest producer of red guavas and India the largest of white guavas (FRANCISCO; BAPTISTELLA; AMARO, 2005). São Paulo is the largest producer of guava in Brazil and its production area expanded by 24% from 2013 to 2017, with production destined for industry (86% of production) and for fresh consumption. The central region of São Paulo is traditional for the production of sugarcane and orange, but the production of guava has been highlighted due to the great stimulus in the replacement of orange by guava resulting from fruit prices, besides the productivity of a guava tree, which is four times larger than one orange. Guava production in this region is destined to the food industry, which receives 1,000 tons per day, having as its main processing industry Predilecta, based in Matão (SP), which has 350 partner producers that cultivate between 900 and 1,000 hectares. Of this total volume received by Predilecta, 70% is destined for the intern market, with 90% used for the production of sweets and the other 30% for pulp production and destined for the foreign market (DE FREITAS and FREDO, 2019). In addition to fresh consumption, the guava produced is intended for the processing industry for the production of juices, pulps, jams, nectars, ingredients for the preparation of yogurts, jellies and sweet-sour sauce (EMBRAPA, 2010).

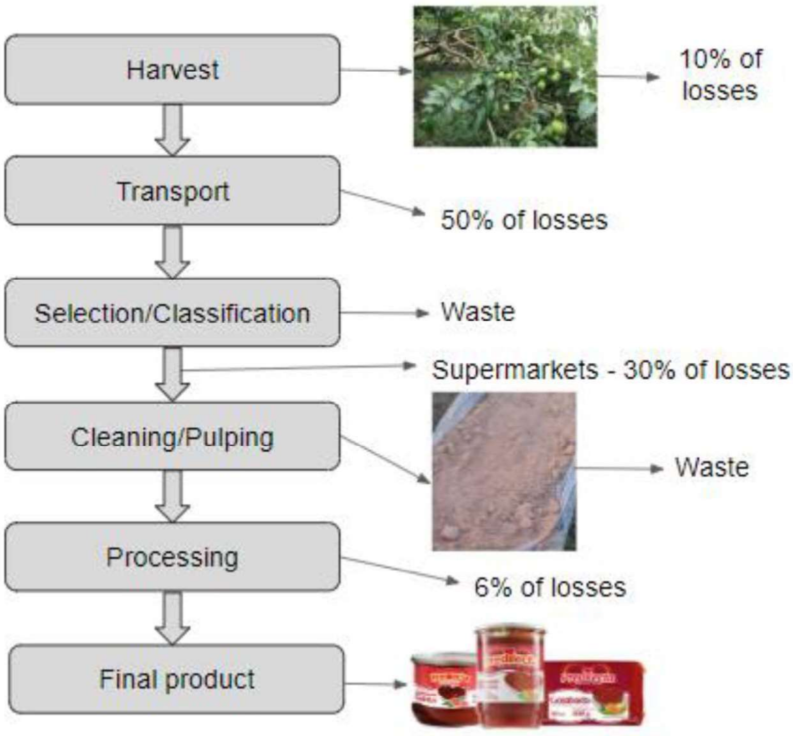
According to information obtained from Supplies Centers (Ceasas from São Paulo, Belo Horizonte and Rio de Janeiro), guava commercialization has grown 500% in the last decade, mainly destined to processing, considering the high degree of perishability of the fruit (CEAGESP, 2008; CEASAMINAS, 2008; CEASARJ, 2008).

The guava processing stages in industry there is waste generation, which constitutes about 8% of its initial mass (SOUZA, 2014). These residues are composed of seeds and some skin and pulp fraction, which are not separated in the physical pulping process and have significant amounts of fatty acids, fibrous matter and other compounds with functional properties in the human diet. This waste is most often destined for animal feed industries, or disposed of in the environment (DA SILVA and JORGE, 2014; MIRABELLA, CASTELLANI and SALA, 2014).

The fresh consumption of fruit in the country is still small, estimated at about 300 grams/per capita/year, at the same time. Guava is one of the most consumed sweets in the country, so the fruit is very representative for processing (PSF, 2016).

The residue generated in the industrial processing of the fruit is composed of seeds, peel and pulp, which are discarded in the production of processed and contains about 5-13% of essential oils and fatty acid rich oil, besides are possible sources of antioxidants (SCHIEBER et al., 2001).

Figure 4 – Generic flow of guava processing steps for derivative production.



Source: author.

The waste generating establishment obliged industry to perform its proper management, transportation, treatment and final disposal. This management of waste increased the demand for control and reuse of the material, in order to reduce the environmental impacts caused by this disposal and for utilization of the nutritional and functional properties of the waste (KRAEMER, 2005).

Orange

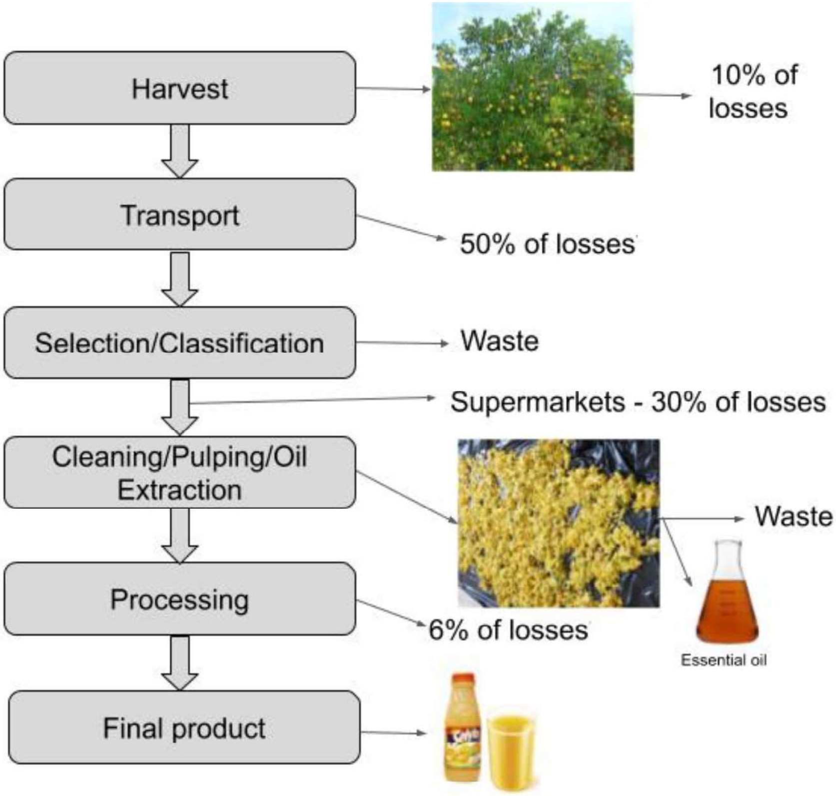
Fresh orange has an increasing intern consumption, guaranteed by the consumption of juice in homes, restaurants and other establishments, as well as the production of pasteurized juices in industry. The domestic orange market has become a major consumer of Brazilian production, consuming around 30% of national production, equivalent to over 100 million orange boxes (40.8 kg).

The Brazilian production corresponds to 50% of the world production of orange juice, besides exporting 98% of the fruits produced and getting an 85% share in the world market. The pear variety is the most consumed in the country, both for its *in natura* acceptability and because the highest soluble solids content (juice). In addition to these factors, it is produced in the period of deficit of other varieties, which add up to 600 worldwide, which makes it reach higher values and, thus, is very interesting for the industry (NEVES, 2011). As a result, about 70% of Brazil's orange production is of the pear variety, which combines flavor with attractive nutritional properties, besides, according to researchers from the Support Center for Research on Food and Nutrition (Napan) and the Faculty of Pharmaceutical Sciences of University of São Paulo (USP), reduce cholesterol, blood glucose, triglycerides and improve the human defense system.

The state of São Paulo is the largest producer of orange pear with the largest producing cities of Limeira, Conchal, Bebedouro, Mogi Mirim, Casa Branca and Pirangi. In 2016, Supplies Center received about 231 thousand tons of this fruit, which is more easily found from August to April, but also available in the market all year round (FORMIGONI, 2018). According to the IBGE report, the harvest of fresh orange in 2017 resulted in a production of 14,653,571 tons. According to the United States Department of Agriculture (USDA, 2019), the Brazilian orange crop projection for 2019/20 (MY 2018/19) is 494 million boxes, an increase of 26% over the previous crop. In the total production of frozen concentrated orange juice, the forecast is 1,397 million metric tons (mmt), a sharp increase over the previous year (1,004 mmt) due to the expected higher availability of fruit for crushing.

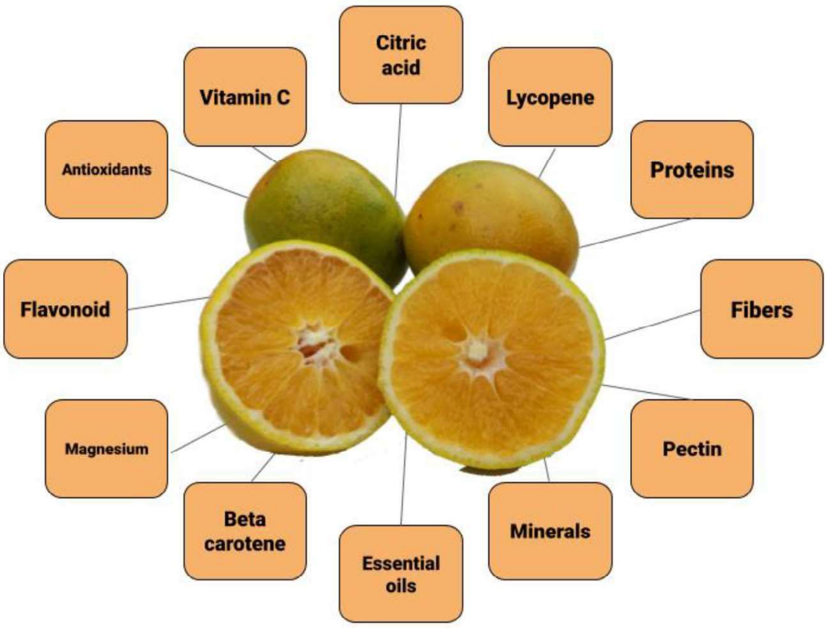
About 50% of the fruit mass is discarded after expelling its juice, in bagasse form. This discarded waste is composed of bark, seed and pulp, usually pressed and pelleted for animal supplementation. This bagasse is rich in fiber and can be used to obtain products with higher added value due to its chemical composition (Cypriano et al., 2016). The orange processing scheme is demonstrated in figure 5, showing process loss. In addition, orange has a number of beneficial compounds, such as antioxidants, phenolic compounds, carotenoids and many soluble sugars and polysaccharides (Figure 6).

Figure 5 - Representation of orange processing from harvest to final product.



Source: author.

Figure 6 - Examples of nutritional components of orange.



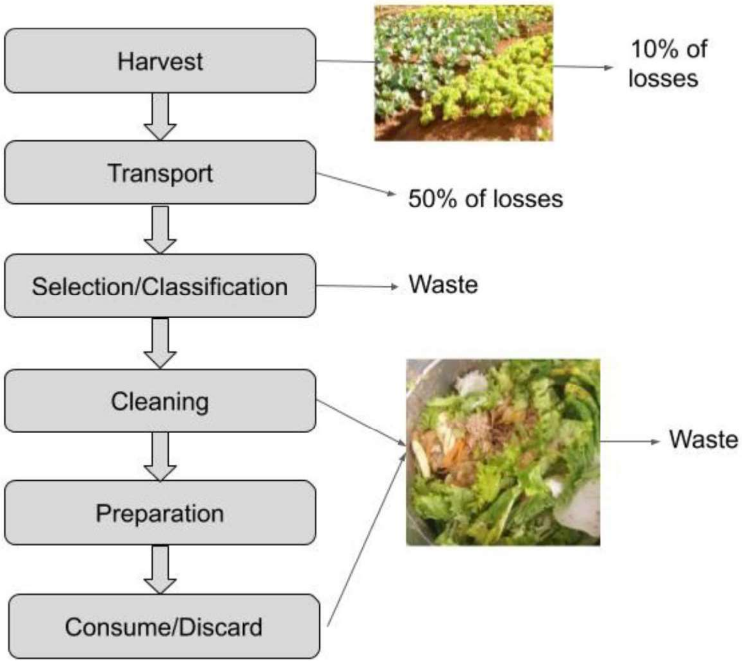
Source: author.

Restaurant residue

The restaurant activity transforms the fruits and vegetables (raw material) using different forms of process and generate a large amount of organic waste, which can cause environmental impacts. One of the first steps of the production process is the food hygiene, and are one responsible in to generate a large part of the waste, where peels and unfit parts for consumption are discarded (VENZKE, 2015).

The amount and variety of food offered in restaurants generally exceeds the need for consumption, leading to leftovers and waste, which increase the generation of solid waste (WRAP, 2013; NASPOLINI et al., 2009). Food companies generate many food leftovers and food waste, which, in Brazil, according to the Brazilian Association of Bars and Restaurants - ABRASEL (2007), account for 15% to 50% of total annual production, which corresponds to about R$ 15 billion that is discarded. This significant amount of solid waste discarded, which is mainly composed of organic waste, is reusable, and as alternatives there is animal feed, composting and incorporation into the soil as organic fertilizer after treatment (SOUZA et. al., 2011; LOPES AND FONSECA, 2013). A representative scheme of food processing involve loss from the harvest to final consume (Figure 7).

