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WILLIAM ROGOSKI

PRODUCTION OF CASSAVA PEEL-BASED XYLOOLIGOSACCHARIDES USING ENDO-1,4-β-XYLANASE FROM *Trichoderma longibrachiatum:* THE EFFECT OF ALKALINE PRETREATMENT

FLORIANÓPOLIS – SC
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Master's dissertation degree presented to the Postgraduate Program in Chemical Engineering at the Federal University of Santa Catarina to obtain the title of Master’s in Chemical Engineering.

Advisor: Prof. Dr. Cristiano José de Andrade.

Co-advisors: Prof. Dra. Débora de Oliveira
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I dedicate...

To my parents, Mr. Marcos and Mrs. Simone, my brothers Thiago and Igor and to my friends who have supported me through this journey.

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ABSTRACT

The lignocellulosic residue cassava peel is an unexplored source of bioactive compounds, such as hemicellulose-based xylooligosaccharides (XOS) that present prebiotic properties. In this sense, the aim of this work was to produce XOS from cassava peels that were pretreated with sodium hydroxide (xylan extraction and lignin removal) followed by enzymatic hydrolysis (endo-1-4-β-xylanase). The cassava peels were pretreated sequentially: starch removal, alkaline hydrolysis, and enzymatic hydrolysis. The aqueous-mechanical reduction and sieving for 15 cycles removed ≈16% of starch (iodometric method). The alkaline pretreatments were carried out with 2, 4, and 6% (w/v) NaOH, 1:100/solid:liquid at 121 °C and 1.1 bar for 30 and 60 minutes. The enzymatic kinetics was evaluated with enzyme concentration at 0.5, 1.5, and 3.0% (v/v). The most suitable alkaline pretreatment for biomass delignication provided around of 34.20% of lignin removal (2% (w/v) NaOH for 30 min). The highest XOS yield was 396.5 mg XOS/g xylan after 48 h and 3.0% enzyme concentration. Regarding the mass balance, from 300 g of cassava peels (an agroindustrial residue), it is possible to obtain up to 3.27% XOS. Therefore, the fractionation of hemicellulose from cassava peels was technically viable from the concepts of biorefinery and bioeconomy, being one of the first researches to approach the extraction of xylan from cassava peel to obtain XOS through enzymatic route with a highly promising yield.

Keywords: Cassava peel, alkaline pretreatment, hemicellulose, xylooligosaccharides, enzymatic hydrolysis.

RESUMO

A casca do resíduo lignocelulósico da mandioca é uma fonte inexplorada de compostos bioativos, como os xilooligossacarídeos à base de hemicelulose (XOS), que apresentam propriedades prebióticas. Neste sentido, o objetivo deste trabalho foi produzir XOS a partir de cascas de mandioca que foram pré-tratadas com hidróxido de sódio (extração de xilanos e remoção de lignina) seguido de hidrólise enzimática (endo-1-4-β-xilanase). As cascas de mandioca foram pré-tratadas sequencialmente: remoção de amido, hidrólise alcalina, e hidrólise enzimática. A redução aquosa-mecânica e peneiração por 15 ciclos removeu ≈16% de amido (método iodométrico). Os pré-tratamentos alcalinos foram realizados com 2, 4, e 6% (m/v) de NaOH, 1:100/sólido:líquido a 121 °C e 1,1 bar durante 30 e 60 minutos. A cinética enzimática foi avaliada com concentração enzimática a 0,5, 1,5, e 3,0% (v/v). O melhor pré-tratamento alcalino para deslignificação da biomassa promoveu em torno de 34,20% de remoção de lignina (2% (m/v) NaOH durante 30 min). O maior rendimento de XOS foi de 396,5 mg XOS/g xilana após 48 h e 3,0% de concentração enzimática. Quanto ao balanço de massa, de 300 g de cascas de mandioca (um resíduo agroindustrial), é possível obter até 3,27% de XOS. Portanto, o fracionamento da hemicelulose das cascas de mandioca foi tecnicamente viável a partir dos conceitos de biorrefinaria e bioeconomia, sendo uma das primeiras pesquisas a abordar a extração da xilana da casca de mandioca para obter XOS por via enzimática com um rendimento altamente promissor.

Palavras-Chaves: Casca de mandioca, pré-tratamento alcalino, hemicelulose, xilooligossacarídeos, hidrólise enzimática.

RESUMO EXPANDIDO

PRODUÇÃO DE XILOOLIGOSSACARÍDEOS A BASE DE CASCA DE MANDIOCA USANDO ENDO-1-4-β-XILANASE DE *Trichoderma longibrachiatum:* EFEITO DO PRÉ-TRATAMENTO ALCALINO

INTRODUÇÃO

Os resíduos agroindustriais têm um grande potencial como forma de matéria-prima de baixo custo e vem ganhando destaque nos últimos anos devido à grande dependência que existe das fontes petroquímicas (Rodrigues et al., 2017). Neste contexto, o conceito de biorrefinaria é essencial, pois está relacionado à conversão de resíduos de base biológica em compostos de alto valor agregado, como produtos farmacêuticos, alimentícios e fertilizantes (Dai et al., 2021; Patrizi et al., 2020).

Em relação ao potencial bioenergético que os resíduos agroindustriais apresentam, os resíduos do processamento da mandioca se destacam. Nos últimos 60 anos, a produção mundial de mandioca cresceu em média 2,3% ao ano. Na África, o avanço foi mais significativo devido ao aumento da área cultivada, especialmente Nigéria (maior produtor), que produz mais de 60 milhões de toneladas de mandioca anualmente. Na Ásia, a produção da raiz aumentou consideravelmente na Tailândia e no Vietnã, sendo os principais exportadores de farinha para o restante do continente. O Brasil é o quarto maior produtor de mandioca do mundo cuja produção anual de mandioca em 2020 foi superior a 20 milhões de toneladas e 538,8 toneladas de farinha (FAO, 2020).

A produção de farinha de mandioca gera, inerentemente, resíduos líquidos (águas residuais de mandioca) e resíduos sólidos (cascas). Grandes instalações de processamento de mandioca, que processam aproximadamente 125 toneladas de mandioca geram até 40 m³ de águas residuais de mandioca. Neste contexto, o processamento da mandioca para produzir 1 kg de farinha gera aproximadamente 0,65 kg de resíduos sólidos e 6 L de resíduos líquidos (FAO, 2016), com potencial para a produção, por exemplo, de xilooligossacarídeos (XOS) e surfactantes, respectivamente.

Os XOS são oligômeros carboidratos com propriedades prebióticas compostos por unidades de xilose. XOS podem ser produzidos a partir de materiais lignocelulósicos (LCMs) que podem ser aplicados em produtos alimentícios e farmacêuticos: composto ativo de alimentos funcionais (Vázquez et al., 2000); em cápsulas; na forma de pó (concentrações de 95%, 35% e 20%); e na forma de xarope (Grafulin, 2018). Os XOS são considerados prebióticos bioativos, pois estimula seletivamente bactérias benéficas, como *Bifidobacterium* spp. e *Lactobacillus* spp. (Poletto et al., 2020a). Estes compostos têm chamado a atenção para as indústrias alimentícias e farmacêuticas devido a sua estabilidade térmica e química (acidez) quando comparados a outros prebióticos como fruto-oligossacarídeos (Courtin et al., 2009).

O pré-tratamento aplicado em resíduos agroindustriais deve ser sustentável e economicamente viável (Wright et al., 2018). Assim, os métodos baseados em álcalis são amplamente utilizados, em particular, devido aos efeitos da deslignificação, aumentando a porosidade da biomassa (Nascimento et al., 2016). No entanto, alternativas como (I) plasma não térmico, no qual as moléculas de ozônio atingem as duplas ligações das ligninas levam a uma branda deslignificação, preservando a celulose e hemiceluloses (Miranda et al., 2019), (II) micro-ondas, que podem ser aplicadas simultaneamente (efeitos sinérgicos) com outros pré-tratamentos, em combinação com compostos químicos (Ethaib, 2015), e (III) ultrassom que levam à erosão superficial de materiais lignocelulósicos (Bussemaker & Zhang, 2013) são passíveis de investigação.

O pré-tratamento alcalino do material lignocelulósico promove a deslignificação, aumentando a produção de XOS devido à maior exposição da hemicelulose durante a hidrólise enzimática (Menezes & Durrant, 2008). Neste sentido, a estratégia mais adequadapara obter XOS é aquela que combina um pré-tratamento eficiente para remover compostos indesejáveis e que preserve a estrutura da hemicelulose para posterior conversão da xilana. As xilanases são as enzimas responsáveis pela hidrólise da xilana, elas podem atuar de forma "endo" hidrolisando elos dentro da cadeia de xilana, formando oligossacarídeos menores ou de forma "exo" hidrolisando a cadeia pelas extremidades liberando monômeros de xilose. Para a produção de XOS o complexo enzimático deve apresentar alta atividade endo-1,4-β-xilanase para minimizar a produção de monossacarídeos (Grafulin, 2018).

OBJETIVOS

O objetivo principal deste trabalho foi a produção de XOS a partir de resíduos oriundos do processamento da mandioca utilizando a técnica clássica de pré-tratamento alcalino com hidróxido de sódio seguido de hidrólise enzimática. Foram avaliados os seguintes parâmetros: influência da concentração de hidróxido de sódio, razão mássica e tempo de hidrólise (em 121 °C) em relação à porcentagem de deslignificação. Além disso, a cinética enzimática em diferentes concentrações de enzimas em uma massa fixa de substrato, caracterização lignocelulósica para determinação do teor de celulose, hemicelulose e lignina; e a quantificação de XOS por HPLC.

METODOLOGIAS

Capítulo 1: Contempla a introdução sobre o tema da dissertação e os objetivos que foram desenvolvidos.

Capítulo 2: Referente à revisão bibliográfica cujos temas abordados englobam processamento da mandioca, cascas de mandioca como resíduo agroindustrial e o potencial biotecnológico associado à produção de farinha, pré-tratamentos relacionados à produção de XOS, composição lignocelulósica das cascas de mandioca (amido, celulose, hemicelulose e lignina), enzimas xilanases e sua aplicação de XOS.

Capítulo 3: Referente à etapa experimental, desde a coleta e armazenagem das cascas, pré-tratamento para remoção de amido, pré-tratamento alcalino (NaOH 2, 4 e 6% (m/v)) em 30 e 60 min para a remoção de lignina e compostos fenólicos, caracterização lignocelulósica para determinação de celulose; hemicelulose; lignina e cinzas, hidrólise enzimática da xilana da hemicelulose estudada e verificada a partir de uma cinética com concentrações de enzima (0,5; 1,5 e 3,0%) em 20 mg de substrato, os pontos da cinética foram coletados em tempos de 6, 12, 24, 48 e 96 horas e o licor foi quantificado em HPLC para determinação de xilose (X1), xilobiose (X2), xilotriose (X3), xilotetraose (X4), xilopentaose (X5) e xilohexaose (X6). Determinação do balanço de massa a partir de 300 g de cascas e balanço econômico preliminar para determinar o custo de produção de XOS a partir de resíduo oriundo do processamento da mandioca.

RESULTADOS E DISCUSSÃO

A remoção de amido foi ≈16% (iodometria). A análise dos resultados obtidos na etapa de hidrólise alcalina indicaram que os teores de lignina solúvel e insolúvel diminuíram mais consideravelmente nos pré-tratamentos alcalinos aplicados com 30 minutos de hidrólise. Além disso, foi identificado que o teor de hemicelulose foi inversamente proporcional à concentração de hidróxido de sódio e tempo de hidrólise. O pré-tratamento alcalino com NaOH 2% (m/v) na proporção 1:100 sólido:líquído durante 30 minutos em autoclave a 121 °C e 1,1 bar resultou em 38,9% de remoção de lignina.

A etapa de hidrólise enzimática foi realizada com o resíduo na condição de maior deslignificação e preservação de hemicelulose. A cinética enzimática foi realizada com concentração fixa de substrato de 1% (20 mg) em temperatura de 50 ºC, pH 6.0, agitação de 180 rpm, no qual amostras foram coletadas nos tempos de 6; 12; 24; 48 e 96 horas, utilizando concentrações de enzima de 0,5; 1,5 e 3,0% (v/v)) em relação ao substrato. Os rendimentos de XOS variaram entre 39,1 mg XOS/g de xilose em 6 horas e 0,5% enzima até 396,5 mg XOS/g de xilana em 48 horas e 3,0% de enzima. A partir da análise estatística (Tukey), os rendimentos nas diferentes concentrações de enzimas nos tempos de 48 e 96 horas não apresentaram diferenças significativas tanto nos valores de conversões e rendimentos. A conversão de XOS em relação à xilana foi de 49,1%. Xilopentose foi o oligômero que se apresentou em maior concentração (119,8 mg/L).

CONSIDERAÇÕES FINAIS

O pré-tratamento alcalino é um método clássico associado à deslignificação de materiais lignocelulósicos, que foi aplicado com sucesso ao resíduo casca de mandioca. Neste contexto, em relação à produção de XOS a partir da casca de mandioca, a presença do amido é um entrave. Porém, por meio do balanço de massa, foi possível, teoricamente, obter até 3,27% de XOS a partir de 300 g de cascas de mandioca.

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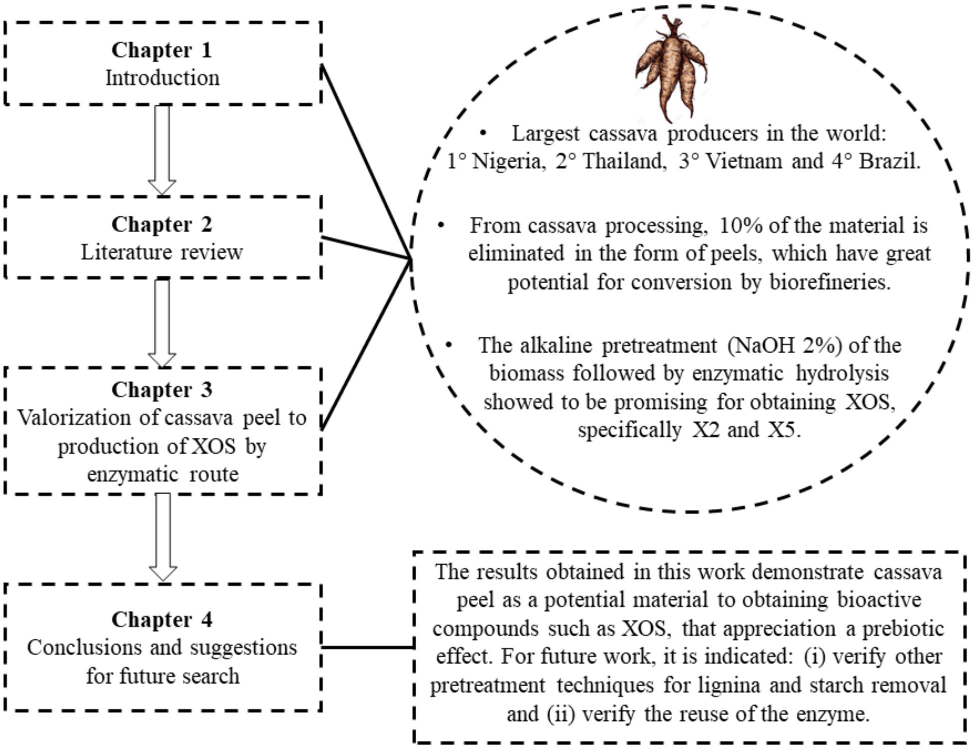
LIST OF ACRONYMS AND ABBREVIATIONS

ALI	Acid-Insoluble Lignin
ALS	Acid-Soluble Lignin
ARS	Aqueous Removal Starch
DP	Degree of Polymerization
EQA	Department of Chemical and Food Engineering
FAPESC	Foundation for the Support of Research and Innovation of the State of Santa Catarina
FAO	Food Organization of the United Nations
HPLC	High Performance Liquid Chromatograph
IBGE	Brazilian Institute of Geography and Statistics
IUB	International Union of Biochemistry
LATESC	Thermodynamics and Supercritical Technology Laboratory
LCMs	Lignocellulosic Materials
LiEB	Biological Engineering Laboratory
NREL	National Renewable Energy Laboratory
pH	Hydrogen Ion Potential
POSENQ	Postgraduate Program in Chemical Engineering
PROFI	Laboratory for Physical Properties of Food
rpm	Rotation Per Minute
U	Unit of Enzyme
UFSC	Federal University of Santa Catarina
UNICAMP	University of Campinas
US$	US Dollar
X1	Xylose
X2	Xylobiose
X3	Xylotriose
X4	Xylotetraose
X5	Xylopentaose
X6	Xylohexaose
XOS	Xylooligosaccharides
(v/v)	Volume/Volume
(w/v)	Weight/Volume

SUMMARY

CONCEITUAL DIAGRAM



Chapter 1
Introduction

Chapter 2
Literature review

Chapter 3
Valorization of cassava peel to production of XOS by enzymatic route

Chapter 4
Conclusions and suggestions for future search

- Largest cassava producers in the world: 1° Nigeria, 2° Thailand, 3° Vietnam and 4° Brazil.
- From cassava processing, 10% of the material is eliminated in the form of peels, which have great potential for conversion by biorefineries.
- The alkaline pretreatment (NaOH 2%) of the biomass followed by enzymatic hydrolysis showed to be promising for obtaining XOS, specifically X2 and X5.

The results obtained in this work demonstrate cassava peel as a potential material to obtaining bioactive compounds such as XOS, that appreciation a prebiotic effect. For future work, it is indicated: (i) verify other pretreatment techniques for lignina and starch removal and (ii) verify the reuse of the enzyme.

1 CHAPTER 1 – INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a woody tuberous root that can be grown in dry infertile soils typical of tropical and subtropical climatic regions (Blagbrough et al., 2010; Mombo et al., 2017). It is a low-cost food crop being an important source of carbohydrates on the African continent (Otekunrin & Sawicka, 2019). Currently, the most cassava producing country in the world is Nigeria contributing about 59.8 million metric tons of cassava annually and more than ten products that can be derived from this root (Adekunle et al., 2016; Sivamani et al., 2018). Annual world production of cassava in 2019 was over 303.6 million metric tons, with Africa accounting for over 60% of this total, followed by Asia and South America, in the same year Brazil produced approximately 17 million metric tons of cassava (Cruz et al., 2021; Kayiwa et al., 2021). The composition of cassava varies depending on the species of the root and type of soil and climate in which it was grown, some studies point out that 45% of the composition of cassava is starch; 23.4% hemicellulose; 14.2% cellulose; 10.9% lignin; 0.3% ash and 3.5% moisture content (Ndongo et al., 2020; O.J. Babayemi, O.J. Ifut, 2010; Pooja & Padmaja, 2015; Sudaryanto et al., 2006).

The cassava flour industry generates large amounts of waste - an environmental threat, if incorrectly disposal (Murata et al., 2021). Among the waste generated are: solids (peels, leaves and roots); liquids (cassava wastewater and washing water) and gases (air emissions from burning the cellulosic content) (Olaoye et al., 2020). The cassava wastewater, due to its high nutrient content, can be exploited to produce surfactants and the washing waters can be used to obtain starch bioproducts (Merico et al., 2020). Solid residues, on the other hand, which constitute up to 20-35% of the tuber and are rich in other carbohydrate polymers, such as cellulose and hemicellulose, can be explored for obtaining bioactive compounds (Aruwajoye et al., 2020).

Therefore, the valorization of agro-industrial residues has been growing in the world scenario for being a source of low-cost raw materials to obtain products with high-added value (Rodrigues et al., 2017). In this sense, cassava waste exhibits a biotechnological potential to be explored aligned with lignocellulosic biorefineries (Xu et al., 2022), resulting in the production of valuable bioproducts, such as cellulose nanofibers (Czaikoski et al., 2020); biogas (Alrefai et al., 2020); lipids (Chaturvedi et al., 2019); oligosaccharides (Ona et al., 2019); biomethane (Alrefai et al., 2020); bioethanol (Awoyale et al., 2021); biofertilizers (Aisien & Aisien, 2020); diverse sugars (Mohammed, 2014; Aruwajoye et al., 2017); enzymes (Salihu et al., 2015) and XOS (Agung et al., 2016).

Cassava peels are lignocellulosic waste composed of cellulose and hemicellulose arranged in a complex crystalline structure encircled by lignin, which is a natural phenolic ramified polymer highly resistant to degradation (Zúñiga-Arias et al., 2022). In this sense, many pretreatments are being developed to promote the removal of lignin present in biomass (Baruah et al., 2018), such as hydrothermal pretreatments; acid, alkaline; oxidative; liquid-ionic; biological; microwave and the most recent non-thermal plasma (Pandey & Negi, 2015; Pereira et al., 2021; Qi et al., 2010). One of the most studied pretreatments is dilute alkali under pressure and temperature conditions, in which lignin is efficiently dissolved by breaking the cross-links that surround lignin with cellulose and hemicellulose (Kim et al., 2016; H. Xu et al., 2015). Pretreatment with sodium hydroxide promotes a saponification reaction resulting in the breaking of the intermolecular ester bonds between lignin and hemicellulose being more effective for obtaining hemicellulosic-based bioproducts (Sun et al., 2016; Zúñiga-Arias et al., 2022).

Hemicellulose is found in plant species and is composed of xylans. The xylan is a pentosan that when hydrolyzed gives rise to a xylose, i.e., xylans are polysaccharides formed by D-xylose units linked by xylosidic bridges of the β-1-4-xylosidic type (Li et al., 2021). One way of converting the xylan present in the hemicellulose of cassava peels occurs with hydrothermal treatment in the presence of alkali followed by enzymatic hydrolysis to obtain xylooligosaccharides, for example. This bioconversion of hemicellulose depends on enzymatic actions such as endo-β-1-4-xylanase (Long et al., 2022). Extracellular endo-β-1-4-xylanases are enzymes that provide catalytic activity required to cleave the β-1-4-xylosidic type bonds in the xylan main chain of hemicellulose which results in obtaining xylose (X1) and different forms of XOS (X2 - X6) (Rahmani et al., 2019). The endo-β-1-4-xylanases are mostly obtained from hemicellulolytic microorganisms such as bacteria and fungi, among them, xylanases from fungi such as *Trichoderma ssp*. show excellent xylanolytic activities (Chen et al., 1997).

XOS are oligomers formed of 2-6 xylose units (X2 - X6) bounded by xyloside interactions and are widely used in the food industry as ingredients and food additives (Ma et al., 2017). XOS obtained from xylan from lignocellulosic sources exhibit a variety of biological properties (Rahmani et al., 2019) such as, prebiotics that stimulate the growth of beneficial bacteria (*Bifidobacterium spp.* and *Lactobacillus spp.*) to the gastrointestinal system, improves calcium absorption in the body, reduces the risk of colon cancer, decreases blood glycerol indices, provides immunological and anti-infectious properties, and promotes antioxidant and microbial activities

(Jagtap et al., 2017). Thus, XOS has drawn the attention of food and pharmaceutical industries, between 1997 and 2009, 32 products containing XOS were launched on the market, in which 38% are food supplements, 25% dairy products, 16% sweets, 13% non-alcoholic beverages and 8% baby foods (Courtin et al., 2009; Grafulin, 2018). There has been only one study developed so far verifying the possible obtainment of XOS from cassava peels. Therefore, this work contemplates the bioconversion of XOS from cassava peels in an unprecedented way by enzymatic hydrolysis of xylan exposed by alkaline treatment. The appreciation of this waste as a biotechnological potential by biorefineries contributes to obtaining bioactive compounds in a sustainable way moving the bioeconomy.

1.1 Aim of the work

Evaluate the alkaline treatment as a first step to use cassava peel as substrate to produce xylooligosaccharides.

1.1.1 Specific aims

- Evaluate the removal of cassava peel starch from the lignocellulosic moiety.
- Identify the best condition for extraction of xylan from hemicellulose by hydrothermal process in alkaline medium.
- Verify the production of XOS by enzymatic hydrolysis from a kinetic using endo-1,4-β-xylanase.
- Prospect the mass and economic balances.

1.1.2 Justification

The valorization of agro-industrial wastes has drawn the attention of the scientific community due to its potential for obtaining value-added bioproducts. Therefore, the fractionation of hemicellulose from cassava peels is strictly related to the concepts of biorefinery and bioeconomy.

1.2 Structure of the dissertation

This dissertation is composed of three chapters - briefly presented below:

Chapter 1: An introduction is presented that contextualizes the general and specific aims and justifies the current scenario and perspectives related to biotechnology.