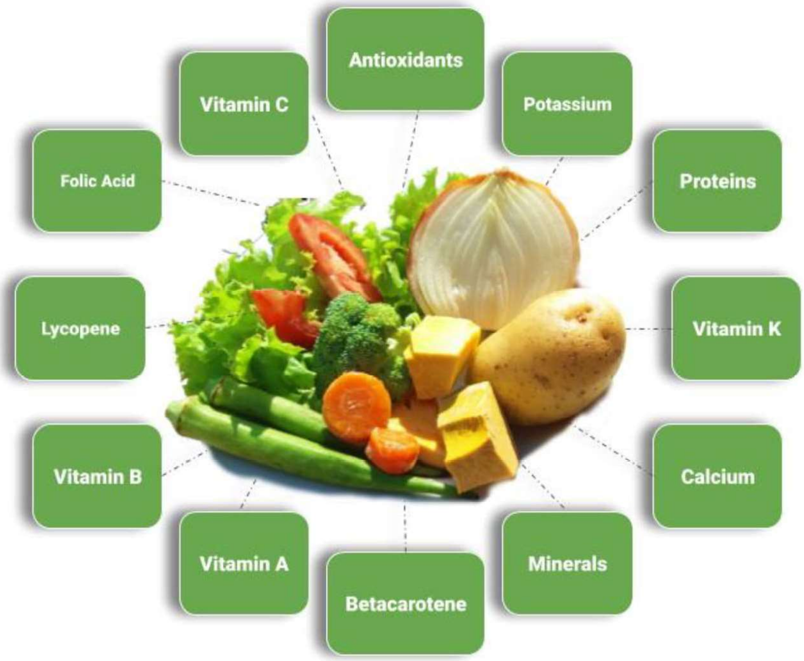
Figure 7 - Scheme representative use of vegetables in a restaurant chain, until the disposal of the consumer.



Source: author.

An important part of restaurant waste is fruits and vegetables (Figure 8), which are important sources of micronutrients, fibers and other components with functional properties. These components are essential in a healthy diet, and have low energy density, favoring the healthy maintenance of body weight through low calories in relation to volume of the food consumed (FIGUEIREDO, 2008).

Figure 8 - Representation of important properties of vegetables.



Source: author.

Concluding remarks

Domestic consumption, food manufacturers, supermarkets and restaurants spend up to 52 billion pounds of food each year, according to the Move For Hunger (MFH, 2018). Household consumption accounts for 43% of food waste, while consumer companies account for 40% of food waste, with 16% coming from farms and 2% from food wasted by manufacturers. This waste occurs at every level of the supply chain, from the farm, to distribution, to the supermarket, and finally at home. Even with these factors, according to the United Nations, it is estimated that by 2050, more than 42 million people will starve (SOBOL, 2018). In addition to the social problem, according to ThinkEatSave, most uneaten food ends up in dumps, and when this waste food decomposes in an oxygen-free landfill, it creates an anaerobic sequence that produces methane, which is about 20 times more harmful to the atmosphere than carbon dioxide. This in turn contributes to the depletion of the ozone layer, which can lead to higher temperatures worldwide. This waste impact the sustainability of food systems and reduces local and global food availability, as well as generating fewer resources for producers and raising prices for consumers. In environmental terms, it also uses natural resources in a non-sustainable way (BENÍTEZ, 2014). The residues generated from food and fruit processing are potential to use as feedstock to produce molecules with high value. New potential uses of these residues, not only based on recycling as animal feed and organic fertilizer, can be developed by new technologies in the biotechnology industry and, thus, in addition to adding value to this material, it would also encourage the preservation of the environment. In addition, a new supply chain planning approach should also be evaluated, considering the large hunger population and the nutrient-rich material that is wasted daily.

CHAPTER 4

XYLOLIGOSACCHARIDES FROM AGROINDUSTRIAL AND RESTAURANT WASTE PRODUCED BY AUTOHYDROLYSIS

Abstract

Waste from agroindustrial and food is increasing and needs search for alternatives to use these biomasses, such as the conversion by autohydrolysis, due to environmental and economic advantages. Moreover, it could be alternative for the pretreatment of lignocellulosic biomass to produce xylooligosaccharides (XOS) and other value added products. This study evaluated the production of XOS via autohydrolysis of agroindustrial and food waste. Application of autohydrolysis pretreatment showed maximum conversion xylan into XOS and xylose of 32.28 % and 5.07 % for banana peel residue, 8.21 % of XOS and 1.99 % of xylose for guava bagasse, 68.53 % XOS and 4.02 % xylose for orange bagasse and 33.42 % XOS and 2.60 % xylose for restaurant residue. Results showed that the ideal conditions to obtain XOS are high temperature and low reaction time (160 °C and 15 min) for banana peel residue, high temperature and medium time (172.43 °C and 35 min) for guava bagasse, medium temperature and time for orange bagasse (130 °C and 35 min) and for restaurant waste, medium temperature and low time (130 °C and 6.72 min). This study determined autohydrolysis conditions that maximized XOS production with low amount of xylose production using agroindustrial and food waste.

Keywords: agroindustrial waste, biomass, autohydrolysis, xyloligosaccharides, prebiotic.

Introduction

Generation of waste from human activities is one of the major environmental concerns, given that much of this waste is discarded without planning (BOCHNIA et al, 2013). In addition to domestic waste and other activities, there is also the disposal of agroindustrial waste. In Brazil, about 35% of agricultural production is wasted, puts the country among the 10 most wasteful in the world (FILHO, 2017). These residual biomasses resulting from industrial food processes are mostly lignocellulosic biomasses, which are basically composed of cellulose, hemicellulose and lignin.

Studies about lignocellulosic materials and the production of XOS by these biomasses are highly important, and generation of XOS are considered an alternative to add value to lignocellulosic biomass. After pretreatment of lignocellulosic biomass, XOS can be produced, biomass is cheap and abundant residue, and XOS have excellent prebiotic properties, are one of the few nutraceutical products, and if was from agroindustrial waste, offers a great potential for food and pharmaceutical industries (SAMANTA et al., 2015). Agroindustrial lignocellulosic materials need to be pretreated to make fermentable sugars available or macromolecule solubilization. Pretreatment effectiveness depends on factors such as lignin content and cellulose degree of crystallinity. Due to their low cost and high availability, these biomasses are a crucial material with an important biotechnological application (CERQUEIRA, 2007; GOELZER, 2009).

XOS are capable of promoting several beneficial health effects due to its immunomodulatory, anti-inflammatory and antioxidant properties and, therefore, have been considered in the treatment of various diseases (ÁVILA et al., 2018). Studies on human dietary supplementation show that the use of prebiotics can provide many health benefits, such as cholesterol reduction, relief of symptoms of gastrointestinal disorders, increased satiety and immunomodulatory effects, even though further studies are needed. Currently accepted prebiotics are those derived from galactooligosaccharides, fructooligosaccharides and inulin fibers, naturally occurring forms in foods such as pulses, grains, fruits and vegetables. In addition, prebiotics can still be isolated and commercially produced for use as functional ingredients and supplements (LOCKYER, 2019). The most prominent activity is as prebiotics, which has attracted great attention among the features of XOS, because it has multi-dimensional effects, especially in the prevention of gastrointestinal disorders (ADEBOLA, 2014). XOS still stand out for their potential for development of growth stimulants in agriculture and for the production of drugs related to the treatment of gastrointestinal infections and obesity control (VÁZQUEZ et al., 2000). If used in food supplementation, these oligomers improve intestinal functions, provide positive effects on the immune system, cardiovascular and triggering anti-inflammatory and antiallergic activities (GROOTAERT et al., 2007; AACHARY and PRAPULLA, 2009; CHUNG et al., 2007). In animal feed, XOS can totally or partially replace the use of antibiotics, acting as prebiotics and preventing the proliferation of pathogens and bacteria by resistance to these antibiotics (CARVALHO et al., 2013).

XOS can be produced by processes such as autohydrolysis, which has advantages of avoid chemical recovery costs, neutralization and decreased of environmental impact (MORAIS, 2015). Autohydrolysis process facilitates the separation of hemicellulose, enabling it to be solubilized and resulting in a liquid rich in derived sugars (xylose and arabinose) or oligosaccharides, and has the advantage of not causing significant cellulose and lignin degradation (MORAIS, 2015). Compared to other methods, XOS obtained from this pretreatment have a more conserved structure compared to the native form of xylan (MOURE et al., 2006).

Food and agroindustrial waste are rich in carbohydrates and can be a source for the production of hemicellulose and its derivatives. In this scenario of waste utilization, the present study aimed to evaluate the production of XOS using autohydrolysis pretreatment. Residues studied were based on the fact that, among the main fruits produced in Brazil, orange leads in volume, totaling 16.7 million tons from orchards in 2015, which corresponded to 40.9% of the total fruit crops (SEAB, 2017). The second most produced fruit in Brazil, in volume, is banana, which had a volume of about 6.7 million tons in 2017, with the main producers being the states of São Paulo and Bahia (ABF, 2018). Another fruit of increasing national production is guava, which grew by 11% in its planted area in the year 2018, while orange increased 2.5% (The Globe, 2018).

Material and Methods

Agroindustrial waste and food waste

Materials used were banana peel, guava bagasse, orange bagasse and restaurant residue. Residues of guava were gently provided by the companie Predilecta and the orange bagasse by Selial companie, which has not been subjected to any process (pectin or oil extraction). Banana peel was collected in local market. Restaurant residue was collected at restaurant of university and was composed by the pre-prepare residue, like onion and garlic peel, vegetable peels and leaves in general. Residues were dried at 60 °C until moisture lower than 10%, ground in knife mill and the material that passed through a 20 mesh sieve was selected.

Physicochemical Characterization

Prior to chemical characterization, moisture, ash and biomass extractives contents were determined. Moisture was measured upon material arrival by placing approximately 1 g of the material in an oven at 105 °C, determined by mass difference.

Ash content was measured by placing approximately 1 g of material in muffle porcelain crucibles at 525 °C for 4h, the ash content was calculated by mass difference. Extractives content was determined by Soxhlet extractor, using the 95% ethanol as solvent for a period of 8 h and subsequently extraction with distilled water for the same period (ABNT NBR 16550-2016).

Chemical characterization of biomasses was performed to determine the content of glucan/cellulose, hemicellulose and lignin. Chemical composition was determined at sulfuric acid reaction with extractive-free samples (ABNT NBR 16550-2016; SLUITER et al., 2010). The soluble fraction was used to determine sugars and acetic acid by high efficiency liquid chromatography (HPLC), and lignin was determined by gravimetry and spectrophotometry. Process was done by hydrolyzing approximately 300 mg of biomass, adding 3 mL of 72 % (m/m) sulfuric acid. Reaction occurred at 45 °C for 7 min. Reaction was stopped with the addition of 84 mL of distilled water. This mixture was autoclaved at 121 °C for 30 min (Gouveia et al., 2009). Contents were filtered through a previously tared porous plate filter n° 4. Solid residue was washed with distilled water, oven dried at 105 °C to a constant weight for the determination of insoluble lignin (Klason). Soluble fraction was used to determine sugar and acetic acid contents by HPLC. Acid soluble lignin content was determined by measuring absorbance at 215 and 280 nm (ABNT NBR 16550-2016).

Glucose, xylose, arabinose and acetic acid concentrations were determined by HPLC using Aminex HPX-87H Bio-Rad column (300 x 7.8 mm) maintained at 45 °C, WATERS 2414 refractive index detector, 0.005 mol/L sulfuric acid mobile phase, 0.6 mL/min flow, 20 µL injected sample volume. The samples were previously filtered through a 0.22 µm syringe filter.

Autohydrolysis

Autohydrolysis of the biomass was performed using a 2^2 experimental design with star rotational points (Table 1). Autohydrolysis variables studied were temperature (°C) and time (min), and as response variable were studied XOS and xylose.

Table 1. Complete factorial design 2^2 with axial, central and coded and real levels of the independent variables for biomass autohydrolysis.

	Coded value		**Real value**	
Assay	**Temperature**	**Time**	**Temperature (°C)**	**Reaction time (min)**
1	-1	-1	100	15
2	+1	-1	160	15
3	-1	+1	100	55
4	+1	+1	160	55
5	-1.41	0	87.57	35
6	+1.41	0	172.4	35
7	0	-1.41	130	6.72
8	0	+1.41	130	63.28
9*	0	0	130	35

* Central point assay in triplicate.

Source: Author.

About 5 g of each biomass were transferred to a 50 mL stainless steel reactors and heated in a thermostatized bath, according to each condition of the experimental design (Table 1). Triplicate was performed with central point conditions. After the reaction time, the reactor was cooled in an ice bath and the hydrolysate was filtered with filter paper, separating the solid and liquid fraction which was prepared for HPLC analysis.

Independent and dependent variables were analyzed using STATISTIC 7 software, to evaluate the influence variables in the production of xylose and XOS yield. Yield of XOS and xylose were calculated by the relation between the mass of generated product and the mass of hemicellulose present in the biomass. Concentration of XOS (mg.mL^{-1}) was obtained by sum the concentrations of X_2+X_3+ X_4+X_5 present in the hydrolysate. XOS content was assessed by liquid chromatography (HPLC, Shimadzu model NEXERA XR) according to literature (MAFEI, 2019). The analysis parameters were BIO-RAD Aminex HPX-87C (300 × 7.8 mm) column; 80 °C oven temperature; ultrapure water (Milli Q) at 0.6 mL min^{-1} as eluent; 20 µL sample volume; refractive index detector at 60 °C (Shimadzu, model RID-20A); and total analysis time of 15 min. Before injection, the pH of the samples was set to 6.5 using NaOH 1 mol/L. Xylose (Sigma), xylobiose (Megazyme), xylotriose

(Megazyme), xylotriose (Megazyme), xylopentaose + xylohexaose (Megazyme), and oligosaccharides with more than 6 xylose units were quantified.