Chapter 2: It presents the fundamental data on cassava, production chain, and lignocellulosic composition of agro-industrial waste, potential pretreatments (chemical, physical and biological) applied in cassava peels to obtain different biobased products, enzymatic approaches, and production of XOS and their application such as prebiotics for pharmaceutical and food industries.

Chapter 3: It details the empirical experiments, including methodology, results and discussions, conclusion, and future trends. Two pretreatments were applied to cassava peels (starch removal and alkaline treatment for delignification). Lignocellulosic biomass was characterized. The production of XOS by enzymatic hydrolysis was evaluated from enzymatic kinetics. The material balance determination was prospected.

1.3 References

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2 CHAPTER 2 – LITERATURE REVIEW [1]

The purpose of this chapter is to give the reader an overview of the main topics covered in this dissertation. The literature review brings topics such as cassava and its production chain application, and lignocellulosic composition of agroindustrial waste, potential pretreatments (chemical, physical and biological) applied in cassava peels to obtain different biobased products, enzymatic aspects, and production of xylooligosaccharides such as prebiotics for pharmaceutical and food industries.

2.1 Cassava peel

Cassava is one of the three most important root and tuber crops in the world. The annual global production is about 270 million tons of unprocessed roots (Ajala et al., 2020). Approximately 60% of the cassava produced is consumed as flour or in fermented products. Animal feed industries consume about 33% of world production, whereas 7% are applied by textile, paper, and food industries (Balagopalan et al., 2018).

It is worth noting that cassava is a low-cost source of carbohydrates, in particular for underdeveloped countries. Cassava can be cultivated in infertile land, with minimal fertilizer, herbicide, and insecticide requirements. In addition, it can be harvested throughout the year, which avoids seasonal shortages of this type of raw material (Phoncharoen et al., 2019; Montagnac et al., 2009). In this sense, ≈ 800 million people worldwide often use cassava-based food as their main source of carbohydrates (Medeiros et al., 2018). According to the Brazilian Institute of Geography and Statistics (BIGS), the Brazilian production of cassava root was 19 million tons that represent 72% out of South American production (FAO, 2016).

The industrial processing of cassava in Brazil is mostly related to the production of flour and starch. Its processing generates a series of solid and liquid wastes such as peels, leaves, sand, washing water, and cassava wastewater (**Figure 1**). The latter has a noteworthy biotechnological potential, in particular as a substrate for biosurfactant production (Mohd Isa et al., 2020).

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[1] *Submitted for publication in Waste and Biomass Valorization*

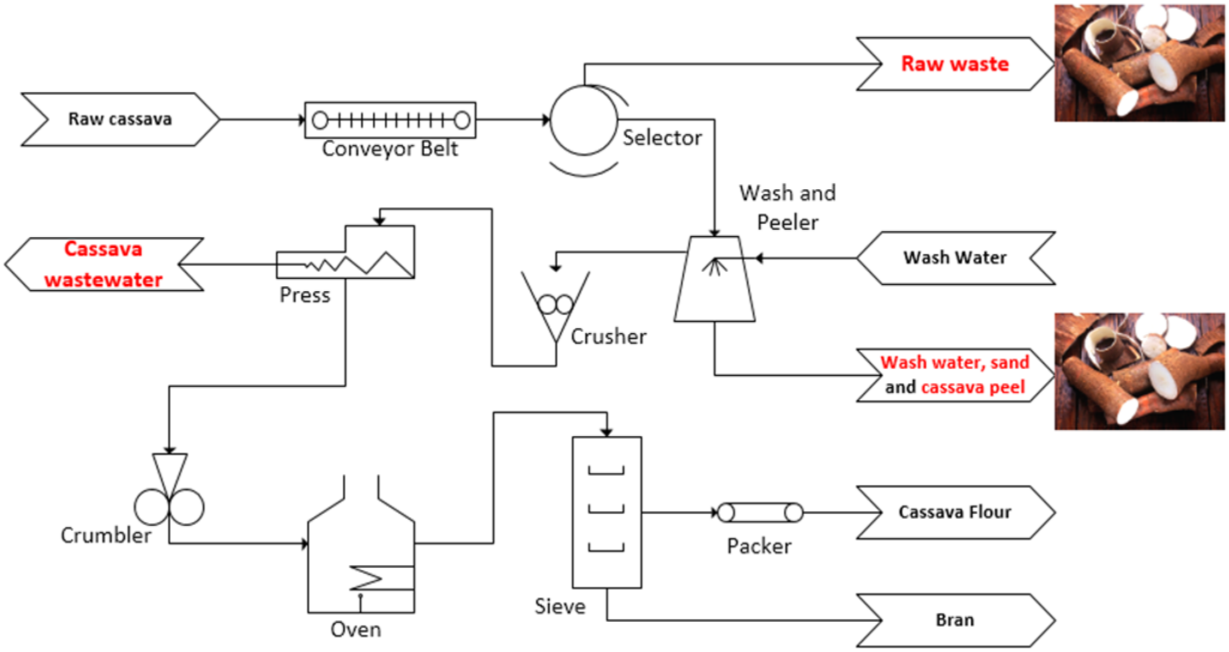


Figure 1. Flowchart of the cassava flour production.

The main residue generated during cassava root processing is cassava peel constituting between 10 to 15% of the tuber weight composition, which implies the generation of 27.7 to 41.5 million metric tons of cassava peel (Kayiwa et al., 2021). Cassava peel consists of three basic polymers: lignin (phenolic structure), cellulose (linear polymer formed by glucose units linked in the β-1-4 form), and hemicellulose that constitutes in heteropolysaccharide derived from several monosaccharides, including xylose, arabinose, mannose and, rhamnose (Yang et al., 2015).

In order to evaluate the recent trends associated with the use of cassava peel, a search was made in three databases (Scopus, Web of Science and Science Direct) with the keywords ("raw cassava" OR "cassava water" OR "cassava peel" OR "cassava husk"). It resulted in 15 review articles. The manuscripts addressed different areas of knowledge and presented themes such as: (I) animal feed, in which the evolution of the diet of goats and sheep fed with cassava-based residues as a substitute for corn was evaluated, as well as evaluating the potential that the roots of cassava tubers provide in poultry feed (Diarra, 2018; Dos Santos et al., 2015; Jiwuba et al., 2021). (II) energy and biofuels, bioethanol production from different lignocellulosic residues (cassava peel, corn cob, sugarcane bagasse, mango peel, sorghum straw, and rice peel) testing amylases as an enzymatic hydrolysis agent, sizing of circulating bed bioreactor from microbial kinetics for alcohol production with cassava crops, i.e., clean and sustainable energy production (Adekunle et al., 2016; A. A. Awoyale & Lokhat, 2019; Giwa et al., 2017; Sivamani et al., 2018); (III) economic, environmental and social topics, utilization of cassava peel-based food waste for sustainable development was verified in addition to the assessment of lignocellulosic biomass management with a focus on environmental sustainability in Nigeria and evaluation of the economic and social impact that cassava causes on the African continent (Abass et al., 2018; Oghenejoboh et al., 2021; Okonko et al., 2009) and (IV) chemical characteristics, from the evaluation of the extraction and functional properties of non-amylaceous polysaccharides extracted from different parts of cassava roots and evaluation of the biochemical properties that cassava flour exhibits (A. A. Awoyale & Lokhat, 2021; Uthumporn et al., 2017).

In this connection, it is noted that the lignocellulosic waste from the processing of cassava (peels) has great potential for bioconversion, although it is clear that the cassava peel is little exploited to obtain products with a focus on the pharmaceutical and food industry. Am opportunity to this biomass would be to produce prebiotics from the enzymatic hydrolysis of hemicellulose, thus contributing to the environment, with new research and with the increase of the concept of biorefineries.

In this context, it is observed that the solid residues (peel) obtained in the processing of cassava have great potential for bioconversion, although it is evident that the cassava peel is unexplored to obtain products focused on the pharmaceutical and food industry. The opportunity for this biomass would be to produce prebiotics from the enzymatic hydrolysis of hemicellulose, thus contributing to the environment by disposing of agricultural residues in a sustainable way and with new research in the area of biological engineering. Therefore, it is unprecedented and promising to take advantage of the potential that cassava peels have (biorefineries concept), to produce XOS by enzymatic hydrolysis in the presence of enzymes such as xylanases.

2.1.1 Starch

Starch is the main polymer present in cassava crops and constitutes 35% to 45% of its composition by mass (Apriyanto et al., 2022). In addition, it is a polysaccharide present in plant cells of tubers such as potato and cassava and grains such as corn and wheat. Starch is formed by the mixture of amylose and amylopectin polymers that contain up to 1,000 α-D-glucose units, joined by α-1,4 glycosidic bonds, as shown in (**Figure 2**). Its multilevel structure exhibits crystalline, semicrystalline, and amorphous lamellar characteristics that confer properties such as, gelatinization and retrogradation (Maniglia, 2017; Zhang et al., 2021)

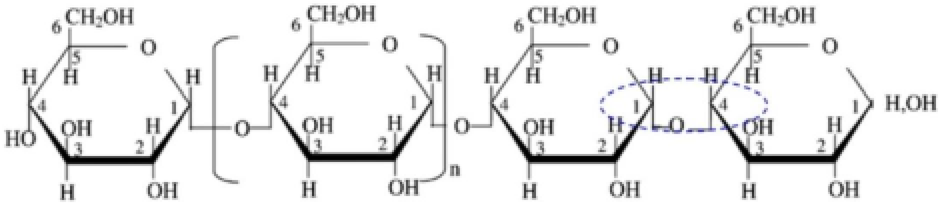


Figure 2. Chemical structure of the glucose polymer present in starch.

Starch properties are affected when subjected to hydrothermal treatments. Heating the system causes the starch granules to absorb extra water and swell. This process is named gelatinization in which the starch loses its natural crystalline structure and becomes amorphous forming a viscous paste with certain rheological properties. As most of the starch sources are used for human food, the scientific community has sought alternatives to investigate obtaining starch from agroindustrial biomass such as cassava peels. Because the methods of starch isolation directly affect the properties of the material it is important to use more appropriate techniques in terms of yield and purity. Industrial starch extraction is done by milling or grating, suspension of the starch in water, centrifugation and drying (Ai & Jane, 2015; Vamadevan & Bertoft, 2015).

2.1.2 Cellulose

It is a linear glucose polymer of high molecular weight formed by β-1-4-glucosidic linkages, insoluble in water, and is the main component of the cell wall of plant biomass. The cellulose polymer is formed by glucose units that range from 15 to 15,000, with an average value of around 3,000 units (George & Sabapathi, 2015). A cellulose molecule may present areas with an ordered, rigid, and inflexible configuration in its structure (crystalline cellulose), and other areas of flexible structures (amorphous cellulose). These conditions influence some aspects such as water absorption and fiber swelling. The hydroxyl groups of the cellulose react with various addition agents, providing the so called: Acid and alkaline celluloses; aminated celluloses and saline celluloses. The hydroxyl groups can also be esterified to form cellulose nitrates, xanthanes, and carboxymethylcellulose (Castro, 2009).

2.1.3 Hemicellulose

Structurally, hemicelluloses are heteropolysaccharides formed by monosaccharides such as xylose, glucose, arabinose, galactose, and mannose. Hemicelluloses have branching and side groups such as acetyl and glycuronic acid units (**Figure 3**) and can be categorized into four general polysaccharide classes, namely xylan, mannan, glucan, and xyloglucan (George & Sabapathi, 2015).

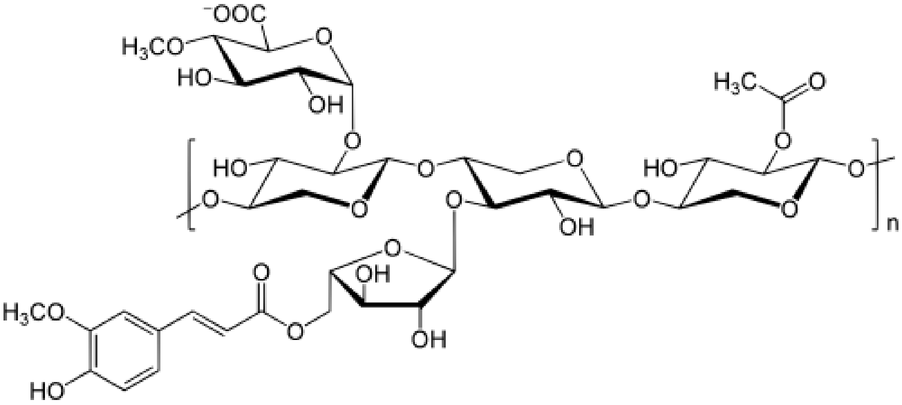


Figure 3. Chemical structure of the hemicellulose

Hemicellulose consists of ᴅ-xylopyranoside units connected by β-(1 → 4) bonds. In their natural state, hemicelluloses are amorphous with a degree of polymerization of only about 200 monomers. Due to the lack of crystallinity and irregular configurations, hemicelluloses easily absorb water. (**Chart 1**) shows the main differences found between cellulose and hemicellulose (Castillo et al., 2020; Castro, 2009; Wu et al., 2010).

Chart 1. Mains differences between a cellulose and a hemicellulose molecule.

CELLULOSE	**HEMICELLULOSE**
It consists of glucose units linked to other.	It consists of different units of sugars linked to other.
It has a high degree of polymerization	It has a low polymerization degree
Form fibrous arrangement	Does not form fibrous arrangement
It is slowly attacked by diluted mineral acid	It is rapidly attacked by mineral acid hot diluted
It is insoluble in alkali	It is soluble in alkali

Fonte: Castillo et al., 2020; Castro, 2009; Wu et al., 2010

2.1.4 Lignin

Lignin is considered one of the most resistant materials in nature. It is an amorphous aromatic polymer of high chemical complexity with three-dimensional cross-linked structure, being a polyphenol formed by phenylpropane units of (*p* -cumaryl alcohol, coniferyl alcohol and sinapyl alcohol). These units are connected mainly by aryl ethers that have hydroxyl (-OH) and methoxy ($-OCH_3$) groups. Lignin is then formed by crosslinking the three phenylpropane units through the (C – C) and (C – O) chemical bonds of the hydroxyl and methoxy groups, as shown in (**Figure 4**) (Cao et al., 2018; Li et al., 2020; Naseem et al., 2016; Ponnusamy et al., 2019; Wei et al., 2022)

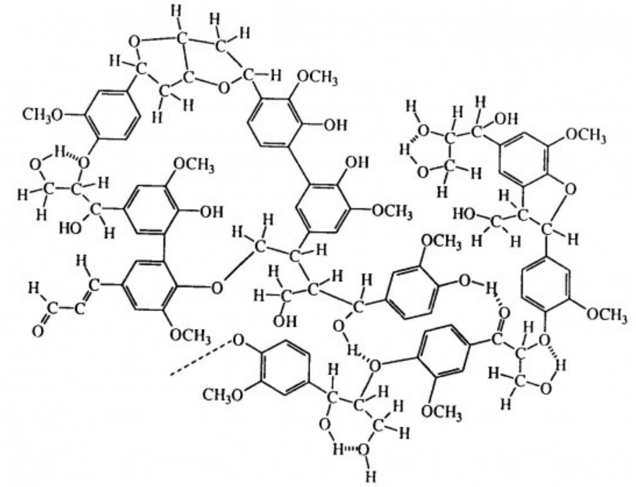


Figure 4. Chemical structure of the lignin

The variety of monomer components and their crosslinking make lignin a heterogeneous polymer of high molecular weight and widely varied structures. However, the complex macromolecular structure induces the low solubility of lignin in most organic solvents, so the need to develop pretreatments that easily remove lignin for its reuse as agricultural waste, in this sense the (**Figure 5**) illustrates possible bioproducts obtained by lignocellulosic biorefineries (Constant et al., 2016; Li et al., 2020).

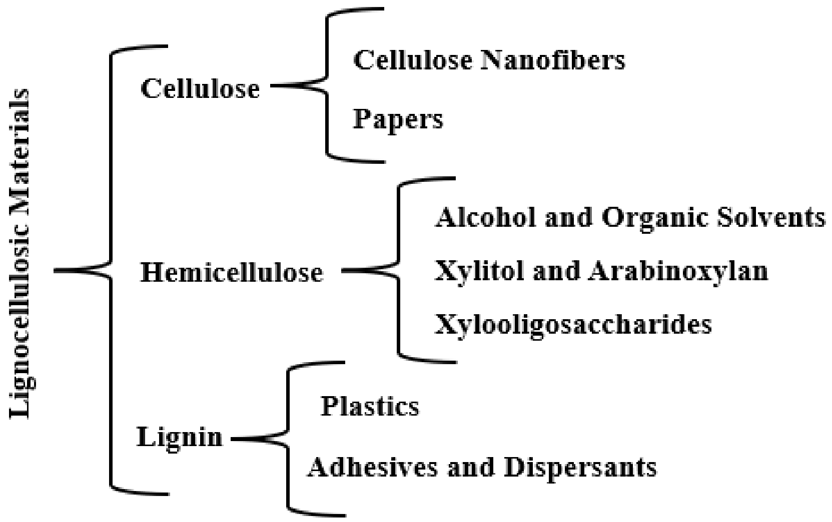


Figure 5. Possible bioproducts obtained from a lignocellulosic biorefinery.

2.2 Biorefineries and pretreatment on cassava peel

The cassava wastes have interesting biotechnological potential, which is aligned to the biorefinery concept (Xu et al., 2022)(Cherubini et al., 2009). Currently, the most used biorefinery classification includes: (I) whole-crop biorefineries, which make use of feedstocks such as corn and cereals, (II) green biorefineries, which use biomass such as, sweet grass or immature cereals, and (III) lignocellulosic feedstock biorefineries that feature cellulose, hemicellulose and lignin-based materials as residues (Kamm & Kamm, 2004).

Search was carried out on databases (Scopus, Web of Science and Science Direct) for the keywords ("raw cassava" OR "cassava water" OR "cassava peel" OR "cassava husk") AND ("pretreatment" OR "pre-treatment"), in order to identify the number of recent publications that evaluated pretreatments on cassava waste to obtain high value-added compounds. (**Figure 6**).

illustrates that from 2010 to the present time 31 studies have been published that have performed different pretreatments on cassava waste. It was found that in recent years the publications based on cassava peel have been increasing, i.e., it is a trend in the concept of biorefinery applied to lignocellulosic waste that has been explored more frequently in recent years due to the need to contribute to environmental matters worldwide and enrich the potential of agroindustrial waste in the bioeconomy chain.

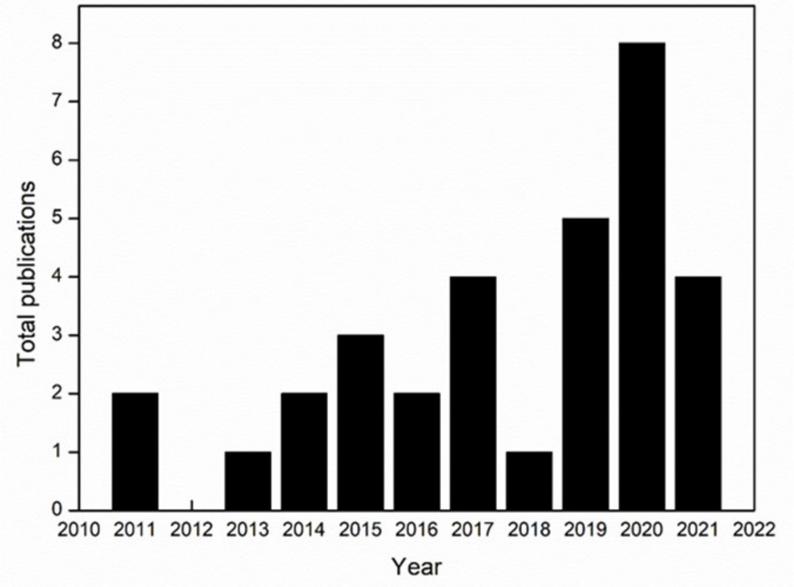


Figure 6. Number of publications related to the pretreatment of cassava peel using the key words ("raw cassava" OR "cassava water" OR "cassava peel" OR "cassava husk") AND ("pre-treatment" OR "pretreatment") in the databases Scopus, Web of Science and and *Science Direct*, over time, from 2010.

Lignocellulosic residues can be used in biotechnological processes, since they have low cost and high availability worldwide. In this sense, lignocellulosic residues often require chemical, physical and/or biological pretreatments. (**Chart 2**) illustrates all publications, including empirical, reviews, chapters, patents, among others, found in the literature, in different databases represented by the graph above, which addressed the obtaining of different bioproducts using cassava and its waste as a source of raw material and justifying the pretreatment method used.

Chart 2. Pretreatments used on cassava peel to obtain high-added value products.

Type of treatment	Treatment method	Enzymes	Application	Reference
Physical	Beating	-	Influence of isolated cassava starch for biogas production by anaerobic digestion	(Alrefai et al., 2020)
Chemical	Acid, alkaline and oxidative pretreatment	-	Production of cellulase enzyme by *Aspergillus Niger*	(Salihu et al., 2015)
Chemical	Methanol with combined acid	-	Bioethanol production	(Afolabi & Ayodele, 2020)
Chemical	Acid and alkaline pretreatment	-	Cellulase and xylanase production by *Trichoderma harzianum*	(Onilude, 1996)
Chemical	Acid and alkaline pretreatment	-	Hydrogen Production thermophilic	(Wang et al., 2013)
Biological	Enzymatic hydrolysis	β-Glucanase with cellulase and hemicellulase activities	Improving the debarking process of different lignocellulosic materials	(Barati et al., 2019a)
Physical and Chemical	Pretreatment by immersion and another in a combination of immersion and boiling at different times	-	Obtaining fermentable sugars	(A. Mohammed, 2014)
Physical and Chemical	Acid hydrolysis and pretreatment and autoclaving	-	Bioethanol production	(Hermansyah et al., 2018)
Physical and Chemical	Microwave-assisted alkali pretreatment and microwave-assisted acid pretreatment.	α-amylase and amyloglucosidase	Evaluation of the biocversion of reducing sugars	(Sudha et al., 2018)
Physical and Chemical	Hydrothermal treatment by immersion in the presence of diluted acid	-	Obtaining fermentable sugars	(Aruwajoye et al., 2017b)

Type of treatment	Treatment method	Enzymes	Application	Reference
Physical and Chemical	Hydrothermal pretreatment with combined acid	-	Bioethanol production	(Kongkiattikajorn & Sornvoraweatn, 2011)
Physical and Chemical	Acid hydrolysis, time-mediated oxidation and ultrasonic disintegration	-	Cellulose nanofiber production	(Czaikoski et al., 2020)
Physical and Chemical	Combined acid-based hydrothermal pretreatment, combined alkali-based hydrothermal pretreatment and hydrothermal pretreatment only	Cellulases and xylanases	Second-generation ethanol production	(W. Awoyale et al., 2021)
Physical and Chemical	Alkali-assisted hydrothermal pretreatment	α-amylase, glucoamylase and cellulase	Ethanol Production	(Papathoti et al., 2021)
Physical and Biological	Acid hydrolysis flowed by microbiological fermentation	-	Bioethanol production by *Saccharomyces cerevisiae*	(Efeovbokhan et al., 2019)
Physical and Biological	Steam explosion followed by enzymatic hydrolysis	Amylases and glucoamylases	Oligosaccharide Production	(Ona et al., 2019)
Chemical and Biological	Alkaline hydrolysis followed by enzymatic hydrolysis	α-amylase and hemicellulases	Combined production of bioethanol and biogas	(Moshi et al., 2015)
Physical, Chemical and Biological	Hydrothermal treatment, microwave exposure in acid paste, and diluted acid treatment with steam under pressure. All followed by enzymatic hydrolysis	Endoglucanase e β-glucosidase	Check the increase in enzymatic degradability	(Pooja & Padmaja, 2015)

2.2.1 Physical pretreatment

Physical pretreatments increase the surface area of the biomass by decreasing the particle size. The smaller particle size increases the access of enzymes in the enzymatic cellulosic and hemicellulosic region, providing more efficient microbiological and biochemistry interactions (Zevallos Torres et al., 2020). Physical pretreatments include standard methods such as ultrasound, microwave, irradiation and mechanical processes (extrusion, disc milling and ball milling) (Rodrigues et al., 2017). The mechanical process, using industrial mixers, is widely applied in agricultural residues and forest crops, as it reduces crystalline cellulose and particle size (Orellana, 2019).