Degradation Products

The conversion into degradation products was measured by reading the biomass samples in their main parameters. The hydrolysates were read by the isocratic method, using a C18 column (NST 150mm x 4.6mm x 0.5µm). As a mobile phase, a mixture of water with acetronitrile, in the proportion 80:10 and 1% acetic acid, was used. The flow rate was maintained at 0.8 mL / min, column at 35 ºC. Readings of the hydrolysates were made in the mildest conditions (100 ºC and 15 min), in the medium condition (130 °C and 35 min) and in the most severe condition (160 °C and 55 min). The parameters measured were hydroxymethylfurfural and furfural content.

Results and discussion

Physicochemical Characterization

Biomasses had a high content of moisture, shown contents of 88.37, 39.06, 79.43 and 99.67% for banana peel, guava bagasse, orange bagasse and restaurant waste, successively (Table 2). Ash content, inorganic material, were 13.22% for banana peel, 1.14% for guava bagasse, 4.50% for orange bagasse and 12.89% for restaurant waste, successively. Total extractives content (in ethanol and water) were successively, 62.97, 27.39, 48.69 and 48.49%, for banana peels, guava bagasse, orange bagasse and restaurant waste, successively. Lignin contents were 30.28% for banana peel, 31.94% for guava bagasse, 21.45% for orange bagasse and 12.10% for restaurant waste.

With sum of xylan contents, arabinosyl and acetyl groups, was possible calculated the amount of total hemicellulose in the biomass. Results were 38.42, 28.33, 31.04 and 20.58 % for banana peel, guava bagasse, orange bagasse and restaurant waste, successively. Glucan/cellulose (anhydroglucose) content was 29.28, 29.93, 24.42 and 50.20 % for banana peels, guava bagasse, orange bagasse and restaurant waste, successively (Table 2).

Table 2. Chemical composition of agroindustrial and food waste. Contents presented as percentage (%, dry basis).

Biomass	Biomass components (g/100g dry basis)			
	Ashes (%)	Lignin (%)	Cellulose (%)	Hemicellulose (%)
Banana Peels	13.22 ± 0.01	20.58 ± 2.62	29.28 ± 0.39	38.42 ± 2.21
Guava Bagasse	1.14 ± 0.00	33.75 ± 2.52	29.93 ± 0.34	28.33 ± 0.53
Orange Bagasse	4.50 ± 0.01	22.74 ± 0.12	24.42 ± 1.96	31.04 ± 0.98
Restaurant waste	12.89 ± 0.08	12.77 ± 0.94	50.52 ± 0.75	20.58 ± 0.32

Source: Author.

Values found in the chemical composition were similar to those found in the literature. For banana peels, the values found were 11.47% for ashes, 89.47% for moisture (Gondim et al., 2015) and 20.61 % for lignin (Souza et al., 2016). For guava bagasse, the values were 1.36% for ashes, 37.20% for hemicellulose (Santos et al., 2011), and 53% for moisture content (Silva et al., 2006). For orange bagasse were 2.90% for ashes, 2.90% for lignin, 26.45% for hemicellulose (Orozco et al., 2014), 38.50% for cellulose and 56.60% for extractives (Moraes and Crestani, 2018). For restaurant waste was reported 10.78, 23.26, 33.62, 4.86, 56.58 and 91.78% of ash, lignin, cellulose, hemicellulose, extractives and moisture, successively (Pereira, 2017).

Xylooligosaccharides (XOS) production

XOS content was determined based on the xylan content found in the chemical characterization of biomasses. XOS produced from banana peels waste was 32.28% with high temperature and low reaction time (160 °C and 15 min); 8.21% for guava bagasse with higher temperature and medium time (172.43 °C and 35 min - rotational star point); 66.13% for orange bagasse at medium temperature and time (130 °C and 35 min); 33.42% for restaurant waste at medium temperature and low reaction time (130 °C and 6.72 min - star point) (Table 3).

Xylose production was 5.07% with banana peels at higher temperature (172.43 °C and 35 min - star point); 1.99% with guava bagasse, 4.02% for orange bagasse at low temperature and medium time (87.57 °C and 35 min - star point); 2.56% with restaurant waste under the medium temperature and high reaction time (130 °C and 63.28 min - star point) (Table 3).

Table 3. Experimental conditions and response variable of the experimental design used to determine the acid hydrolysis conditions of biomass.

			Conversion xylan into XOS and Xylose (%)							
			Banana peels		Guava bagasse		Orange Bagasse		Restaurant waste	
Cond	**Temp. (ºC)**	**Time (min)**	**Xylose**	**XOS**	**Xylose**	**XOS**	**Xylose**	**XOS**	**Xylose**	**XOS**
1	100	15	1.73(-)	14.35(-)	0.03	3.62	0.02	5.43	0.60	21.54
2	160	15	4.97	32.28(+)	0.10	5.34	0.74	26.87	0.61	24.71
3	100	55	4.94	25.41	0.02(-)	3.25(-)	0.04	34.29	0.00(-)	17.74(-)
4	160	55	4.71	24.85	0.56	3.58	0.33	5.17(-)	1.62	25.40
5	87.57	35	1.89	18.71	0.21	3.52	4.02(+)	39.10	0.00(-)	18.33
6	172.43	35	5.07(+)	29.35	1.99(+)	8.21(+)	0.00(-)	9.05	0.00(-)	31.35
7	130	6.72	2.13	22.53	0.23	3.59	0.23	35.33	1.09	33.42(+)
8	130	63.28	2.31	20.15	0.17	4.43	0.10	61.51	2.56(+)	33.09
9*	130	35	3.08	28.49	0.04	3.99	0.13	66.13(+)	1.44	28.58

*Central point in triplicate; (-) Lower value; (+) Highest value.

Source: Author.

In comparison with others biomasses, with the same pretreatment however with different experimental conditions, xylan conversion into XOS was lower, with values of 27.10% for barley husks, 24.80% for corncobs, 18% for rice husks and 15.40% for *Eucalyptus* wood material (Parajó et al., 2004). conversion xylan into XOS were reported for corncobs, almond shells, olive stones, rice husks, wheat straw, and barley straw as 60%, 55%, 30%, and 43%, successively (Nabarlatz et al., 2007). With corn fiber obtained the maximum total conversion xylan into XOS 18.60% (Samala et al., 2012).

Statistic Analysis

Models of hemicellulose hydrolysis are based on acid-catalyzed breakdown of long chains of hemicellulose to form shorter oligomers that continue to break down to monomeric sugars. Hemicellulose is hydrolyzed to xylose, that if subjected to acid concentration for long times, turn breaks down to degradation products, like furfural (Jacobsen and Wyman, 2000).

Response variables used for the analysis were conversion xylan into XOS and xylose. These contents are directly linked to the potentiality of the residue, since the XOS content can provide the generation of prebiotics and the xylose content can disrupt this purpose, and the ideal for the purpose of the study is a high production of XOS and low xylose. Therefore, the aim of the study was to define an autohydrolysis condition that would allow an effective release of XOS with a small generation of xylose, besides analyzing the potentiality of the biomasses chosen for this purpose, since not enough studies of these biomasses were found for this purpose. Statistically significant effects of the independent variables temperature and reaction time of biomass pretreatment and XOS as the dependent variable (Figure 1).

From the design statistical, the polynomial regression model relating the percentage of conversion (Y) with independents variables, temperature (T) and time (t), where Y is the predicted percentage xylooligosaccharides conversion and equation 1 represents banana peels, 2 represents guava bagasse, 3 represents orange bagasse and 4 represents restaurant residue, was generated as follows:

$$Y_1 = (8.05 * T) - (3.42 * T^2) + (0.07 * t) - (6.3 * t^2) - (9.24 * T * t) \quad (1)$$

$$Y_2 = (2.19 * T) + (1.36 * T^2) - (0.24 * t) - (0.51 * t^2) - (0.69 * T * t) \quad (2)$$

$$Y_3 = -(13.46 * T) - (50.77 * T^2) + (11.06 * t) - (27.42 * t^2) - (25.28 * T * t) \quad (3)$$

$$Y_4 = (7.16 * T) - (7.15 * T^2) - (0.9 * t) + (1.15 * t^2) + (2.24 * T * t) \quad (4)$$

The coefficient with linear terms of temperature (T) and time (t) shows the effect of that particular factor for the autohydrolysis of biomasses. On the contrary, the coefficient multiplied by two factors (T*t), depicts the interaction effects on the responses. Second order terms related to T^2 and t^2 represent the quadratic effect. A positive sign indicates a synergistic effect, whereas a negative sign indicates an antagonistic effect.

ANOVA (Tables 4 and 5) showed that the variable temperature (T), as well as its square effect (T (Q)), together with the temperature and time interaction variable (T + t), had a significance in the conversion of XOS dependent variable for all biomasses. It can be noted that the standardized value of the temperature effect is the most pronounced for all biomass when compared to the effects obtained by the other independent variables.

Table 4. Analysis of variance (ANOVA) for autohydrolysis of biomass of banana peels and guava bagasse with confidence level of 95%.

Banana Peels					
	SS	**df**	**MS**	**F**	**P**
T - Temp (°C) (L)	129.14	1	129.14	387.41	0.002
T - Temp (°C) (Q)	23.06	1	23.06	69.17	0.014
t - Time (min) (L)	0.01	1	0.01	0.02	0.896
t - Time (min) (Q)	60.41	1	60.41	181.23	0.005
T*t	81.00	1	81.00	243.00	0.004
Lack of Fit	13.48	3	4.49	13.48	0.069
Pure Error	0.67	2	0.33		
Total SS	291.64	10			
R²	0.951				
Guava Bagasse					
	SS	**Df**	**MS**	**F**	**P**
T - Temp (°C) (L)	7.33	1	7.33	5.50	0.144
T - Temp (°C) (Q)	0.98	1	0.98	0.74	0.481
t - Time (min) (L)	0.50	1	0.50	0.37	0.603
t - Time (min) (Q)	1.92	1	1.92	1.44	0.353
T*t	0.00	1	0.00	0.00	1.000
Lack of Fit	4.17	3	1.39	1.04	0.524
Pure Error	2.67	2	1.33		
Total SS	18.73	10			
R²	0.635				

**SS is the Sum of Squares, df is the degree of freedom, MS is the Mean Squares.*

*** (Q) refers to quadratic parameters and (L) to linear parameters.*

Source: Author.

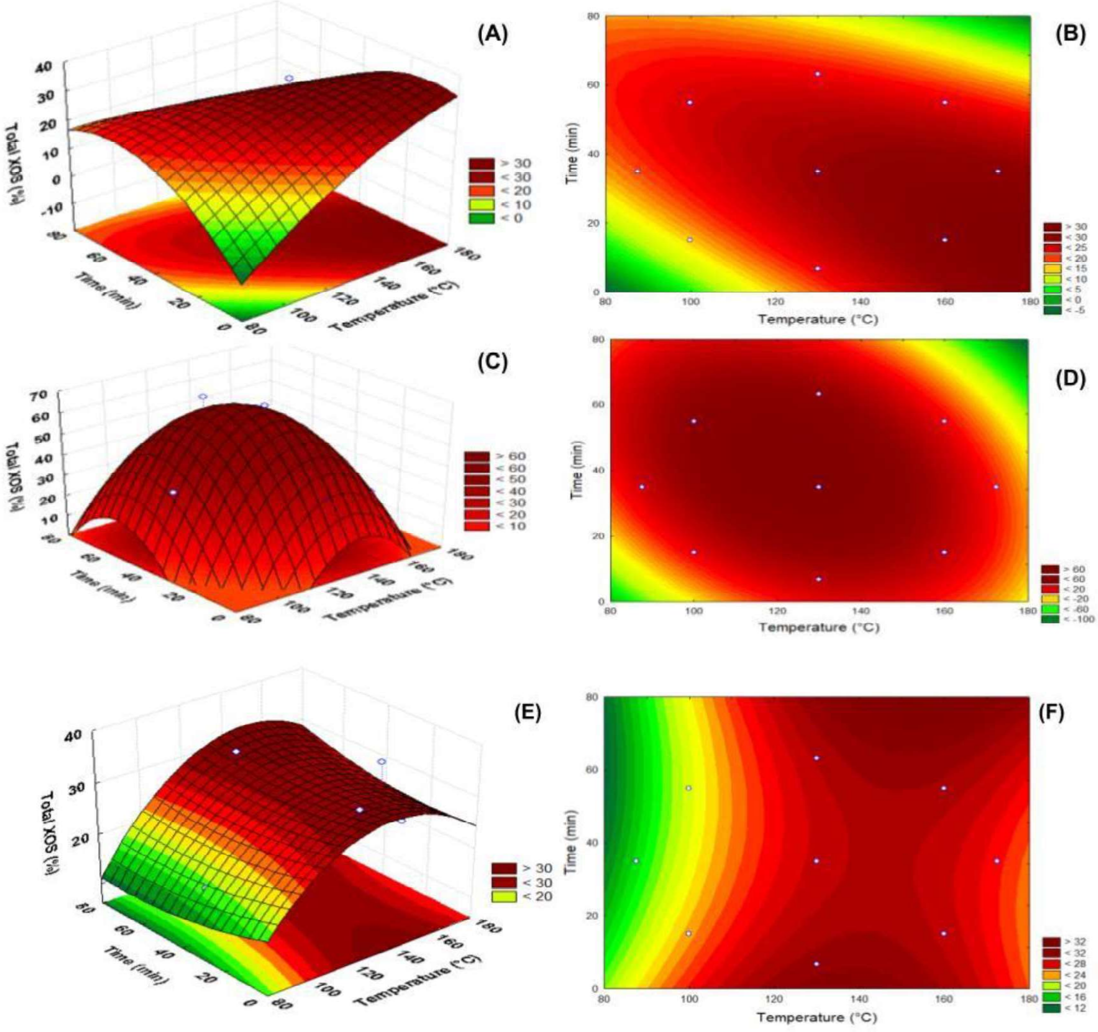
Table 5. Analysis of variance (ANOVA) for autohydrolysis of biomass of orange bagasse restaurant residue with confidence level of 99%.

Orange Bagasse					
	SS	**df**	**MS**	**F**	**p**
T - Temp (°C) (L)	305.39	1	305.39	70.47	0.0139
T - Temp (°C) (Q)	3.64	1	3.64	840.41	0.0012
t - Time (min) (L)	239.45	1	239.45	55.26	0.0176
t - Time (min) (Q)	1.01	1	1.01	233.94	0.0042
T*t	650.25	1	650.25	150.06	0.0066
Lack of Fit	933.81	3	311.27	71.83	0.0138
Pure Error	8.67	2	4.33		
Total SS	5.99	10			
R²	0.8428				
P	99%				
Restaurant Residue					
	SS	**df**	**MS**	**F**	**p**
T - Temp (°C) (L)	103.53	1	103.53	310.58	0.003
T - Temp (°C) (Q)	78.15	1	78.15	234.46	0.004
t - Time (min) (L)	1.14	1	1.14	3.41	0.206
t - Time (min) (Q)	1.30	1	1.30	3.90	0.187
T*t	2.25	1	2.25	6.75	0.122
Lack of Fit	92.91	3	30.97	92.91	0.011
Pure Error	0.67	2	0.33		
Total SS	298.55	10			
R²	0.6866				
P	99%				

**SS is the Sum of Squares, df is the degree of freedom, MS is the Mean Squares.*
*** (Q) refers to quadratic parameters and (L) to linear parameters.*
Source: Author.

Production of XOS with banana peel residue was higher in the region of higher temperatures and reaction time and longer times and lower temperatures, within the established range of conditions (Figure 1AB). With orange bagasse residue it was observed an optimal range, with a higher generation under the medium conditions (Figure 1CD). With restaurant waste it was observed a higher generation under the conditions of higher temperature (Figure 1EF).

Figure 1. Response surface and contour plot as a function of temperature, time of hydrolysis and conversion xylan into xylooligosaccharides (XOS).

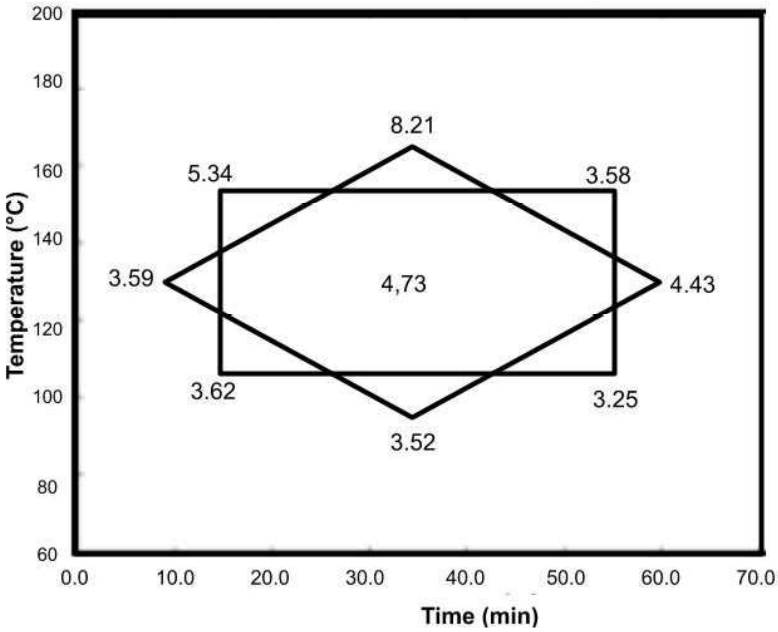


**(A) and (B) represents the banana peel residue, (C) and (D) the Orange bagasse and (E) and (F) the restaurant waste.*

Source: Author.

With guava bagasse residue the model did not present a pattern, but it was possible to observe through yield analysis that a higher generation of XOS occurred under higher temperature conditions (Figure 2).

Figure 2. Conversion xylan into xylooligosaccharides (XOS) as a function of temperature and reaction time in the autohydrolysis of guava bagasse.



Source: Author.

With the obtained results, it was possible to observe that the increase of the temperature combined with average pretreatment times contributed to the hydrolysis of the biomass, increasing the release of oligomers.

Degradation Products

The results of the conversion into degradation products reaffirmed that the chosen interval and the parameters used were effective in converting XOS, considering that all biomasses presented low furfural and hydroxymethylfurfural values, which shows that there was no great breakdown of the oligomers and xylose, as can be seen in the table 6.

Table 6 – Results of degradation products conversion in main conditions.

		Autohydrolysis		
Biomass	**%**	**100 °C and 15 min**	**130 °C and 35 min**	**160 °C and 55 min**
Banana Peel	**HMF**	0.02	0.04	0.21
	Furfural	0.00	0.01	0.04
Guava Bagasse	**HMF**	0.00	0.00	0.02
	Furfural	0.00	0.00	0.01
Orange Bagasse	**HMF**	0.00	0.01	0.05
	Furfural	0.00	0.00	0.05
Restaurant Residue	**HMF**	0.01	0.00	0.02
	Furfural	0.00	0.00	0.00

Conclusion

Evaluation of autohydrolysis pretreatment allowed compared biomass in order to XOS production. Conditions used in the autohydrolysis study resulted in low xylose generation, what is an advantage for XOS production. Production of XOS was about 8 times higher than the production of xylose with banana peel residue, 59 times higher with guava bagasse, 319 higher with orange bagasse and 25 times higher with restaurant waste. Higher amount of XOS compared to xylose showed that biomasses submitted to the pretreatment condition determined potential process. The most advantageous biomass was the orange bagasse, which presented a higher XOS production, with low xylose production. XOS were produced with waste biomasses pretreatment with medium temperature and reaction time as one of the most advantageous condition.

CHAPTER 5

XYLOOLIGOSACCHARIDES PRODUCTION FROM FRUIT WASTE BY ACID HYDROLYSIS

Abstract

Brazil has a large role in the world fruit market, having an expressive production volume. A significant part of this production is wasted annually, a material of high value without use, provoking environmental and social damages. These residues from agroindustrial processes or that can no longer be used in the market (last fruit), are lignocellulosic biomass that can generate value-added products by pretreatments. One of the important pretreatments is acid hydrolysis, which can produce xylooligosaccharides (XOS) from these biomasses, a product of great commercial value in the food and pharmaceutical markets, mainly due to its prebiotic potential. Bananas, oranges and guava generate a large volume of waste, represent much of Brazil's fruit production. The 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 material and pretreatment, the ideal conditions to produce XOS are at high temperature, low time and high acid concentration for banana peel residue (160 °C,15 min and 3% H_2SO_4) , low temperature and time, and high acid concentration for guava bagasse (100 °C,15 min and 3% H_2SO_4), high temperature and acid concentration with low time for orange bagasse (160 °C,15 min and 3% H_2SO_4) and high temperature and time and high acid concentration for restaurant waste (160 °C, 55 min and 3% H_2SO_4). The study identified acid hydrolysis conditions that maximized XOS production with low amount of xylose production using agroindustrial and food residues, also showing the high potential of the chosen residues through the high yields of XOS production.

Keywords: agroindustrial waste, biomass, acid hydrolysis, xylooligosaccharides, prebiotic.

Introduction

Total waste generation in Brazil in 2017 reached 214,868 t / day, according to the 2017 Panorama of Solid Waste in Brazil, prepared by the Brazilian Association of Public Cleaning and Special Waste Companies (ABRELPE), number 1% higher than of the year 2016. Of this total, approximately 52% is made up of organic material (fruit, vegetables, legumes and food in general), and 6.9 million tons of waste were not collected and thus disposed of incorrectly. The big waste of food, solid waste generation and a lack of proper food management plan have become an increasing problem in the food industry. In general restaurants, the variety and quantity of food offered exceeds 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 it is increasingly essential to properly plan food preparation and conscious use of resources, implementing preventive measures for solid waste generation or an ideal reuse of waste generated, and can add value to it from the use and obtaining of products with higher added value. In addition to household disposal, industrial production also generates a large volume of waste, with a loss of 30 to 40% estimated in the fruit production market (DIAS, 2003). Brazil has great representation in the fruit market, which results in a large volume of fruits being wasted annually.

In a recent survey of 2015, the indices had a fruitful production of 40.9 million tons. Among the main fruits produced in Brazil, orange leads in volume, totaling 16.7 million tons from orchards in 2015, which corresponded to 40.9% of the total fruit crops. Among the Brazilian states, São Paulo leads the production, with 12.3 million tons, representing 73.3% of the volume produced (SEAB, 2017). The second most produced fruit in Brazil, in volume, is banana, which had a volume of about 6.7 million tons in 2017, with the main producers being the states of São Paulo and Bahia (ABF, 2018). Another fruit of increasing national production is guava, which grew by 11% in its planted area in the year 2018, while in sugarcane and orange, the increase was, respectively, 0.5% and 2.5% (O Globo, 2018). In addition to this growth, the greatest diversity of guava species is found in the country, which occupies a prominent position among the world's guava producers (POMMER, 2006).

The use of lignocellulosic biomass is fundamentals as economical and sustainable alternative. However, the development of fractionation processes is necessary for the use of compounds present in biomass (MARCONDES, 2018). In order to fractionate the lignocellulosic material, several methods are used, such as pretreatments in acid, alkaline, organic solvents and ionic liquids, each with its advantages and disadvantages (AGBOR et al.,2011). Given the costs involved and sugar recovery at the end of the process, dilute acid, steam explosion and alkaline pretreatments are the most used for biomass fractionation (HENDRIKS; ZEEMAN, 2009). In addition, acid pretreatment has the advantage of short reaction time but, in contrast, degradation products (furfural) and monosaccharide (mainly xylose) formation occurs (AKPINAR; ERDOGAN; BOSTANCI, 2009). Pretreatment with diluted acid allows the fractionation of hemicellulose into oligosaccharides and monosaccharides by cleavage of the xylan chain glycosidic bonds. This breakdown depends on variables such as the type of acid used, the concentration, reaction time and temperature (BRIENZO et al, 2016).

Xylooligosaccharides are a product of great interest and can be obtained industrially from xylan-rich lignocellulosic materials such as agroindustrial by-products (abundant sources of lignocellulosic biomass) (Freitas et al., 2019). For the solubilization and/or hydrolysis of xylan present from the hemicellulosic fraction, different processes can be performed, among which is acid hydrolysis of biomass (QING et al., 2013; VÁZQUEZ et al., 2000). The mainly fruit/food wastes produced in Brazil were studied: banana peel, guava bagasse, orange pomace and restaurant residue. The biomasses were submitted to diluted acid pretreated to produce XOS, determining the experimental conditions that maximize XOS with low xylose production.

Materials and methods

Agroindustrial and food waste

The materials used were banana peel, guava bagasse, orange bagasse and restaurant residue. Residues of guava were gently provided by the companie Predilecta and the orange bagasse by Selial companie, which has not been subjected to any process (pectin or oil extraction). Banana peel was collected in local market.

Restaurant residue was collected at restaurant of university and was composed by the pre-prepare residue, like onion and garlic peel, vegetable peels and leaves in general. Residues were dried at 60 °C until moisture lower than 10%, ground in knife mill and the material that passed through a 20 mesh sieve was selected.

Acid hydrolysis

The acid hydrolysis of the biomass was performed from a 2^3 experimental design with star rotational points (Table 1). The acid hydrolysis variables studied were temperature (°C), time (min) and acid concentration (H_2SO_4 % m/V), and as response variable were studied xylooligosaccharides and xylose.

Table 1 – Experimental design 2^3 with rotational star points, central and coded and real levels of the independent variables for biomass acid hydrolysis.

	Codded Value			Real Value		
Assay	**Temperature**	**Time**	**H2SO4**	**Temperature (°C)**	**Time (min)**	**H_2SO_4 (% m/V)**
1	-1	-1	-1	100	15	1
2	+1	-1	-1	160	15	1
3	-1	+1	-1	100	55	1
4	+1	+1	-1	160	55	1
5	-1	-1	+1	100	15	3
6	+1	-1	+1	160	15	3
7	-1	+1	+1	100	55	3
8	+1	+1	+1	160	55	3
9	-1.41	-1.41	0	79.55	35	2
10	+1.41	+1.41	0	180.45	35	2
11	0	0	0	130	1.36	2
12	0	0	0	130	68.64	2
13	0	0	-1.41	130	35	0.32
14	0	0	+1.41	130	35	3.68
15*	0	0	0	130	35	2

*Central point assay in triplicate

Source: author.

Around 5 g of each biomass were placed in 50 mL stainless steel reactors and heated in a thermostatized bath, according to each condition of the experimental design (Table 1). Acid hydrolysis was performed with 15 different conditions with v triplicates under central point conditions. After the reaction time, the reactor was cooled in an ice bath and the hydrolyzate was filtered with filter paper, separating the solid and liquid fraction which was prepared for HPLC analysis.

The yield in XOS was calculated by the relation between the mass of generated oligomers and the mass of hemicellulose present in the biomass. The conversion of hemicellulose to xylose was calculated by the relationship between the generated xylose mass and the initial hemicellulose mass. XOS content was assessed by liquid chromatography (HPLC, Shimadzu model NEXERA XR) according to literature (MAFEI, 2019). The analysis parameters were BIO-RAD Aminex HPX-87C (300 × 7.8 mm) column; 80 °C oven temperature; ultrapure water (Milli Q) at 0.6 mL min^{-1} as eluent; 20 µL sample volume; refractive index detector at 60 °C (Shimadzu, model RID-20A); and total analysis time of 15 min. Before injection, the pH of the samples was set to 6.5 using NaOH 1 mol/L. Xylose (X_1; Sigma), xylobiose (X_2; Megazyme), xylotriose (X_3; Megazyme), xylotriose (X_4; Megazyme), xylopentaose (X_5) + xylohexaose (X_6)(Megazyme), and oligosaccharides with more than 6 xylose units were quantified. The concentration of XOS (mg/mL) was obtained by sum the concentrations of X_2+X_3+X_4+X_5 present in the hydrolyzate, determined by liquid chromatography and converted to percentage.