Regarding physical pretreatments on cassava peel, the immersion and boiling were applied at different times to obtain fermentable sugars, where immersion reconciled to boiling for 2 hours removed the greatest amount of cyanides and increased the amount of carbohydrates produced (A. Mohammed, 2014). Mechanical pretreatment - beating - was applied to evaluate the cassava starch-based biogas production by anaerobic digestion of the residue in a biodigester. This pretreatment was carried out in a Holland beater for 5 minutes with 1:9 (w/v), cassava:water, respectively. The authors concluded that the cassava starch did not affect the quality of the biogas. In addition, the beating pretreatment provided low biogas production, 3.83 L (0.85 methane), when was used 2.22 kg of cassava peel and, sludge at 50% (Alrefai et al., 2020).

Thus, it can be seen that, in general, physical pretreatments have the advantages of increasing the total surface area of lignocellulosic materials, defibrillation and reducing the degree of polymerization and crystallization, reducing the crystallinity of cellulose and not producing compounds toxic, all without altering the biochemical composition of the biomass. Despite this, it’s a pretreatment method that can present high energy expenditure and difficulty in recovering the mass of materials in addition to high losses.

2.2.2 Chemical pretreatment

The chemical pretreatments mainly involve acid, alkaline, oxidative or organic solvent hydrolysis, in parallel with variations in physical parameters, such as pressure and temperature. Among the chemical pretreatments, acid hydrolysis is one of the most used methods for lignocellulosic materials, since it induces delignification (higher hemicellulose solubilization)

(Karp et al., 2013). The efficiency of obtaining the monossacharide xylose from the acid hydrolysis of hemicellulose can reach values of 75% (Jie Zhang et al., 2018). On the other hand, the alkaline hydrolysis, in particular using NaOH and KOH, lead to high delignification yields, i.e., the softening and solubilization of lignin. Thus, alkaline hydrolysis is widely used to concentrate hemicellulose which is quite interesting to the production of prebiotics, for example (Rodrigues et al., 2017).

Chemical pretreatment was studied using 100 mL of hydrochloric acid (HCl) in cassava pulp and peels in the proportion of 20% (w/v) followed by microbiological hydrolysis with *Aspergillus niger*, to obtain reducing sugars and subsequent bioethanol production in the presence of *Saccharomyces cerevisiae*. As a result, the biomass pretreated with hydrochloric acid followed by biological hydrolysis showed an average concentration of reducing sugars of 102.6 g/L. And as a result of the fermentative process, the maximum ethanol yield obtained was 54.8% by means of *Saccharomyces cerevisiae* (Efeovbokhan et al., 2019). On the other hand, different agricultural wastes such as corn peels, sorghum peels, soybean peels, peanut peels, banana peels, cassava peels, sugarcane bagasse, sawdust and rice straw were treated with 1N sulfuric acid, 1N sodium hydroxide and 1N hydrogen peroxide with 10% (w/v) solids, loading in an autoclave at 121 °C and 15 psi for 20 minutes. Then, pretreated samples were dried, and used as substrate for cellulase production by *Aspergillus niger* at 30 °C for 96 hours. As a result, the alkaline treatment on soya peels led to the highest production of cellulases (9.91 U/g) (Salihu et al., 2015). Similarly, cassava waste (root peels and fiber) was pretreated in three different forms for the production of cellulase and xylanase enzymes by solid state fermentation (*Trichoderma harzianum*): (I) the biomass was treated with 1% (w/v) sodium hydroxide solution at 120 °C and 20 minutes, (II) solution containing 96% ethyl alcohol with 8% (w/v) sodium hydroxide at 170 °C for 30 minutes and (III) sulfuric acid solution at 120 °C for 20 minutes. As a result, it was found that alkaline thermal pretreatment - 1% (w/v) NaOH at 120 °C - increased the cellulose content in the biomass by 155%. Moreover, most pretreatment processes also led to higher hemicellulose content, with the exception of H_2SO_4 treatment, which reduced the hemicellulose content of cassava husk by 25.3% (Onilude, 1996). Furthermore, the alkaline treatments (KOH, NaOH, and NH_4OH), when applied to lignocellulosic residues are advantageous, when compared to acid methods since the alkaline treatments lead to the swelling of the biomass fibers, directly influencing the chemical bonds of cellulose and hemicellulose, removing lignin easily and efficiently (Rodrigues et al., 2017).

2.2.3 Integrated pretreatment

Integrated pretreatments or combined pretreatments are those that use physical, chemical, and/or biological agents simultaneously or in sequence, for example, alkaline hydrolysis followed by enzymatic hydrolysis for the oligosaccharide production (Ona et al., 2019). Moreover, integrated pretreatment methods are characterized by high efficiency in removing unwanted compounds and as a consequence, higher bioconversion. Cassava peels, with a particle size of 850 µm, were treated in 200 mL of sodium hydroxide (0.5 M) and autoclaved at 121 °C for 15 minutes in order to produce bioethanol. After pretreatment, the samples were hydrolyzed, enzymatically, by α-amylase from *Bacillus* sp. at 70 °C for 1 hour and under 96 rpm agitation. The alkaline treatment promoted an improvement in biofuel yield by 56% (Moshi et al., 2015).

It was verified the application of biological pretreatment with enzymes in order to increase the efficiency of cassava peeling evaluating the enzymatic hydrolysis of a β- glucanase with cellulase and hemicellulase activities, the greatest catalytic effect observed occurred under conditions of pH 4.5, the temperature of 49.8 °C, incubation time of 3.9 hours and enzyme dose of 1.25 mL per 1 g of cassava peel (Barati et al., 2019b).

Three different pretreatments of cassava peel to verify the increase of enzymatic degradability of the residue were checked. Firstly, hydrothermal treatment was used. A second treatment applied was microwave exposure in acid paste (samples prepared in 0.75% (v/v) sulfuric acid were kept in a microwave oven at 300 W for 5, 10, 15 and 20 minutes). The third treatment studied was with diluted acid in contact with steam under pressure (samples were also prepared in 0.75% (v/v) sulfuric acid and exposed to a temperature of 121 °C for 30 minutes at a pressure of 15 psi). All samples treated in different ways were incubated at 60 °C for 120 hours in a multienzyme complex with endoglucanase and β-glucosidase activities. In conclusion, the hydrothermal treatment of biomasses was the most suitable technique to enhance biodegradation by cellulolytic enzymes (Pooja & Padmaja, 2015).

The physicochemical processes usually used acid or alkali solutions combined with some physical disturbances such as agitation, temperature, pressure, ultrasound, and microwave. In this context, pretreatments such as acid hydrolysis (sulfuric acid 30% (v/v) at 60 °C and 90 minutes) and oxidation (hydrogen peroxide) combined with physical ultrasound treatment (power 300 W for 20 minutes) on cassava peel for the production of cellulose nanofibers can be performed. The results showed that the acid treatment was more efficient in producing cellulose nanofibers with greater aspects and quantities (Czaikoski et al., 2020).

Three other distinct pretreatments (combined acid-based hydrothermal (0.75 M sulfuric acid), combined alkali-based hydrothermal (NaOH) and only hydrothermal pretreatment (boiled biomass for 1 h) were applied to cassava peel to produce second-generation ethanol. The post-treated samples were neutralized to pH 5.5 and incubated xylanases and cellulases for 2 h at 80 °C and as a result, it was possible to notice a greater conservation of sugars when performed the alkaline treatment (A. A. Awoyale & Lokhat, 2021).

Lignocellulosic biomass was chemically pretreated by immersion in dilute hydrochloric acid (3.6% v/v) at varying temperatures to obtain fermentable sugars was applied to cassava peel as well feedstock. It was found that the immersion treatment increased the accessibility of the diluted acid solution to the starch granules resulting in the cleavage of the macromolecule (Aruwajoye et al., 2017a).

Physicochemical pretreatment from acid hydrolysis of biomass (cassava peels) with sulfuric acid (10% (v/v)) followed by autoclaving at 121 °C for 30, 45 and 60 minutes was studied aiming at bioethanol production in the presence of yeast isolated from Durian (*Durio zhibetinus*). As a result of this pretreatment, one can see that the optimal sulfuric acid concentration of the hydrolysis process occurred in 30 min and the duration of the fermentation process by stirring in the incubator was 8 days (Hermansyah et al., 2018).

Cassava peel was also submitted to physicochemical pretreatment, in which the ground peels were autoclave ranged from 110 to 130 °C, from 15 to 30 min; and sodium hydroxide from 0.25 to 1.00% (w/v)). Then, enzymatic hydrolysis was carried out with cellulases and amylases at different doses to obtain ethanol by fermentative process presenting 86.11% of fermentation efficiency with pretreated peels (Papathoti et al., 2021). A comparison between two microwave pretreatments was carried out, using cassava pulp as substrate, in the presence of an aqueous solution of sodium hydroxide or sulfuric acid between 120 - 160 W at 20 - 60 min (intervals). It was realized that the amount of reducing sugar produced after enzymatic hydrolysis of cassava pulp pretreated in the presence of alkali in microwave showed 2.7 times more sugar compared to acid pretreatment (Sudha et al., 2018). Cassava peels pretreated with distilled water at 135 °C, for 30 min and under pressure of 1.03 bar can produce ethanol with high yield as those of pretreatment in the presence of dilute sulfuric acid under the same conditions (Kongkiattikajorn & Sornvoraweatn, 2011).

Hence, these studies show the importance and relevance of pretreatment for cassava peel to obtain high-added products as XOS, a necessary step to evaluate a pretreatment that can help reduce the recalcitrance of cassava peel for subsequent enzymatic hydrolysis and, consequently, obtaining oligomers with prebiotic properties.

The production of oligosaccharides from cassava peels using steam explosion pretreatment of the biomass followed by enzymatic hydrolysis with amylases and glucoamylases has been verified in the literature, but the steam explosion treatment did not show a high production of fermentable sugars (Ona et al., 2019).

Therefore, it is essential to further research on efficient pretreatments for the production of different oligomers, such as XOS In addition, the production of XOS using integrated pre-treatments such as a physical or chemical pre-treatment followed by a biological one by enzymatic hydrolysis proved to be very promising and efficient, as there is a higher yield in the production of the desired biocompound. In this sense, the next topics discuss the production of XOS data from different biomasses, including pretreatments, impurity removal, and XOS purification.

2.3 Enzymology

Most enzymes are proteins, while others are ribonucleic acids. They exhibit biocatalytic activity and specificity for different types of substrates, some of them require co-factors to perform their functions, the named co-enzymes (Tao et al., 2020). To simplify the understanding of enzymes, the International Union of Biochemistry (IUB) established a Commission on Enzymes that classified them into 7 groups according to the type of reaction catalyzed **(Chart 3).**

Xylanases (EC 3.2.1.8) are hydrolase enzymes that degrade xylan from hemicellulose, they act on the xylan main chain by randomly cleaving internal regions where there are β-1,4-xylosidic bonds (Malgas et al., 2019). Xylanolytic enzymes are a diverse group of enzymes that depolymerize xylan by an attack of the main chain (Puchart et al., 2021). The endo-β-1,4-xylanases have found important applications in various industrial sectors such as, in bleaching of cellulose by paper industries (Gangwar et al., 2014), decrease of viscosity of some cereals in baking area (Juturu & Wu, 2012) and conversion process of xylan into xylooligosaccharides, used as functional food additives and prebiotics (Samanta et al., 2015).

Chart 3. Classification of enzymes according to the Enzyme Commission of the International Union of Biochemistry (IUB).

Classes	**Function**
Oxidoreductases (EC 1)	Catalyze oxireduction reactions by transferring electrons or protons (H^+)
Transferases (EC 2)	They transfer chemical groups between molecules.
Hydrolases (EC 3)	They use water as a receptor for functional groups
Lyases (EC 4)	Form or destroy double bonds by removing or adding functional groups such as water, ammonia, and carbon dioxide
Isomerases (EC 5)	They transform a molecule into its isomer.
Ligases (EC 6)	They form chemical bonds by condensation reactions, consuming energy in the form of ATP.
Translocases (EC 7)	Catalyze the movement of ions or molecules across membranes, usually cell membranes.