Degradation Products

The parameters measured were hydroxymethylfurfural and furfural content, that was measured by reading the biomass samples in their main parameters and shows the conversion into degradation products. The hydrolysates were read by the isocratic method, using a C18 column (NST 150mm x 4.6mm x 0.5µm). As a mobile phase, a mixture of water with acetronitrile, in the proportion 80:10 and 1% acetic acid, was used. The flow rate was maintained at 0.8 mL / min, column at 35 ºC. Readings of the hydrolysates were made in the mildest conditions (100 ºC and 15 min), in the medium condition (130 °C and 35 min) and in the most severe condition (160 °C and 55 min).

Design of a response surface methodology experiment for biomass pretreatment

The results were analyzed using STATISTIC 7 software, generating 3D and 2D graphs and ANOVA tables, that show the influence variables in the generation of xylose and xylooligosaccharides.

Results and discussion

XOS production

The value of xylooligosaccharides production was reported as the percentage by mass of xylan that was converted into XOS. The highest yield was obtained under the following conditions: for banana peels, the highest XOS value was 37.69%, at high temperature, low time and high concentration of acid (160 °C,15 min and 3% H_2SO_4); for guava bagasse the yield was 59.60% of XOS at low temperature, low time and high concentration of acid (100 °C,15 min and 3% H_2SO_4); for orange bagasse the highest XOS yield occurred at high temperature, low time and high concentration of acid (160 °C,15 min and 3% H_2SO_4), which was 28.70%; for restaurant waste, the highest XOS yield was 49.64%, occurring under the highest experimental conditions (160 °C, 55 min and 3% H_2SO_4).

Compared to sugarcane bagasse submitted to the same type of pretreatment, biomasses showed higher potential, considering that for sugarcane, a maximum XOS yield found was 44.5% and for sugarcane pulp residual hemicellulose levels of 45.18% XOS were found (Zhou and Xu, 2019; Wang et al, 2018). Under the optimal conditions for brewery's spent grain (Temperature of 170 °C, acetic acid concentration of 5% and reaction time of 30 min), yield of 6.9% of XOS and 8.9% of xylose were found (Wen et al., 2019). With tobacco stalk (Temperature of 100 °C, acid concentration of 0.25 mol/L of H_2SO_4 and reaction time of 30 min) a yield of 13% of XOS and 14% of monosaccharides were reported (Akpinar et al., 2010). These results showed a great potential of the fruit waste biomasses used in the present study, since the optimum conditions for each biomass presented higher XOS contents with much lower xylose concentrations.

With the objective of XOS production it is desired low formation of xylose. For this reason, it is important to optimize the acid hydrolysis to maximize XOS with low xylose production. In this study, acid was applied at diluted condition considering the percentage of mass of acid per mass of material. For xylose yield, the maximum value for banana peel was 2.64%, at high temperature, low time and high concentration of acid (160 °C,15 min and 3% H_2SO_4); for guava bagasse, the highest content was 5.69% at high temperature, low time and high concentration of acid (160 °C,15 min and 3% H_2SO_4), for orange bagasse, the highest xylose content occurred at medium temperature and time and at high acid concentration, being 3.10% (130 °C, 35 min and 3.68% H_2SO_4 - star point) and for restaurant waste, the highest value was 4.58%, occurring under the highest conditions (160 °C, 55 min and 3% H_2SO_4).

For sugarcane bagasse, contents of 7 to 13% of xylose were found (Jacobsen and Wyman, 2002); and 8.9 % of xylose were reported for brewery's spent grain (Wen et al., 2019), higher values than those found in the study, which shows the potentiality of the studied biomass, considering that the lower xylose formation is advantageous for the study objective.

Table 2 – Xylooligosaccharides and xylose yield by acid hydrolysis of biomasses using a factorial design 2³ with acid concentration (%, m/V, time (min) and temperature (°C) variation.

				Banana Peels		Guava Bagasse		Orange Bagasse		Restaurant Waste	
				Yield (%, base dry mass)							
Assay	**Temp (°C)**	**Time (min)**	**H_2SO_4 (%)**	**Xylose**	**XOS**	**Xylose**	**XOS**	**Xylose**	**XOS**	**Xylose**	**XOS**
1	100	15	1	0.21	7.72	0.00	2.29	0.14	2.03	0.00	9.27
2	160	15	1	0.96	18.94	1.77	3.13	0.50	0.79	0.05	6.92
3	100	55	1	0.29	4.64	0.05	3.36	0.03	0.60	0.00	5.34
4	160	55	1	1.11	5.75	3.53	2.67	1.98	3.61	0.78	11.76
5	100	15	3	0.25	30.07	0.03	59.60	0.03	17.11	0.00	26.86
6	160	15	3	2.64	37.69	5.69	28.07	3.03	28.70	0.00	22.68
7	100	55	3	0.37	30.75	0.26	31.52	0.25	17.69	0.25	26.87
8	160	55	3	2.11	32.23	2.94	28.88	3.00	24.65	4.58	49.64
9	79.55	35	2	1.21	16.72	0.18	40.75	0.05	24.29	0.00	14.62
10	180.45	35	2	0.94	17.41	0.53	14.78	1.34	11.75	1.80	16.16
11	130	1.36	2	0.00	0.00	0.00	15.81	0.06	19.76	0.07	13.05
12	130	68.64	2	1.60	16.11	3.16	17.47	0.48	16.35	0.21	9.92
13	130	35	0.32	0.34	2.59	0.00	1.85	0.81	9.47	0.00	1.58
14	130	35	3.68	1.93	17.22	2.65	14.31	3.10	15.10	1.14	17.40
15*	130	35	2	0.84	16.25	0.70	15.69	1.02	11.34	0,30	12.78

*Central point in triplicate.

Source: Author.

Central composite design model fitting

From the design statistical, the polynomial regression model relating the percentage of conversion (Y) with independents variables, temperature (T), time (t) and sulfuric acid concentration (H_2SO_4), where Y is the predicted percentage xylooligosaccharides conversion and equation 1 represents banana peels, 2 represents guava bagasse, 3 represents orange bagasse and 4 represents restaurant residue, was generated as follows:

$$Y_1 = (3.31 * T) + (6.65 * T^2) + (0.88 * t) + (0.27 * t^2) + (17.33 * H_2SO_4) + (1.59 * H_2SO_4{}^2) - (4.06 * T * t) - (0.81 * T * H_2SO_4) + (2.87 * t * H_2SO_4) \quad (1)$$

$$Y_2 = -(11.38 * T) + (9.13 * T^2) - (3.49 * t) + (1.26 * t^2) + (23.09 * H_2SO_4) - (4.8 * H_2SO_4{}^2) + (6.84 * T * t) - (8.58 * T * H_2SO_4) - (6.97 * t * H_2SO_4) \quad (2)$$

$$Y_3 = -(0.11 * T) + (2.72 * T^2) - (1.14 * t) + (2.75 * t^2) + (13.28 * H_2SO_4) - (1.34 * H_2SO_4{}^2) - (0.09 * T * t) + (4.19 * T * H_2SO_4) - (1.21 * t * H_2SO_4) \quad (3)$$

$$Y_4 = (3.7 * T) + (3.97 * T^2) + (3.31 * t) + (1.2 * t^2) + (17.49 * H_2SO_4) - (0.19 * H_2SO_4{}^2) + (8.93 * T * t) + (3.63 * T * H_2SO_4) + (6.51 * t * H_2SO_4) \quad (4)$$

The coefficient with linear terms of temperature (T), time (t) and sulfuric acid concentration (H_2SO_4), shows the effect of that particular factor for the acid hydrolysis of biomasses. On the contrary, the coefficient multiplied by two factors like T*t, depicts the interaction effects on the responses. Second order terms related like T^2 for example, represent the quadratic effect. A positive sign indicates a synergistic effect, whereas a negative sign indicates an antagonistic effect.

Tables ANOVA (Tables 3 and 4) showed that the acid concentration variable had a significant positive effect on the content of the XOS dependent variable for all biomasses and, in the case of restaurant residue, the interaction of Temperature and time variables also had positive significance. The effect of acid concentration had its most pronounced standardized value for all biomasses when compared to the effects obtained by the other independent variables. These results could show that for banana peels, guava bagasse and orange bagasse, the model using the axial points did not lead to the best result, being a first order model more appropriate for the analysis of these biomasses. With data at work, a first-order equation better explained the results.

With first order model, for banana peel, it was possible to observe the significance of acid concentration, temperature, time and curvature. Although the curvature is significant, the analysis with the rotational points did not lead to a second order model adjustment. For this reason, the statistical analysis followed with the standard design data. For guava bagasse, temperature, acid concentration and the interaction between the two parameters had significance. The same happened for orange bagasse.

For the analysis, the response variables used were the contents of the oligomers of interest (xylooligosaccharides and xylose) in the hydrolyzed material. The contents of these oligomers are directly linked to the potentiality of the residue, since XOS content can provide prebiotic generation and xylose content can disrupt this objective, and ideally for the purpose of the study is a high production of XOS and low xylose content. Thus, the study focused on the analysis of acid hydrolysis conditions that would allow an effective release of XOS with a small generation of xylose, besides analyzing the potentiality of the biomass chosen for this purpose, since not enough studies of these biomass were found for this purpose. The statistically significant effects of XOS generation as the dependent variable and the independent variables (T, t and acid concentration) of greatest pretreatment significance for each biomass are presented in Figures 2 and 3.

Table 3 - Analysis of variance (ANOVA) for acid hydrolysis of biomass of banana peels and guava bagasse with confidence level of 95%.

	Banana Peels				
	SS	df	MS	F	p
Curvature	65.00	1	65.00	27.86	0.034
T - Temp (°C)	55.12	1	55.12	23.62	0.039
t - Time (min)	55.12	1	55.12	23.62	0.039
H_2SO_4 (%)	1081.12	1	1081.12	463.34	0.002
T*t	36.12	1	36.12	15.48	0.058
T+H2SO4	1.12	1	1.12	0.48	0.559
t+H2SO4	15.12	1	15.12	6.48	0.126
Lack of Fit	1.12	1	1.12	0.48	0.559
Pure Error	4.67	2	2.33		
Total SS	1314.54	10			
R^2	0.9956				

	Guava Bagasse				
	SS	df	MS	F	P
Curvature	3.19	1	3.19	9.56	0.0906
T - Temp (°C)	10.12	1	10.12	30.37	0.0314
t - Time (min)	0.12	1	0.12	0.37	0.6026
H_2SO_4 (%)	1596.12	1	1596.12	4788.37	0.0002
T*t	1.12	1	1.12	3.37	0.2076
T+ H_2SO_4	15.12	1	15.12	45.37	0.0213
t+ H_2SO_4	1.12	1	1.12	3.37	0.2076
Lack of Fit	3.12	1	3.12	9.37	0.0922
Pure Error	0.67	2	0.33		
Total SS	1630.73	10			
R^2	0.9977				

*SS is the Sum of Squares, df is the degree of freedom, MS is the Mean Squares.

** (Q) refers to quadratic parameters and (L) to linear parameters.

Table 4 - Analysis of variance (ANOVA) for acid hydrolysis of biomass of orange bagasse with confidence level of 95% and restaurant residue with confidence level of 99%.