Fonte: Tao et al., 2020

2.4 Production of xylooligosaccharides

XOS are considered promising prebiotics since they selectively stimulating the growth of specific probiotic bacteria, such as *Bifidobacterium*, and *Lactobacillus*, whereas other prebiotics as fructooligosaccharides, and galactooligosaccharides, stimulation both probiotic and some pathogen bacteria (Poletto et al., 2020a). Thus, XOS can be used by the food and pharmaceutical industries (Jingzhi Zhang et al., 2019). In this sense, there is an increasing demand on the production of XOS (Poletto et al., 2020a). According to Global Info Research, it is expected a prebiotic ingredient market ≈ US$130 million in 2024, which represents an increase of 5.3% in the coming years. XOS can be produced from many agro-industrial residues rich in xylans, such as corn peel (Samanta et al., 2016), coffee husk (Ávila et al., 2020), beer residue (Amorim et al., 2019), sugarcane bagasse (Kaur et al., 2019) and cassava peel (Agung et al., 2016).

The search on Scopus, Web of Science, and Science Direct databases with keywords "Lignocellulosic materials" AND "Xylooligosaccharides", showed a total of 44 empirical scientific articles, summarized in (**Table 1**).

Table 1. The production of xylooligosaccharides using agroindustrial residues.

Residue	Pretreatment	Conversion Factor in XOS	Reference
Walnut shell	Despectination (HCl)/ Delignification (sodium chlorite and acetic acid)/alkaline extraction (KOH)	90.0%	(Cebin et al., 2021)
Pea pod	Despectination (HCl)/ Delignification (sodium chlorite and acetic acid)/ alkaline extraction (KOH)	85.4%	(Cebin et al., 2021)
Eucalyptus wood	Steam explosion	50.0%	(Cebreiros et al., 2021)
Empty fruit bunches	Alkaline hydrolysis (NaOH)	41.1%	(Wijaya et al., 2020)
Corn cob	Alkaline hydrolysis (NaOH)/ acid precipitation (HCl)	75.0%	(Jie Zhang et al., 2018)
Coconut shells	Alkaline hydrolysis (NaOH)/ Steam	93.1%	(Jnawali et al., 2018)
Hazelnut shells	Hydrothermal (150 – 200 °C)	62.0%	(Surek & Buyukkileci, 2017)
Rice straw	Hydrothermal (150 – 240 °C)	40.1%	(Moniz et al., 2014)
Sugarcane	Treatment with hydrogen peroxide, glacial acetic acid and trifluoroacetic acid	91.8%	(Bragatto et al., 2013)
Corn stalk	Hydrothermal (196 °C) / acid hydrolysis (H_2SO_4)	70.0%	(Garrote et al., 2001)

In this sense, materials with high cellulose and/or lignin contents usually show the lowest XOS yields. The lignin involves the biomass, making its extraction difficult, while large amounts of cellulose could incorporate the hemicellulose chain. Thus, it is extremely important to know the composition of the material, as this influences the extraction of hemicellulose (Poletto et al., 2020a). Regarding cassava peel composition, values between 23.4 to 32.3% of hemicellulose, 9.7 to 14.2% of cellulose, 10.8 to 16.9% of lignin and 3.7 to 11.3% of ash were reported (Pondja Jr. et al., 2017). Therefore, the challenges related to this topic are to develop more efficient pre-treatment methods that allow a greater removal of the lignin content from the biomass and in the steps of enzymatic recovery and purification of XOS.

2.5 Pretreatments correlated with xylooligosaccharides production

A wide range of pretreatments can be carried out on lignocellulosic materials for subsequent enzymatic hydrolysis, for instance, chemical pretreatments such as acid (Aguilar et al., 2002), alkali (Nascimento et al., 2016), ionic liquid (Vancov et al., 2012) and hydrothermal (Monteiro et al., 2021), physicochemical as a steam explosion (Jacquet et al., 2015), also the biological ones through direct fermentation (Amorim et al., 2019), the physical methods as microwaves (Ethaib, 2015), ultrasound (Bussemaker & Zhang, 2013), and also the little investigated methods as non-thermal plasma (Pereira et al., 2021). XOS is produced by chemical and hydrothermal treatments followed by enzymatic hydrolysis (Poletto et al., 2020a) (**Figure 7**).

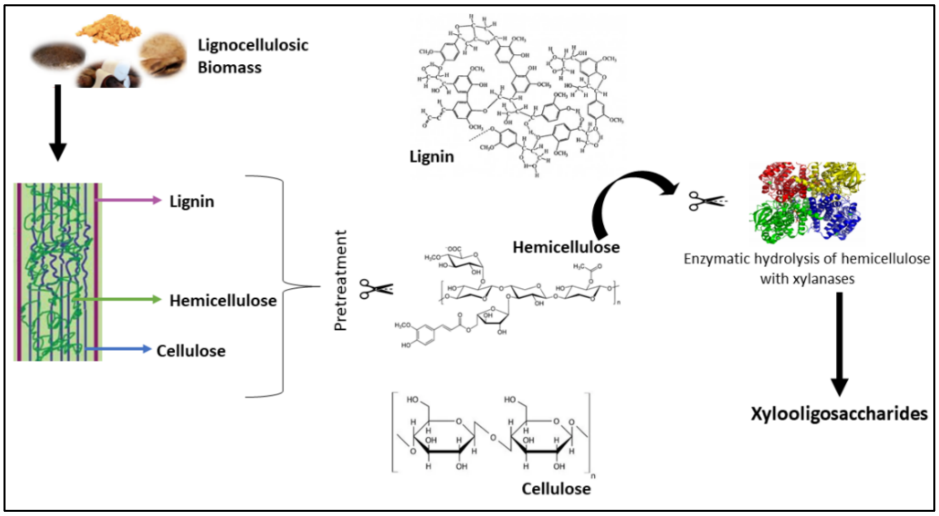


Figure 7. Chemical-enzymatic xylooligosaccharides production.

In this sense, dilute acid and alkaline pretreatments stand out. The solubilization of hemicellulose during acid treatment occurs due to cleavage of glycosidic bonds in the hemicellulose chain, releasing oligosaccharides and monosaccharides (de Freitas et al., 2019). Acid treatment is a quick method that requires high temperatures and pressures, which favors the formation of toxic compounds and requires a purification step of the extracted xylan besides reducing the concentration of hemicellulose and generating undesirable by-products such as furfural. Xylan can be purified by different methods such as frozen-thawed fractionation, Fehling method fractionation and ion-exchange column fractionation (Cipriani, 2003). On the other hand, acid pretreatments, including mineral and organic acids, have been shown to effectively break the recalcitrant crystalline structure of the cellulosic matrix by efficiently enhancing the enzymatic hydrolysis step (Dai et al., 2021) The alkaline method is generally performed at 121 °C and 1.1 bar (Karp et al., 2013).

Regarding the alkaline treatment, the efficient removal of lignin from biomass occurs through the use of alkalis, increasing the internal surface area and the distension of fibers in the waste, facilitating the enzymatic activity (de Freitas et al., 2019), i. e., the alkaline pretreatment has as its main effect on the biomass the delignification (solubilization of lignin), turning the hemicellulose and cellulose more accessible to subsequent hydrolysis processes. The use of alkaline agents promotes swelling in the fibers, affecting the hemicellulose and cellulose bonds, thus increasing biomass porosity (Haghighi Mood et al., 2013). The disadvantage is the formation of salts, which makes it necessary to add purification steps.

The hydrothermal procedure is performed by exposing the biomass to high temperatures (160 to 230 °C) and pressure (2.5 – 10 MPa) (Rabemanolontsoa et al., 2016). The exposure of lignocellulosic biomass to water leads to its penetration into cell structures and, consequently, to cellulose hydration and hemicellulose depolymerization (Poletto et al., 2020b). It is an interesting method since there is no need for dangerous or expensive reagents, as only water is used. Despite the low consumption of chemicals, the process requires high energy consumption due to the pressure and temperature conditions used (Karp et al., 2013).

In addition to these chemical and hydrothermal processes, new strategies are being studied as an alternative to improve the accessibility of the enzyme for high-pressure depolymerization of hemicellulose (Pereira Ramos, 2003) and ionic liquids (Mäki-Arvela et al., 2010). The high-pressure methodology can be applied in biomass to promote delignification without modifying the covalent bonds of the hemicellulosic fraction since ionic liquids can be an interesting strategy in the dissolution of cellulose and lignin.

Another technology that has been explored as a pretreatment technique in lignocellulosic materials is the use of non-thermal plasma (Pereira et al., 2021), considered an environmentally correct technique, where the plasma is produced by means of dielectric barrier discharge to improve a later step of access of the enzymatic complex to the lignocellulosic matrix (Kriegseis et al., 2011). Therefore, many pretreatments can be used in different lignocellulosic materials to potentially obtain products with prebiotic properties such as XOS.

Enzymatic hydrolysis does not result in toxic catalysis compounds such as acetic acid, furfural, and hydroxymethylfurfural (Brienzo et al., 2009). The purification of XOS through this route is facilitated by pretreatment applied to lignocellulosic biomass and the specific action of endo-β-1-4-xylanases (Gullo et al., 2006). The enzymatic pathway is carried out by endo-β-xylanases that catalyze the hydrolysis of glycosidic bonds of the β-1,4 xylan type. Therefore, for the production of XOS, xylanolytic preparations with high activity of endo-β-1-4-xylanases and low activity of exo-xylanases and/or β-xylosidases are desired, in order to avoid the formation of xylose that can inhibit the endo-xylanase activity (Vázquez et al., 2000).

Regarding enzymatic hydrolysis, a new search was carried out in the same databases with the keywords ("raw cassava" OR " cassava water" OR "cassava peel" OR " cassava husk") AND "xylooligosaccharides"). Only one report was found related to the application of cassava peel and waste as raw materials for xylooligosaccharide production using endoxylanase from *Bacillus subtilis* of soil termite abdomen (Agung et al., 2016).

In this study, the focus was on the enzymatic production of XOS from cassava peel, where first, the xylan was extracted by immersion in 10% sodium hydroxide (NaOH) for 24 h, followed by pH adjustment to 7 by addition of 5% (w/v) hydrochloric acid (HCl). Then, cassava peel xylan was hydrolyzed using endoxylanase (2.21 U/mL) from *Bacillus subtilis* from the abdomen of soil termites at pH 5 and 50 °C for 15 h. As a result, it was obtained the production of XOS, especially X3 (degree of polymerization), X4, and X5, were also found; nevertheless X2 was not found. The X2 (xylobiose) production is quite desirable since it shows remarkable prebiotic properties. It is worth noting that the alkaline method was used to expose the xylan and assist in the enzymatic hydrolysis step. Based on this argument, (**Table 2**) was elaborated with the objective of correlating the XOS production by alkaline pretreatments from lignocellulosic residue.

Table 2. XOS production from different lignocellulosic materials using alkaline pretreatment method combined with enzymatic hydrolysis.

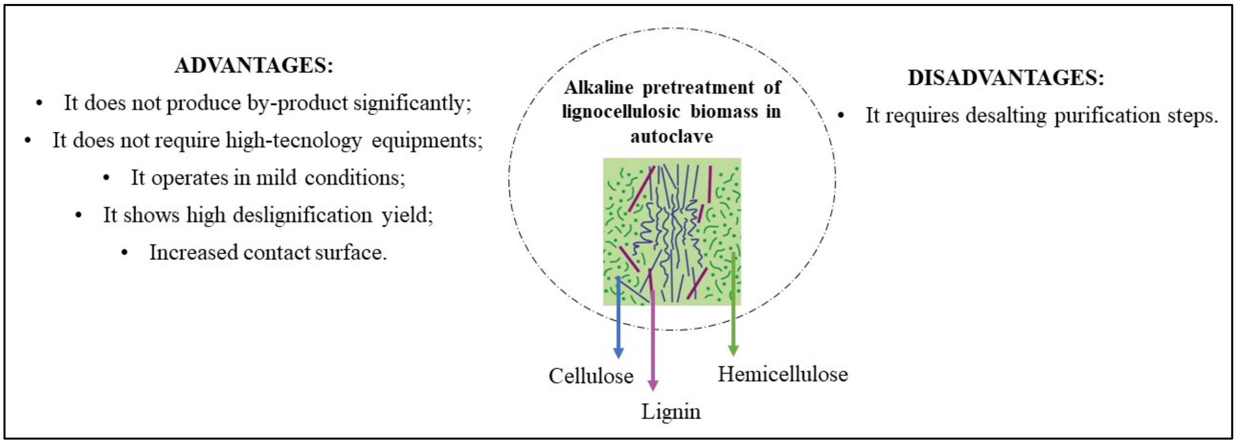
Raw material	Pretreatment	Purpose of pretreatment	Enzymatic Hydrolysis	XOS Production	References
Cassava peel	10% (NaOH) and (HCl) 5%	Assist in xylan recovery	Endoxilanase from*B. subtilis*	Mainly xylopentoses	(Agung et al., 2016)
Sugarcane bagasse	KOH 24% (3 h/ 35 °C) including $NaBH_4$ 1% (w/w).	Aid in the recovery of arabinoxylan	Xylanases from *A. fumigatus*	0.88%	(Flavia et al., 2020)
Brewery grain	NaOH 0.38 M, 172 °C, ultrasound assistance	Obtaining arabinoxylans	Endoxylanase	3.58% AX total	(Lucas et al., 2020)
Rice peel	12% NaOH, 30 min vaporization time and 133.64 °C	Assist in xylan recovery	Commercial xylanase	1.73%	(Khat-udomkiri et al., 2018)
Empty fruit bunch fiber	0.5% NaOH (121 ºC, 20 min)	Assist in xylan recovery	Xylanase-producing bacterial isolates	0.78%	(Suvarna et al., 2012)

Different lignocellulosic materials have already been pretreated with the alkaline method to obtain XOS. Rice peel, for example, when treated alkaline under ideal conditions of 12% NaOH, at 133 ºC, combined with a 30 min vaporization, obtained xylan recovery in 54.4 ± 0.6, which resulted in 17.3 ± 0.3 mg of XOS per mL of xylan after enzymatic hydrolysis (Khat-udomkiri et al., 2018). On the other hand, when sugarcane bagasse was treated for 3 h at 35 °C with 24% KOH including 1% NaBH4 (w/v) and subsequent enzymatic hydrolysis with xylanase from A. fumigatus, a 72.1% yield was obtained when compared to the hydrogen peroxide method (Flavia et al., 2020). In empty fruit bunches, pretreatment with 0.5% NaOH for 15 minutes at 121 ºC was considered the best, as it made the xylan highly accessible for xylanase and subsequent production of XOS (Suvarna et al., 2012). The brewery residue, when treated with microwaves and 0.38 M NaOH at 172 °C, obtained a pre-hydrolysate with 17 g/L arabinoxylans, which was later hydrolyzed by endo-xylanases, which significantly increased the concentration of oligomers with 2–6 xylose units (Lucas et al., 2020).

Therefore, the alkaline treatment method is a classic method of pretreatment in lignocellulosic materials, which has numerous advantages, such as not producing undesirable by-products in large amounts of monosaccharides and requiring little specialized equipment (Suvarna et al., 2012), in addition to requiring mild conditions, when compared to other pretreatment methods (Badiei et al., 2014). Some disadvantages as high power consumption are reported (Chen et al., 2017), which does not prevent further investigation of this method for the residue proposed in this review, aiming to increase the availability of xylan from cassava peels to obtain XOS.

2.6 Future trends and conclusions

As a proposal for future works, a more detailed investigation of classic pretreatment methods such as alkaline in cassava peel. This review has addressed numerous advantages (**Figure 8**). to use this method, such as not producing undesirable by-products in large amounts of monosaccharides and requiring little specialized equipment, requiring less severe conditions (pressure, temperature, and time) compared to other pretreatment methods. Another great advantage that the alkali pretreatment promotes is the more efficient removal of lignin from the biomass, increasing the internal surface area and fiber distension in the residues, facilitating the enzymatic activity. However, alkaline hydrolysis can promote the unwanted formation of residual salts in the biomass, requiring, in occasional cases, purification steps. In addition to, regarding the alkaline pretreatment, more specifically the base of sodium hydroxide and being more effective to remove lignin, the use of sodium hydroxide also eliminates acetyl and uronic acid groups existing in hemicellulose, which, if not treated, result in compounds toxic in the process.



ADVANTAGES:

- It does not produce by-product significantly;
- It does not require high-tecnology equipments;
- It operates in mild conditions;
- It shows high deslignification yield;
- Increased contact surface.

DISADVANTAGES:

- It requires desalting purification steps.

Figure 8. The main advantages and disadvantage of alkaline treatment method for delignification.

Another alternative would be to work with non-thermal plasma as a pretreatment on cassava peel to delignify this material and make access to xylanases easier and more assertive to obtain XOS with different degrees of polymerization that have prebiotic properties. An interesting aspect in relation to this innovative technique is that, until now, it has not been used as a pretreatment to obtain XOS, only in some studies to obtain bioethanol. This technique is considered non-toxic, environmentally correct as it does not use chemical products, in addition to the possibility of working with air as a plasma gas, which results in the minimal generation of costs to the process. Some studies have already applied this technique to lignocellulosic waste such as beer waste, where about 36% of lignin was removed using this technique as a pretreatment in 10 min (Ravindran et al., 2019), sugarcane bagasse, with 58% of delignification after 2 h (Miranda et al., 2019), and wheat straw, where there was 74% delignification after 7 h (Schultz-Jensen et al., 2011).

Other techniques such as high-pressure hydrothermal methods can also be an excellent option to treat lignocellulosic materials and assist in conversion to value-added products. Maga kernel, for example, when treated at 2.5 MPa and 15 min provided a high hemicellulose conversion of approximately 40%, divided into 8.2% XOS (X2-X6) and 31.1% soluble xylan (X > 6) being an alternative for reusing the mango seed shell (Monteiro et al., 2021). Finally, the application of xylooligosaccharides in food and pharmaceutical compounds will be a future trend due to their potential for human health.

The processing of cassava to obtain flour and starch generates huge amounts of waste: liquids (cassava wastewater) and solids (peels), which when disposed of incorrectly cause great environmental impacts. In view of this, the valorization of agroindustrial biomasses has gained prominence in recent years due to the strong potential they have in being reused to obtain hemicellulosic-based compounds such as xylooligosaccharides. Cassava peel is a lignocellulosic residue that can be converted into high value-added bioproducts such as sugars, fertilizers, biofuels and bioactive compounds. For this to be possible, different pretreatments are applied for greater bioconversion. In this sense, the alkaline pretreatment has the highest delignification efficiency compared to other chemical methods (acids and organic solvents) and physical (mechanical methods) because the alkaline pre-treatment promotes greater lignin removal and less xylan loss, which guarantees a higher yield in the enzymatic hydrolysis step.

Also, alkaline pretreatment can improve substrate digestibility, the main desired characteristic in the production of XOS by enzymatic hydrolysis. In addition, other pretreatments, such as non-thermal plasma and ionic liquid, should also be further studied because they are environmentally friendly and little explored techniques. Therefore, lignocellulosic residues have a strong potential for bioconversion by industrial biorefineries, which contributes to an increase in bioeconomy and sustainability, which reduces dependence on petrochemical sources.

2.7 References

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3 CHAPTER 3 – OBTAINING XYLOOLIGOSACCHARIDES BY ENZYMATIC HYDROLYSIS [2]

3.1 Introduction

Agroindustrial biomass has been gaining prominence as an excellent feedstock for the production of many different industrially essential biocomposites for the contemporary world (Jain et al., 2022). The use of these renewable wastes as alternative culture media is very considerable due to their high availability and the growing industrialization of the agricultural sector. The annual production of agroindustrial biomass is a challenging, since the incorrect disposal of residues can lead to environmental impacts such as, water pollution, and air pollution (Raina et al., 2022).

Agrobiomass can be found mainly in the form of pulp, peels, seeds, sugarcane bagasse, rice straw, and corn cob. Such biomasses have a great potential as a low-cost raw material. This approach has been drawing to attention the industrial and scientific community (Zevallos Torres et al., 2020). In this context, the concept of biorefinery can lead to the conversion of agricultural wastes into high value-added compounds such as pharmaceuticals, food, fertilizers, among others (Dai et al., 2021; Patrizi et al., 2020).

Cassava peels are lignocellulosic waste composed of cellulose and hemicellulose arranged in a complex crystalline structure encircled by lignin, which is a natural phenolic ramified polymer highly resistant to degradation (Zúñiga-Arias et al., 2022). In this sense, many pretreatments were evaluated to promote the removal of lignin present in biomass (Baruah et al., 2018), such as hydrothermal pretreatments; acid, alkaline; oxidative; liquid-ionic; biological; microwave and the most recent non-thermal plasma (Pandey & Negi, 2015; Pereira et al., 2021; Qi et al., 2010). One of the most studied pretreatments is the alkali, thermal and pressurized system, in which lignin is efficiently dissolved by breaking the cross-links that unite lignin with cellulose and hemicellulose (Kim et al., 2016; H. Xu et al., 2015).

Alkaline pretreatment of lignocellulosic material induces to delignification, enhancing XOS production due to the cellulose and hemicellulose exposure (Menezes & Durrant, 2008). In this sense, the most assertive strategy to obtain XOS is one that combines an efficient pretreatment to remove unwanted compounds, such as starch and lignin, and to concentrate hemicellulose for the convert the exposed xylan into XOS. Once the xylan has been isolated or degraded by the

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[2] *Submitted for Publication in Biomass Conversion and Biorefinery*

pretreatments, it will be available for the enzymatic hydrolysis step (Menezes & Durrant, 2008). The xylanases are the enzymes responsible for the hydrolysis of xylan, they can act in an "endo" way, hydrolyzing links inside the polymer, forming smaller oligosaccharides (endo-1,4-β-xylanase) or in an "exo" way, hydrolyzing the xylan chain by the exterminates, releasing xylose monomers (β-xylosidase), for XOS production the enzyme complex should ideally have high endo-1,4-β-xylanase activity to minimize monosaccharide production (Grafulin, 2018).

Therefore, the enzymatic production of **cassava peel-based** XOS is an interesting approach, as it is environmentally friendly, besides it presents high efficiency and specificity, that is, it leads to low amount of xylose and undesirable by-products (Wan Azelee et al., 2016). Due to the increased interest by the food and pharmaceutical industries for XOS, the necessity of valorization of agricultural wastes and the few that has been addressed in the literature about cassava peels as raw material for production of XOS. This study has great highlight for contributing to the use of biotechnological techniques to obtaining a bioactive of high economic interest.

3.2 Material and methods

The experimental stage of this project is represented by **Figure 9**.

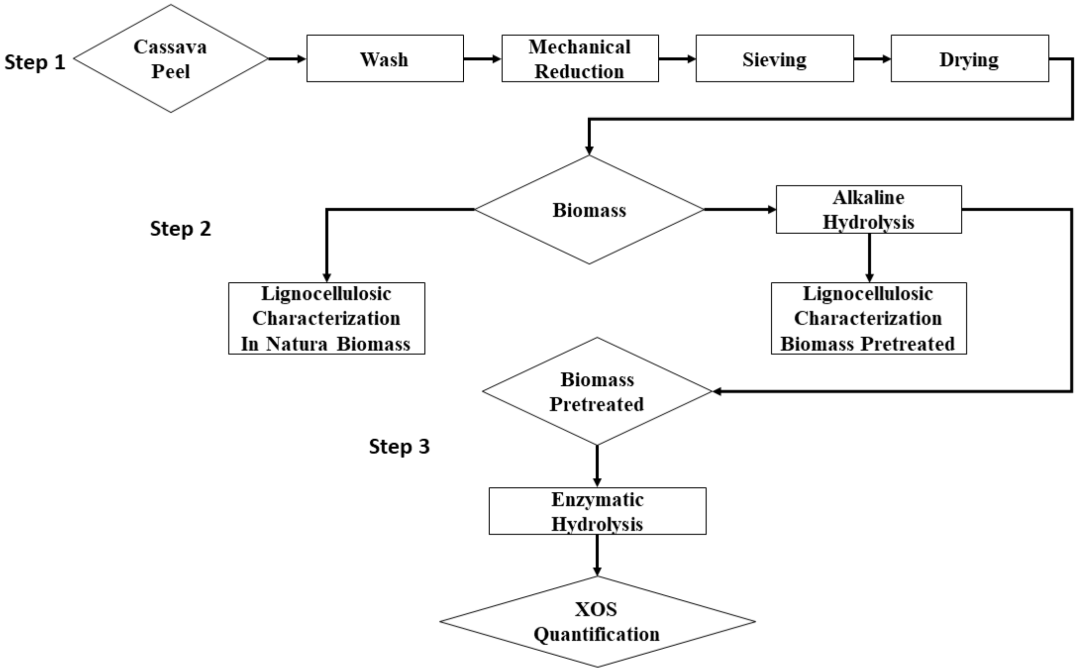

Figure 9. Flowchart of the experimental step to obtain XOS.