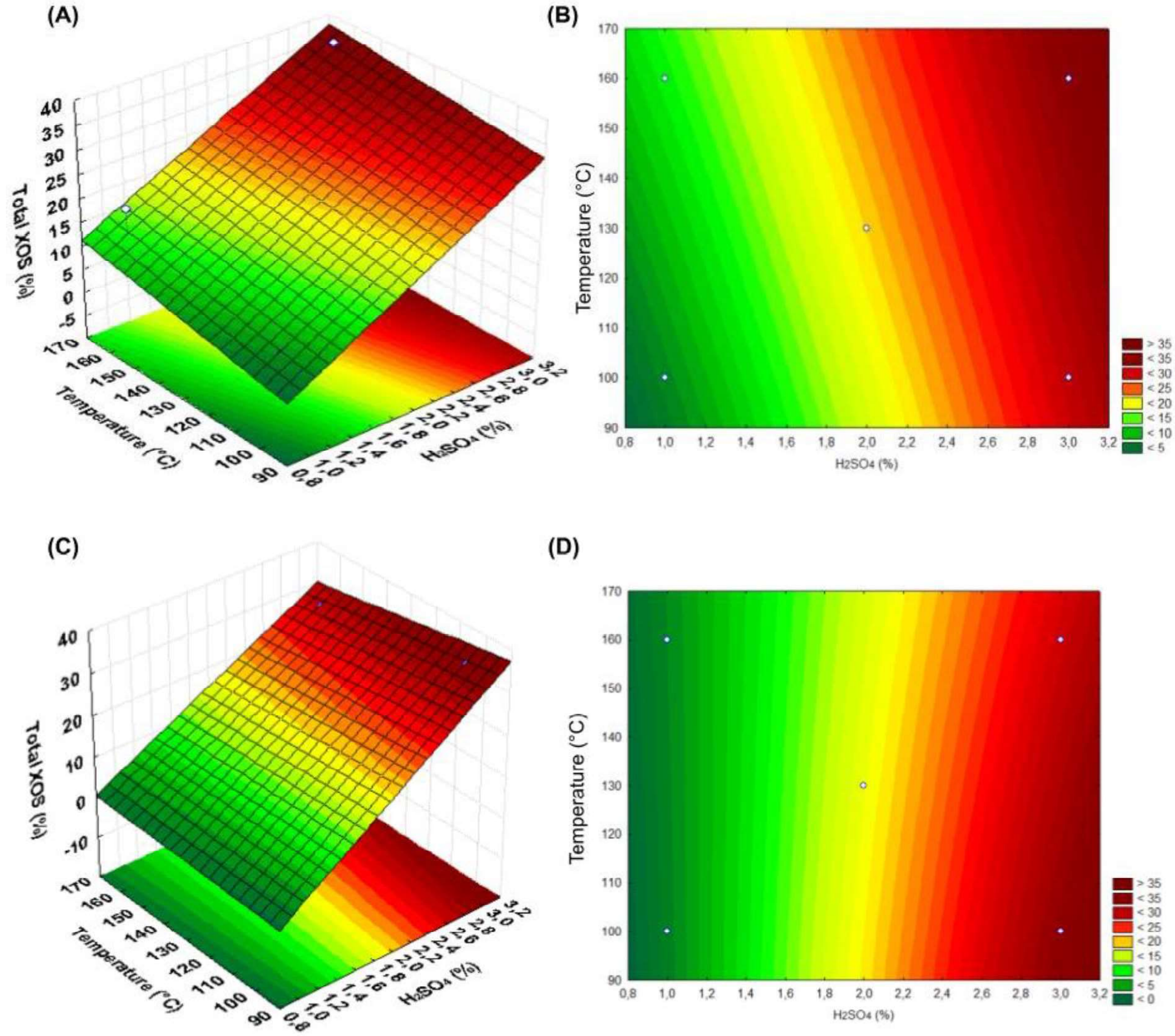
	Orange Bagasse				
	SS	**df**	**MS**	**F**	**p**
Curvature	2.76	1	2.76	2.76	0.2384
T - Temp (°C)	55.12	1	55.12	55.12	0.0177
t - Time (min)	0.12	1	0.12	0.12	0.7575
H_2SO_4 (%)	820.12	1	820.12	820.12	0.0012
T*t	0.12	1	0.12	0.12	0.7575
T+ H_2SO_4	36.12	1	36.12	36.12	0.0266
t+ H_2SO_4	3.12	1	3.12	3.12	0.2191
Lack of Fit	10.12	1	10.12	10.12	0.0862
Pure Error	2.00	2	1.00		
Total SS	929.63	10			
R^2	0.987				
	Restaurant Residue				
	SS	**df**	**MS**	**F**	**p**
T - Temp (°C) (L)	48.29	1	48.29	36.22	0.0265
t - Time (min) (Q)	71.73	1	71.73	53.80	0.0181
t - Time (min) (L)	38.58	1	38.58	28.93	0.0329
t - Time (min) (Q)	13.82	1	13.82	10.37	0.0844
H_2SO_4 (L)	1041.32	1	1041.32	780.99	0.0013
H_2SO_4 (Q)	1.84	1	1.84	1.38	0.3609
T*t	162.00	1	162.00	121.50	0.0081
T+ H_2SO_4	24.50	1	24.50	18.37	0.0503
t+ H_2SO_4	84.50	1	84.50	63.37	0.0154
Lack of Fit	503.39	5	100.68	75.51	0.0131
Pure Error	2.67	2	1.33		
Total SS	1980.94	16			
R^2	0.7445				

*SS is the Sum of Squares, df is the degree of freedom, MS is the Mean Squares.

** (Q) refers to quadratic parameters and (L) to linear parameters.

For restaurant waste, the model using the axial points resulted in to the best yield, as second order model more appropriate for the analysis of this biomass acid hydrolysis for XOS production. The parameters that had significance for this biomass were the acid concentration and the interaction between temperature and time. From these analyzes were generated the surface graphs, which allowed the observation in the analysis of the generation of XOS according to the parameters that showed significance. Figure 2A and 2B represents the XOS production from banana peel residue, where it is possible to observe a higher yield in the combination regions of the highest acid concentrations and medium to high temperatures, confirmed by the contour plot showed in figure 2B. For guava bagasse residue (Figure 2C, 2D), the best conditions of XOS production were under the conditions of highest acid concentrations and lower to medium temperature. For orange bagasse residue (Figure 3E, 3F), the higher XOS production was at the highest concentrations of acid and temperature. For restaurant waste (Figure 3G, 3H), the higher XOS production was under the conditions of higher temperature and acid concentration.

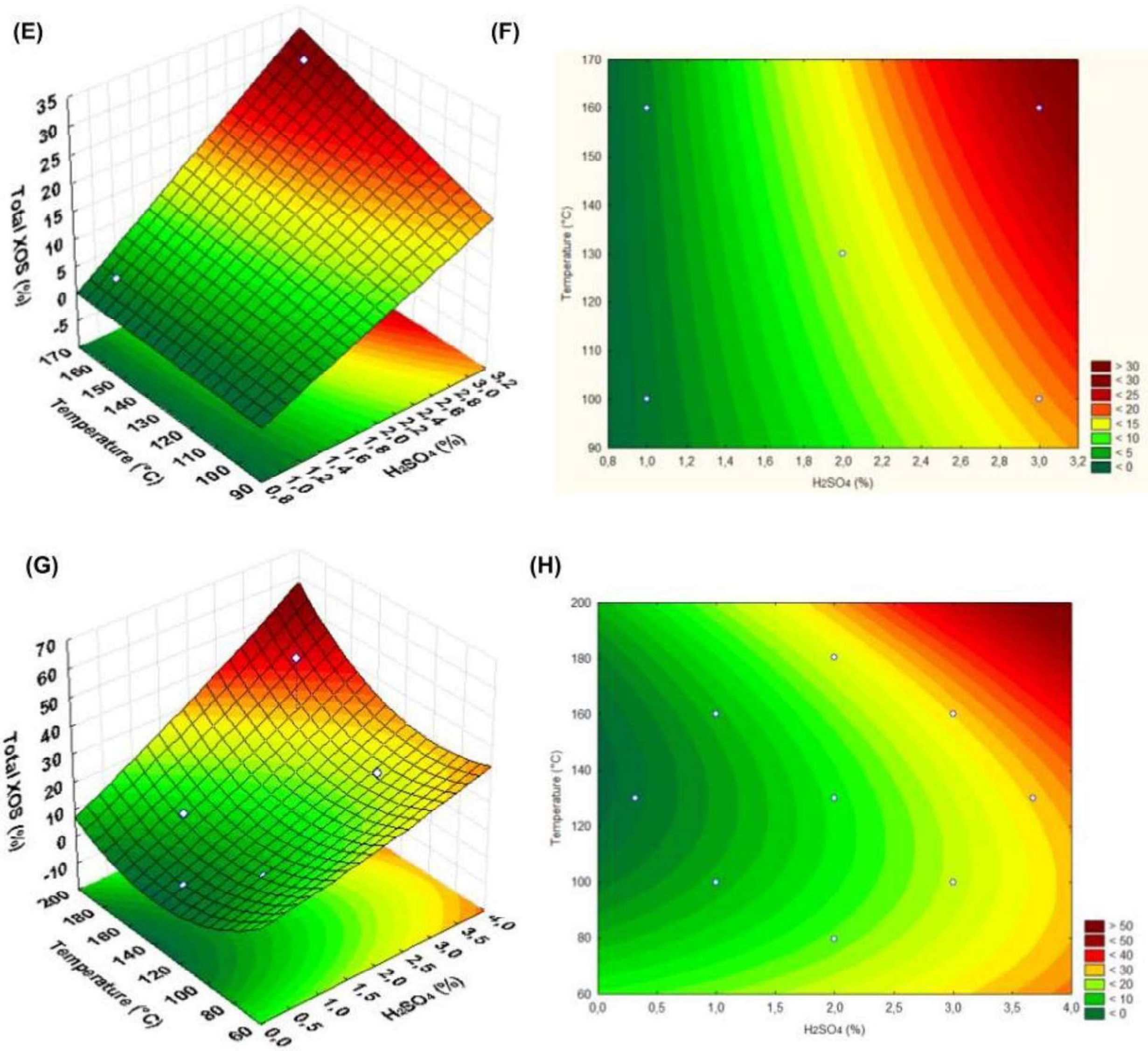
Figure 2 - Variation of total XOS production (%) in acid hydrolysis as a function of temperature (°C) and acid concentration (H_2SO_4 % m/V) for biomass of banana peels and guava bagasse.



*(A) and (B) represents the banana peel residue, (C) and (D) the guava bagasse.

Source: Author.

Figure 3 – Variation of total XOS production (%) in acid hydrolysis as a function of temperature (°C) and acid concentration (H2SO4 % m/V) for biomass of orange bagasse and restaurant residue.



*(E) and (F) represents the Orange bagasse and (G) and (H) the restaurant residue.

Source: Author.

The combination between acid concentration and temperature with higher level in acid pretreatment could contribute to the banana peels, orange bagasse and restaurant residue hydrolysis increases the release of XOS. For guava bagasse to increase acid concentration and decrease temperature could result in a better XOS yield by dilute acid pretreatment.

Degradation Products

The results of the conversion into degradation products reaffirmed that the chosen interval and the parameters used were effective in converting XOS, considering that all biomasses presented low furfural and hydroxymethylfurfural values, which shows that there was no great breakdown of the oligomers and xylose, as can be seen in the table 5.

Table 5 – Results of degradation products generation in main conditions.

		Acid Hydrolysis		
Biomass	**%**	**100 °C, 15 min, 1% H2SO4**	**130 °C, 35 min, 2% H2SO4**	**160 °C, 55 min, 3% H2SO4**
Banana Peel	**HMF**	0.10	0.39	0.37
	Furfural	0.01	0.01	0.48
Guava Bagasse	**HMF**	0.00	0.07	0.14
	Furfural	0.01	0.01	1.24
Orange Bagasse	**HMF**	0.00	0.00	0.24
	Furfural	0.00	0.00	0.78
Restaurant Residue	**HMF**	0.00	0.16	0.49
	Furfural	0.00	0.02	0.26

Conclusion

The acid hydrolysis resulted in a high production potential of XOS with low xylose yield. Acid concentration was the most significance variable, since the highest XOS percentages occurred under the conditions of higher acid concentrations. XOS maximization in relation to xylose production is a fundamental point to use acid hydrolysis. The production of XOS was about 25 times higher than the production of xylose for banana peel residue, 147 times higher for guava bagasse, 94 higher for orange bagasse and 38 times higher for restaurant waste, which showed that biomasses submitted to this pretreatment have great potential. It was also possible to observe that all biomasses presented high XOS yields, with emphasis on guava bagasse, which presented higher XOS production, with low xylose production in relation to the percentage of XOS produced.

CHAPTER 6

XYLAN SOLUBILIZATION FROM FOOD AND AGROINDUSTRIAL WASTE AND XOS PRODUCTION BY ENZYMATIC HYDROLYSIS

Abstract

A large volume of lignocellulosic biomass waste is discarded daily, and the use of alkaline and enzymatic treatments is an alternative it to reuse and generate value added products. In this study four agroindustrial residues banana peel, guava bagasse, orange bagasse and restaurant residue were used. The biomasses were subjected to three types of alkaline treatment: 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 *Aspergiullus 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). The results showed that each of the biomass needed an ideal condition of pretreatment. The ideal conditions to obtain xylan are with NaOH treatment for banana peels and guava bagasse, and with KOH for orange bagasse and restaurant waste. To obtain xylooligosaccharides the best biomasses were treated with peroxide for banana peels and orange bagasse, and with potassium hydroxide for guava bagasse and restaurant waste, with 12 and 48 h of enzymatic hydrolysis.

Keywords: agroindustrial waste, biomass, alkaline treatment, xyloligosaccharides, xylose.

Introduction

A wide variety of residues such as forest and agroindustrial can be a source of lignocellulosic materials. These materials are basically composed of three major polymers, namely lignin, cellulose and hemicellulose (MELATI, 2019). Hemicelluloses are much smaller molecules (lower degree of polymerization) than celluloses, composed mainly of xylose, arabinose, rhamnose, galactose and mannose residues. The sugar units that form hemicelluloses can be subdivided into components such as pentoses, hexoses and uronic acids. (BRIENZO et al., 2016). Among the hemicelluloses, xylan stands out as the most abundant polysaccharide in grass hemicelluloses and, consequently, the one with higher incidence in agricultural and agroindustrial residues (GÍRIO et al, 2010). The main structure of isolated xylan present in plants is the same, which is composed of xylose residues, differing by pendant groups and branched residues (MADEIRA JUNIOR et al, 2017; ÁLVAREZ et al, 2017). Hemicellulose can be use as feedstock to several industrial interest molecules, applying chemical and enzymatic treatments.

For the production of XOS, the extraction of hemicellulose is necessary to obtain in isolated form and subsequent hydrolysis for the production of oligomers. The extraction method depends on the final purpose and application of this xylan, and may be based on the desired hemicellulose final characteristics. Some important characteristics to consider are the degree of polymerization, degree of branching/substitution, solubility, reactivity and impurities such as residual lignin. Considering the recovery of hemicellulose based on the polysaccharide or monosaccharide form, the concepts of acid and alkaline treatments can be successfully applied. Acid extraction and its derived processes lead to end products based on monosaccharides and oligosaccharides. Alkaline extraction and its derivative processes lead to end products based on high polymerization macromolecules (BRIENZO et al., 2016). For obtaining XOS, both methods can be applied, with differences in the final product, since for acidic methods low XOS and high xylose content with degradation products are obtained, while for alkaline methods, acid processes or enzymatic hydrolysis are required to produce XOS. Despite the many possibilities for obtaining XOS, industrial scale production is not yet economically and technically feasible (CARVALHO et al., 2013). Hemicellulose has great potential and applicability such as in the manufacture of biofilms and additives in papermaking. Hemicellulose can be applied in its hydrolyzed form as oligosaccharides for therapeutic and analytical purposes, or for xylitol production in its monomer form. The use of hemicellulose has been highlighted in the health area, as the search for a healthy lifestyle and care with food are increasing, boosting the use of components that stimulate the proper functioning of the body, such as xylose oligosaccharides, which can have several beneficial health effects such as lowering serum cholesterol levels, stimulating bifidobacteria in the gastrointestinal tract and preventing caries (BRIENZO et al., 2016).

Xylooligosaccharides (XOS) has great potential for improving human health and well-being by maintaining intestinal microflora. They can be incorporated into many food products, and their properties can offer a new dimension to the development of functional foods (AACHARY and PRAPULLA, 2011). Because of their varied influence on human health, as well as their potential to prevent gastrointestinal problems, XOS are gain space in the prebiotic market. Still stand out the advantages of being produced from lignocellulosic waste, abundant and low-cost material, which are not suitable for human consumption and are available in large quantities. In addition, agricultural waste is still underutilized and the fact that xylan of these materials can be converted into higher value-added products meets the need for use of this wide variety of waste (SAMANTA et al., 2015).

The use of enzymes for XOS production is considered a cleaner form of production as it does not require high temperatures, pressure or harmful chemicals. In addition, XOS recovery is still possible as it does not produce by-products in the process (AKPINAR, 2007). This enzymatic production, through the action of endoxylanases, allows the low formation of sugar monomers, the absence of byproducts generated during hydrolysis, and does not require special equipment or high pH and temperature conditions. At the same time, this process has the disadvantage of being easily inhibited by compounds present in lignocellulosic biomass, requiring some pretreatment to remove these compounds, in addition to the cost of enzymes (AACHARY; PRAPULLA, 2011; QING et al., 2013). The production of XOS from enzymatic hydrolysis can be done through the action of endoxylanase in the xylan chain. It is important that hemicellulose extraction from lignocellulosic biomass is made and that the enzyme extract is free of β-xilosidase, enzyme that hydrolyzes XOS to xylose, since the presence of the enzyme β-xylosidase is harmful, since it performs the hydrolysis of XOS in xylose, reducing the yield of XOS production (FREITAS, 2019). Thus, enzymatic hydrolysis of extracted xylan should be performed by applying the enzyme endo-β-1,4-xylanase, with little or no activity of the enzyme β-xylosidase, given that the objective is the production of oligomers rather than monosaccharides, such as xylose. In contrast, there is no possibility of controlling the degree of polymerization of the produced XOS, since xylanases with different substrate specificities generate different hydrolysis products (AKPINAR et al, 2010).

From this, the study aimed to analyze the xylan solubilization from alkali pre-treatments using different agroindustrial lignocellulosic biomasses, which are the banana peels, guava bagasse, orange bagasse and restaurant residue to, from this solubilized xylan, optimize the obtaining xyloligosaccharides from enzymatic hydrolysis using pure enzyme.

Materials and methods

Agroindustrial and food waste

The materials used were banana peel, guava bagasse, orange bagasse and restaurant residue. Residues of guava were gently provided by the companie Predilecta and the orange bagasse by Selial companie, which has not been subjected to any process (pectin or oil extraction). Banana peel was collected in local market. Restaurant residue was collected at restaurant of university and was composed by the pre-prepare residue, like onion and garlic peel, vegetable peels and leaves in general. Residues were dried at 60 °C until moisture lower than 10%, ground in knife mill and the material that passed through a 20 mesh sieve was selected.

Chemical Characterization

Chemical characterization of biomasses was performed to determine the content of glucan/cellulose, hemicellulose and lignin. Chemical composition was determined at sulfuric acid reaction with extractive-free samples (ABNT NBR 16550-2016; SLUITER et al., 2010). The soluble fraction was used to determine sugars and acetic acid by high efficiency liquid chromatography (HPLC), and lignin was determined by gravimetry and spectrophotometry. Process was done by hydrolyzing approximately 300 mg of biomass, adding 3 mL of 72 % (m/m) sulfuric acid. Reaction occurred at 45 °C for 7 min. Reaction was stopped with the addition of 84 mL of distilled water. This mixture was autoclaved at 121 °C for 30 min (Gouveia et al., 2009). Contents were filtered through a previously tared porous plate filter n° 4. Solid residue was washed with distilled water, oven dried at 105 °C to a constant weight for the determination of insoluble lignin (Klason). Soluble fraction was used to determine sugar and acetic acid contents by HPLC. Acid soluble lignin content was determined by measuring absorbance at 215 and 280 nm (ABNT NBR 16550-2016).

Glucose, xylose, arabinose and acetic acid concentrations were determined by HPLC using Aminex HPX-87H Bio-Rad column (300 x 7.8 mm) maintained at 45 °C, WATERS 2414 refractive index detector, 0.005 mol/L sulfuric acid mobile phase, 0.6

mL/min flow, 20 µL injected sample volume. The samples were previously filtered through a 0.22 µm syringe filter.

Hemicellulose solubilization

Previously to the solubilization the biomass was washed with 0.2% (m/v) ethylenediaminetetraacetic acid (EDTA) for 1h at 90 ºC to remove metal cations, such as iron manganese ions. These ions promote the decomposition of hydrogen peroxide reducing its delignification performance (MONTE et al., 2011). The xylan solubilization was evaluated with and without washing.

The biomasses were subjected to xylan extraction using three reaction solutions: hydrogen peroxide in alkaline medium (pH 11.6), sodium hydroxide (NaOH) and potassium hydroxide (KOH). About 10 g samples of biomass were treated with alkaline peroxide (6%, m/v) in a reaction volume of 200 mL. In the case of hydrogen peroxide, before the addition of biomass, the solution was pH adjusted to 11.6 with 6 mol/L NaOH solution and was shaking at 120 rpm at room temperature and. The solubilization with sodium hydroxide (NaOH) and potassium hydroxide (KOH) in a thermostated bath at 70 ºC and shaking at 70 rpm were used. After 4 h, the hydrolysate was filtered and the insoluble fraction was discarded. The supernatant liquid was adjusted to pH 6 with 6 mol/L HCl and then concentrated to about one third of its volume under air circulation at 45 °C. The concentrated sample was added three volumes of 95% ethanol to precipitate hemicellulose, which was then washed about four times with 70% ethanol. The decanted hemicellulose was air dried at 45 ºC. The yields were calculated based on the sum of the biomass anhydroarabinan and anhydroxylan contents, from their initial chemical characterization.

Enzymatic hydrolysis of xylan

Enzymatic hydrolysis was performed with endoxylanase produced by *Aspergillus versicolor* and purified by ion exchange chromatography and gel filtration techniques, and with hemicellulose extracted from banana peel, guava bagasse, orange bagasse and restaurant residue biomasses in alkaline medium with H_2O_2, NaOH and KOH 6% (m/v). Enzymatic hydrolysis was performed by varying the parameter of reaction time in order to evaluate the best conditions for xylooligosaccharides production according to the extraction reagent and reaction time.

The hydrolysis was conducted in 50 mL centrifuge tubes containing 5 mL of 50 mmol/L sodium phosphate buffer (pH 6) at 55 °C in a water bath with 120 rpm agitation. The reaction was conducted with *A. versicolor* purified endoxylanase produced in wheat bran, applying 30 UI/g (Freitas, 2019). The enzymatic hydrolysis analyzed parameters such as reaction time and xylan solubilization type (2 to 48h, NaOH, KOH and H_2O_2). At time intervals the tubes were collected to monitor the xylooligosaccharides content, removing an aliquot of 0.1 mL heated in boiling water for 5 min (BRIENZO et al., 2009). The hydrolysate was filtered with a 0.22 µm syringe filter prior to HPLC injection for XOS quantification. XOS content was assessed by liquid chromatography (HPLC, Shimadzu model NEXERA XR) according to literature (MAFEI, 2019). The analysis parameters were BIO-RAD Aminex HPX-87C (300 × 7.8 mm) column; 80 °C oven temperature; ultrapure water (Milli Q) at 0.6 mL min^{-1} as eluent; 20 µL sample volume; refractive index detector at 60 °C (Shimadzu, model RID-20A); and total analysis time of 15 min. Before injection, the pH of the samples was set to 6.5 using NaOH 1 mol/L. Xylose (Sigma), xylobiose (Megazyme), xylotriose (Megazyme), xylotriose (Megazyme), xylopentaose + xylohexaose (Megazyme), and oligosaccharides with more than 6 xylose units were quantified.

Results and discussion

Characterization of biomasses

The moisture content was 88.37% for banana peel, 39.06% for guava bagasse, 79.43% for orange bagasse and 99.67% for restaurant waste (Table 1). The ash content, inorganic material, was 13.22% for banana peel, 1.14% for guava bagasse, 4.50% for orange bagasse and 12.89% for restaurant waste. The total extractives content (in ethanol and water) was 62.97% for banana peels, 27.39% for guava bagasse 48.69% for orange bagasse and 48.49% for restaurant waste. The result of lignin content was 30.28% for banana peel, 31.94% for guava bagasse, 21.45% for orange bagasse and 12.10% for restaurant waste.

The amount of total hemicellulose in the biomass was calculated with sum of xylan contents, arabinan and acetyl groups. The sum of the components resulted in the average of hemicellulose, that was 38.42, 28.33, 31.04 and 20.58% for banana peel, guava bagasse, orange bagasse and restaurant waste. The glucan/cellulose (anhydroglucose) content was 29.28, 29.93, 24.42 and 50.20% for banana peels, guava bagasse, orange bagasse and restaurant waste (Table 1).

Table 1 - Chemical composition of agroindustrial and food waste.

(%)	Banana Peels	Guava Bagasse	Orange Bagasse	Restaurant waste
Ashes	13.22 ± 0.01	1.14 ± 0.00	4.50 ± 0.01	12.89 ± 0.08
Lignin	30.77 ± 2.62	33.75 ± 2.52	22.74 ± 0.12	12.77 ± 0.94
Cellulose	29.28 ± 0.39	29.93 ± 0.34	24.42 ± 1.96	50.52 ± 0.75
Hemicellulose	38.42 ± 2.21	28.33 ± 0.53	31.04 ± 0.98	20.58 ± 0.32

Source: Author.

The values found for the chemical composition were similar to those found in the literature. For banana peels, ash was reported as 11.47% and moisture of 89.47% (Gondim et al., 2015), and lignin of 20.61 % (Souza et al., 2016). For guava bagasse ash was reported as 1.36% and hemicellulose of 37.20% (Santos et al., 2011), and moisture content of 53 % (Silva et al., 2006). For orange bagasse was 2.90% of lignin and 26.45% of hemicellulose (Orozco et al., 2014), and 38.50 and 56.60 of cellulose and extractives, respectively (Moraes and Crestani, 2018). For restaurant waste was reported 10.78, 23.26, 33.62, 4.86, 56.58 and 91.78% of ash, lignin, cellulose, hemicellulose, extractives and moisture, successively (Pereira, 2017).

Hemicellulose solubilization

The biomass hemicellulose was extracted with different agents, and the extracted hemicelluloses were used in the enzymatic hydrolysis step for XOS production, using *Aspergillus versicolor* endo-xylanase purified (FREITAS, 2019). The hemicellulose biomasses extraction was evaluated with washed and not washed with EDTA.

The hemicellulose solubilization with hydrogen peroxide resulted in highest yields with orange bagasse and restaurant waste. The extraction with sodium hydroxide, the highest yields were with guava bagasse and restaurant waste washed with EDTA. Extraction with potassium hydroxide showed highest yields with restaurant waste, washed with EDTA and not washed form (Table 1). The maximum yields obtained from xylan extraction were 74.20% with banana peel (washed with EDTA and treated with NaOH), 88.01% with guava bagasse (not washed and treated with NaOH), 80.81% with orange pomace (washed with EDTA and treated with KOH) and 91.74% with restaurant waste (not washed and treated with H_2O_2).

Xylan extraction high yields were obtained with the most effective reagent as potassium hydroxide, with the biomasses washed with EDTA. In addition, the highest yielding biomass was 91.74% with the restaurant waste, which showed a high potential for xylan production.

Table 2 - Xylan solubilization yield obtained with hydrogen peroxide, sodium and potassium hydroxide

	Xylan Yield (%)					
	H2O2		**NaOH**		**KOH**	
Biomass	*Washed (%)	Not Washed (%)	Washed (%)	Not Washed (%)	Washed (%)	Not Washed (%)
Banana Peels	41.98 ± 5.51	20.68 ± 6.73	74.20 ± 11.63	63.68 ± 6.52	73.67 ± 4.61	50.77 ± 1.12
Guava Bagasse	44.89 ± 6.18	46.41 ± 2.20	33.97 ± 0.83	88.01 ± 4.33	25.31 ± 2.75	43.35 ± 3.12
Orange bagasse	30.67 ± 3.77	58.66 ± 0.91	42.10 ± 3.51	50.01 ± 2.71	80.81 ± 9.82	31.12 ± 3.46
Restaurant residue	40.97 ± 6.86	91.74 ± 12.18	84.13 ± 10.42	81.79 ± 24.48	90.70 ± 6.88	82.13 ± 5.77

*Washed: biomass previously washed with EDTA 0.2% (m/v).

Source: Author.

The hemicellulose extraction yields obtained were higher than those founds in the literature, that showed, for example, 33.3% with extraction conditions of 12% of NaOH, 80 °C and 4 h of reaction and 21.4% with 2.0% of KOH, 75 °C and 3 h of reaction of recovery of crude xylan from the sweet sorghum bagasse (Wei, 2018). For pigeon pea stalk, the recovery of xylan for treatment with 12% NaOH, room temperature and overnight incubation was 12.63% and with 12% KOH, room temperature and overnight incubation was 12.35% (SAMANTA et al., 2013). For sugarcane bagasse, with H_2O_2 in alkaline medium (6%, m/v, 60 °C, 16h), the extraction yield was 60.8%, and with 1 M NaOH, 40 °C and 18 h was 62.10% (BRIENZO et al.,2009; XU et al.,2006).

The characteristics of each biomass, especially chemical composition, influence the optimal hemicellulose extraction conditions and the final purpose of the extraction process. In order for hemicellulose to be used in the conversion of new products, after their separation from the other components, regardless of the extraction method, it must be in specific and desirable quality conditions (FREITAS, 2019).

In oxidative pretreatment, oxidizing compounds are used on biomass suspended in water. Hydrogen peroxide is an effective oxidizing agent for breaking/removing lignin and hemicellulose, capable of increasing cellulose accessibility and isolating and bleaching fibers. In addition, a combination of alkaline reagents and oxidants such as peroxide is effective for pretreatment and may result in a high conversion of lignocellulosic biomass to sugars following enzymatic depolymerization of cell wall polysaccharides. (BRAGATTO; SEGATO; SQUINA, 2013; TEIXEIRA; LINDEN; SCHROEDER, 2000; MONTE et al., 2011).

Enzymatic hydrolysis for XOS production

Xylanases are the most important enzymes to perform xylan hydrolysis and are responsible to reduce the degree of polymerization of the β-1,4 glycosidic bonds of the xylan main chain by hydrolysis thereof (BATTAN et al, 2007; POLIZELI et al., 2005). The enzymes responsible for hydrolysis of xylan chains are endo-1,4-β-xylanases and β-xylosidises (GOSWAMI; RAWAT, 2015). Endo-β-1,4-D-xylanases (EC 3.2.1.8) act on the xylan main chain, forming xylooligosaccharides, while β-xylosidases hydrolyze xylobiose and short-chain xylooligomers, releasing xylose units (COLLINS et al, 2005; SOMERA et al, 2009). To maximize XOS production is necessary to apply purified endo-xylanase avoiding xylose production (Table 4).

The solubilized xylan was used as substrate for enzymatic hydrolysis with endo-xylanase produced by *A. Versicollor* in order to produce XOS. As expected, no xylose content was detected in any of the analyzes, which proves the effectiveness of xylan extraction by alkaline treatment, the absence of β-xilosidases and the efficient purification of xylanase enzyme. The results were showed in Table 4 and 5 and showed that the predominant oligomers generated were xylobiose (X_2), xylotriose (X_3), xylotetrose (X_4).

Table 4 – XOS produced by enzymatic hydrolysis with different reaction times for banana peels and guava bagasse pre-treated with 3 different reagents.

		Banana peels				Guava Bagasse			
Treatment agent	**Time of reaction (h)**	**X_2 (%)**	**X_3 (%)**	**X_4 (%)**	**XOS (g/L)**	**X_2 (%)**	**X_3 (%)**	**X_4 (%)**	**XOS (g/L)**
	2	0.00	0.06	9.36	1.87 ± 0.49	0.00	0.02	12.57	2.50 ± 0.05
	4	0.00	0.00	4.98	0.98 ± 0.12	0.00	0.03	12.74	2.52 ± 0.13
	6	0.00	0.03	6.11	1.21 ± 0.46	0.00	7.35	8.98	3.25 ± 0.11
H_2O_2	8	0.07	15.46	17.89	6.68 ± 0.32	0.00	16.82	19.12	7.16 ± 0.30
	12	0.24	16.64	17.77	6.92 ± 0.94	0.02	20.09	22.06	8.42 ± 1.23
	24	0.18	24.21	27.86	10.45 ± 0.96	0.10	22.20	23.13	9.74 ± 0.89
	48	0.91	26.21	27.01	10.83 ± 0.81	0.00	25.76	24.83	9.85 ± 0.90
	2	0.01	0.00	12.46	2.48 ± 0.48	0.00	0.01	5.80	1.15 ± 0.35
	4	0.00	3.46	12.03	3.08 ± 0.91	0.02	0.00	9.15	1.82 ± 0.01
	6	0.23	0.00	23.00	4.62 ± 0.51	0.00	18.37	20.43	5.53 ± 0.53
NaOH	8	0.00	18.37	20.43	7.73 ± 1.04	0.40	16.81	22.36	7.91 ± 0.48
	12	0.08	20.03	22.04	8.42 ± 1.65	0.43	22.30	33.42	10.06 ± 0.75
	24	0.65	22.11	24.70	8.49 ± 0.76	0.21	20.36	36.81	11.42 ± 0.42
	48	0.68	22.56	22.93	9.23 ± 1.02	0.20	21.03	38.83	11.96 ± 0.83
	2	0.00	0.00	12.21	2.42 ± 0.35	0.03	0.00	10.13	2.03 ± 0.31
	4	0.00	6.33	9.72	3.20 ± 0.50	0.22	3.30	9.65	2.63 ± 0.56
	6	0.00	15.32	18.46	6.73 ± 0.22	0.42	15.04	21.08	7.31 ± 0.01
KOH	8	0.19	17.97	21.24	7.88 ± 0.94	0.51	18.88	21.50	8.18 ± 0.04
	12	2.16	23.65	27.21	10.61 ± 0.80	0.46	21.17	21.72	8.67 ± 0.63
	24	1.50	24.85	27.03	10.68 ± 0.50	0.62	22.03	22.82	9.10 ± 0.71
	48	1.46	25.46	26.67	10.72 ± 1.38	0.04	22.91	23.79	9.33 ± 0.70

*X2: xylobiose; X3: xylotriose; X4: xylotetraose; XOS: xylooligosaccharides

Source: Author.

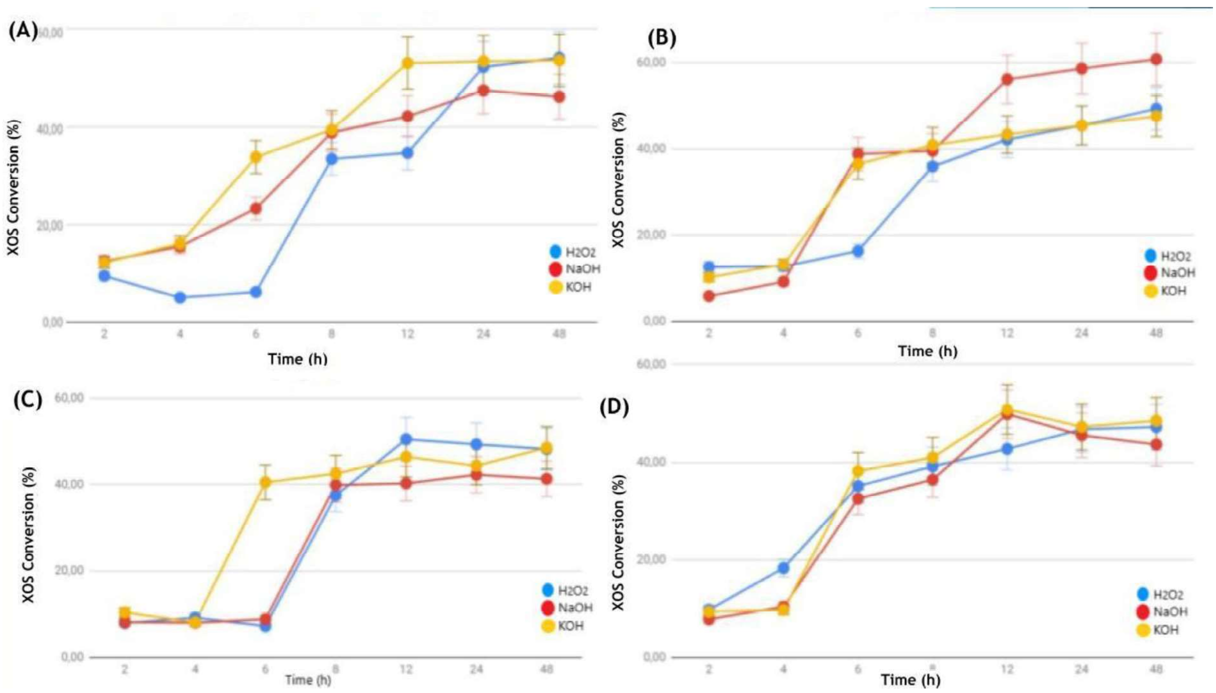
Table 5 – XOS produced by enzymatic hydrolysis with different reaction times for Orange bagasse and restaurant residue pre-treated with 3 different reagents.

		Orange Bagasse				Restaurant Residue			
Pre Reagent	Time of reaction (h)	X_2 (%)	X_3 (%)	X_4 (%)	XOS (g/L)	X_2 (%)	X_3 (%)	X_4 (%)	XOS (g/L)
H_2O_2	2	0.00	0.05	7.95	1.59 ± 0.12	0.00	0.03	9.77	1.94 ± 0.28
	4	0.00	0.11	9.10	1.83 ± 0,08	0.00	3.88	14.40	3.64 ± 0.27
	6	0.00	0.06	7.24	1.12 ± 0.46	0.00	13.56	21.54	6.99 ± 0.00
	8	0.00	18.68	18.75	7.47 ± 0.41	0.00	17.88	21.28	7.81 ± 0.52
	12	0.00	24.75	25.67	10.06 ± 0.37	0.00	18.88	21.18	8.32 ± 0.73
	24	0.00	26.40	22.84	9.82 ± 0.56	1.74	22.79	22.23	9.35 ± 0.77
	48	0.00	25.59	22.56	9.60 ± 0.78	0.00	24.54	22.69	9.81 ± 0.43
NaOH	2	0.06	0.00	8.15	2.17 ± 0.03	0.00	0.00	7.84	1.55 ± 0.61
	4	0.00	0.00	8.04	2.05 ± 0.11	0.05	0.00	10.35	2.06 ± 0.17
	6	0.00	0.02	8.81	1.74 ± 0.05	0.00	15.09	17.40	7.68 ± 0.45
	8	0.00	18.78	21.05	7.94 ± 0.27	0.24	17.70	18.50	6.77 ± 0.73
	12	0.07	19.52	20.62	8.03 ± 0.42	0.44	22.63	26.77	9.97 ± 0.77
	24	0.21	21.46	20.55	8.45 ± 0.18	0.64	22.36	22.52	9.10 ± 1.71
	48	0.00	22.07	19.22	8.23 ± 0.23	0.57	22.46	20.60	8.73 ± 0.33
KOH	2	0.00	0.00	10.43	2.07 ± 0.16	0.00	0.00	9.39	1.86 ± 0.06
	4	0.00	0.00	8.12	1.60 ± 0.17	0.06	0.00	9.69	1.93 ± 0.75
	6	0.14	18.55	21.79	8.10 ± 0.32	0.12	16.68	21.40	8.17 ± 0.63
	8	0.11	19.10	23.25	8.49 ± 0.15	0.17	18.98	21.84	8.20 ± 0.02
	12	1.46	22.18	22.74	9.29 ± 1.44	0.83	23.68	26.30	10.15 ± 0.44
	24	1.80	21.22	21.33	8.87 ± 0.36	0.51	22.07	24.67	9.45 ± 0.33
	48	1.48	25.05	21.93	9.69 ± 0.84	0.96	24.19	23.32	9.69 ± 0.27

*X_2: xylobiose; X_3: xylotriose; X_4: xylotetraose; XOS: xylooligosaccharides

Source: Author.

Figure 1 – Total XOS yield obtained from xylan enzymatic hydrolysis with endo-xylanse from *A. versicollor*.



*(A) represents the banana peel residue, (B) the guava bagasse, (C) the Orange bagasse and (D) the restaurant waste.

Source: Author.

The biomass with the highest generation of total XOS was guava bagasse, showing potassium hydroxide as the best treatment and presented maximum value with NaOH treatment (60.82% and 48h). Banana peel and restaurant residues also presented KOH as the best treatment, with the highest average XOS yields around 50% yield. Orange bagasse was best treated with hydrogen peroxide, with an average yield of around 50% (Figure 1).

Even the lowest values were higher than those found in the literature, which presented higher values of 37.6% of XOS for the solubilized hemicellulose of sugarcane bagasse (was treated for 3 h at 35 °C with 24% KOH including 1% (by mass per volume) $NaBH_4$.) in 48-72 h of reaction using *Aspergillus fumigatus* M51 (35.6 U/mL) in a reaction mixture of 60–500 U of xylanases or crude enzymes from selected fungi per g of substrate in a total volume of 10 mL of 100 mM acetate buffer (pH=5.0) in a 20-mL glass test tube and 1–2% of hemicellulose (by mass per volume (Carvalho et al.,2015). With residual hemicelluloses of the dissolving pulp, the highest XOS yield found was 42.96%, with 120 UI g^{-1} substrate enzyme loading for 8 h, also smaller than those found in the project (Wang et al., 2018). A study with the same xylanase catalyst of

Aspergillus niger, after hydrolysis of beechwood xylan (20 mg/mL), with 22.7 U of total enzyme activity and 55 °C for 24 h, using as a biomass powdered corn, had the highest yield of 62% XOS (ARAGON, 2018), yield similar to those obtained in the study, but still lower than the maximum value obtained with guava bagasse.

Conclusion

The solubilization of xylan presented high yield values for all the biomasses, compared to other residues found in the literature, especially restaurant waste, which showed a high yield for all treatments used. On the other hand, the chemical characterization of this xylan presented low yields in relation to the initial material.

Guava bagasse biomass presented the best XOS production, with a maximum yield of about 60% (12 g/L), with sodium hydroxide as xylan extraction agent and 48 h reaction time. For banana peel residue, the maximum yield of XOS was 54.14% (10.83 g/L) (peroxide solubilized, 48 h of reaction), for orange bagasse was 50.42% (10.06 g/L) (peroxide solubilized,12 h of reaction) and 50.80% (10.15 g/L) with restaurant residue (potassium hydroxide solubilized, 12 h of reaction).

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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