3.2.1 Material

Cassava waste was kindly donated by Rocha Alimentos® (Santa Catarina, Brazil) on August 2021 as a heterogeneous sample containing peels and roots. Thawing of the material occurred at room temperature followed by submerged sanitization in 10^3 mg/L sodium hypochlorite (NaClO) solution for 15 minutes and washed with abundant flow water for 30 minutes to remove impurities (Czaikoski et al., 2020). The analytical reagents sodium hydroxide (NaOH), chloridric acid (HCl), sodium thiosulfate pentahydrate ($Na_2S_2O_3.5H_2O$), sodium carbonate (Na_2CO_3), chloroform ($CHCl_3$), potassium dichromate ($K_2Cr_2O_7$) and potassium iodide KI) were obtained from NEON Reagents® (Brazil). Sulfuric acid (H_2SO_4) was obtained from Sigma-Aldrich® (USA) and the endo-1,4-β-xylanase enzyme (E.C 3.2.1.8) from *Trichoderma longibrachiatum* was obtained from Megazyme International® (Ireland).

3.2.2 Pretreatments on cassava peel

Different pretreatments were applied sequentially to cassava peels: (i) washing, mechanical reduction and drying to remove starch; (ii) alkaline pretreatment to remove lignin and acid hydrolysis for lignocellulosic characterization; and (iii) enzymatic hydrolysis to obtain XOS, as represented in Fig. 9.

3.2.2.1 Starch removal

The aqueous removal of starch (ARS) was applied to cassava peels. Briefly, a mixture 1:4 (cassava peels: distilled water) was mechanical reduced in a blender (Philips Walita 600 W) at maximum speed for 2 minutes and then the mixture was separated by sieves of 150 and 106 mm particle size. The permeate (washing water) was collected alternately from one wash to the other and frozen for later starch quantification by iodometric method. The retained material was reprocessed (15x), dried at 70 °C for 24 hours and then stored in polypropylene bottles at ambient conditions until use in the hydrolysis steps. The biomass yield was determined by the mass difference after drying and the value was used for the material balance calculations (Czaikoski et al., 2020). The removed starch was measured by iodimetry assay from the amount of titrating agent consumed in relation to the first (V_1) and the fifteenth (V_f) washing water, according to Eq. 1.

Moreover, it was assumed that the average amount of starch in cassava peels is 45%, as predicted in the literature (Andrade, 2001; Gangola et al., 2021).

$$[ARS] = \frac{V_f}{V_1} \,.\, 100\% \qquad \text{Eq. 1}$$

3.2.2.2 Alkaline pretreatments

The cassava peels with reduced starch content were subjected to alkaline treatment with sodium hydroxide (NaOH) at different concentrations (2, 4 and 6% (w/v)) and hydrolysis time (30 and 60 minutes) at 121 °C and 1.1 bar pressure. The alkaline treatments were conducted in 250 mL Schott flasks with a ratio solid: liquid of 1: 100 in triplicate. After alkaline pretreatment of the lignocellulosic content the phase separation was performed by vacuum filtration; the liquid stream was collected and frozen and the solid material was stove dried for 24 hours at 100 °C (Salihu et al., 2015; Singh & Bishnoi, 2013). After drying time, the material was triturated in a knife mill and its content of cellulose, hemicellulose, lignin, moisture content and ash was determined according to the methodology described in Section 3.2.3.

3.2.3 Lignocellulosic characterization

The lignocellulosic centesimal composition of the solid samples was quantified according to the National Renewable Energy Laboratory (NREL) methodology. The determination of extractives was performed after sequential extraction in Soxhlet with osmosis water and anhydrous ethanol (99.8%) according to procedure (NREL/TP-510-42.619) as part of the determination of the *in natura* material (Sluiter et al., 2004). The cellulose, hemicellulose, lignin, and ash contents were quantified starting from an acid hydrolysis, in which 0.3 g of the material treated in the previous step was suspended in 3 mL of 72% (v/v) sulfuric acid in 250 mL Schott flasks and placed in a shaker for 60 minutes at 30 °C and 120 rpm, every 15 minutes the samples were manually homogenized with a glass rod. In the sequence the samples were diluted to 4% with the addition of 84 mL of distilled water and autoclaved at 121 °C and 1.1 bar. Subsequently, the separation of the phases occurred by gravimetric filtration, a part of the collected liquor was analyzed in a spectrophotometer for determination of the acid-soluble lignin content in quartz cuvette and wavelength of 240 nm, with results within a range of 0.700 - 1.000 absorbance (Pereira et al., 2021). The other part of the liquor obtained in the same liquid stream was quantified for glucose, xylose, arabinose, acetic acid, and formic acid contents by High Performance Liquid Chromatography (HPLC) using the Shimadzu Prominence LC-20A (Shimadzu, Tokyo, Japan) chromatograph with a Bio-rad Aminex HPX-87H column, detection by refractive index (RI). The injection of 10 µL of sample was eluted with 5 mmol/L of H_2SO_4 at 0.5 mL/min flow rate. The oven temperature was maintained at 35 °C and the run time was 30 min. The solid retained on the filter paper was washed with abundant warm distilled water to remove all acid present and placed in a stove at 100 °C for 24 hours for determination of insoluble lignin. After being weighed, the dry solid was taken to the muffle furnace at 575 °C for 24 hours and burned for ash determination, according to the protocols of NREL (Sluiter et al., 2004).

3.2.4 Enzymatic hydrolysis and kinetics

The cassava peels with reduced starch and lignin content (highest percentage of delignification) were selected for the enzymatic hydrolysis step. The experiment was performed with endo-1,4-β-xylanase (3.2 U/mL). It was decided to fixe substrate condition of 1% (w/v) in a 2 mL reaction volume with 0.5 mol/L sodium phosphate buffer pH 6 in polystyrene plates with cell culture wells (Agung et al., 2016). Different enzyme concentrations (0.5, 1.5, and 3.0% (v/v)) were added to the wells with substrate and incubated in a shaker at 50 °C and 180 rpm. The reaction products (liquor + sample) were collected in duplicate at time intervals of (6, 12, 24, 48 and 96 hours) and transferred to microcentrifuge tubes, the samples were placed in a boiling bath for 5 minutes followed by an ice bath for another 5 minutes to the enzyme by denaturation. The, the samples were centrifuged for 5 minutes at 1,000 rpm, the liquid stream was collected, pressure filtered (0.22 µm), and quantified for xylose (X1) and XOS contents.

3.2.4.1 Quantification of xylose and xylooligosaccharides

The quantification of monosaccharides (xylose) and polysaccharides (XOS) was performed on a high-performance anion exchange column (HPLC-PAD) using the supernatants resulting from the enzymatic hydrolysis, as described in the methodology (Ávila et al., 2020). The chromatographic analysis was performed on a Dionex DX-500 (Sunnyvale, CA, EUA) with a column CarnoPac PA1 (4 mm x 250 mm), a guard column CarboPac PA1 (4 mm x 50 mm), and an electrochemical detector, adopting a linear gradient of A (NaOH 100 mM) and B (NaOH 100 mM; NaOAc 300 mM). The integrated peak areas were adjusted based on the patterns acquired from the Megazyme® (Bray, County Wicklow, Ireland): xylose (X1), xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5) and xylohexaose (X6). The yields of xylooligosaccharides (mg XOS/g xylan) were calculated using Eq. 2 and Eq. 3 (Ávila et al., 2020).

$$Yield\ (xos) = \left(\frac{[XOS_{\ total}]\ .\ 0.88}{f_{(xylose)} \cdot \frac{S}{L}}\right) \tag{Eq. 2}$$

$$f_{(xylose)} = \left(\frac{[\ xylose\ HPLC]\ .\ 0.88\ .\ 0.08673}{ODW}\right) \tag{Eq. 3}$$

Where: [XOS total] are the concentrations of X2 - X6 (g/L) for yield; [S/L] is the solid/liquid ratio used in the enzymatic hydrolysis step (g residue/L solution); f (xylose) is the fraction of xylose in the dry material (g xylose/g residue); [xylose HPLC] is the concentration of xylose determined in the characterization of the material after pretreatment (g/L); ODW is the dry mass of the residue used in the lignocellulosic characterization step (g); 0.08673 (L) value of the lignocellulosic characterization equivalent to the dilution of sulfuric acid in distilled water (NREL); and 0.88 is the correction factor used for pentoses to obtain sugars in polymeric form.

3.2.5 Enzymatic activity

The activity of the enzyme endo-1-4-β-xylanase was determined from 250 µL of pre-equilibrated buffered enzyme preparation at 40 °C was added to 1% (w/v) substrate solution (azo-xylan birchwood) in microcentrifuge tubes under vortex agitation. The samples were immediately incubated in a 40 °C water bath for a time of 10 min. Successively 1.25 mL of 95% (v/v) ethanol was added followed on a vortex mixer to precipitate the unhydrolyzed material, the tubes were allowed to stand for 5 min at room temperature and shaken again. The samples were centrifuged at 3,000 rpm for 10 min, then the absorbance was determined at 590 nm. One unit of enzyme activity was defined as the amount of enzyme required to release one µmol D-xylose reducing arabinoxylan at pH 6.0 per minute at 40 °C.

3.2.6 Statistical analysis

Statistical analysis of the data was performed with OriginPro 8.5 software. The results were evaluated from the mean values and standard deviations of the calculated data (ANOVA one-way) and significant differences were determined by the Tukey test at a probability level of less than 5% ($p < 0.05$).

3.3 Results and discussion

This section will present the results obtained during the experiments and the comparative discussion with other studies found in the literature. The effect of starch removal by mechanical method in liquid medium, the effect of alkaline treatment on lignin removal and exposure of xylan from hemicellulose of cassava peels and the yield of XOS obtained by enzymatic hydrolysis of biomass.

3.3.1 Effect of aqueous starch removal

The biomass after processing (**Figure 10 – A**) and drying (**Figure 10 – B**) showed an average yield ≈5.16%, i.e., from 300 grams of peels it is possible to obtain ≈15.5 grams of material. Thus, ≈95% represent starch and in particles smaller than 106 mm that were not retained on the sieves. Cassava peels are made up of approximately 45% starch. For 300 grams of peels used in the experiment, 135 grams corresponds to starch, which was removed by washing and quantified by titulometry (Ndongo et al., 2020).

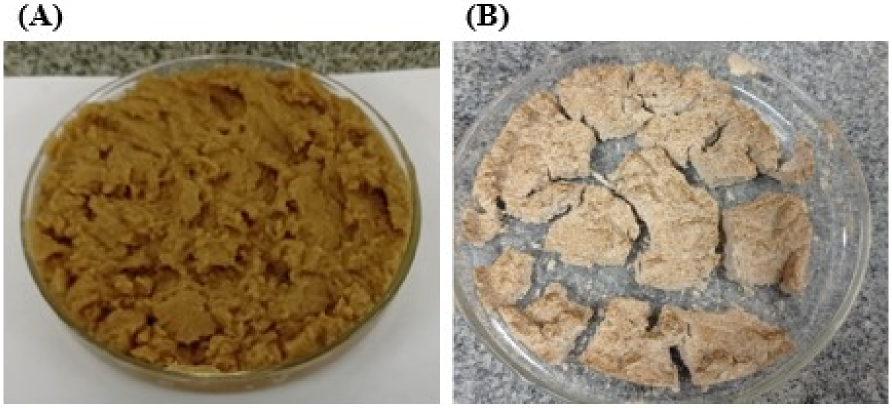

Figure 10. (A) Biomass after washing for 15 cycles and (B) Dry biomass after 24 hours in an oven at 70 °C.

From the amount consumed in volume of the titrating agent during iodometry was determined the content of starch removal, the results obtained in triplicate resulted in a removal of 16.3%, qualitatively represented by (**Figure 11**). Similarly, reported the extraction of starch from fibrous residue of cassava processing with water and mechanical reduction obtaining removal of 19%. Moreover, the removal obtained when compared with the result exposed by (Maniglia, 2017) was lower, which obtained a starch removal yield in turmeric residue by extraction in water and mechanical reduction of 31%. However, it is necessary to consider the composition of each biomass, because in the case of turmeric residue there is 64% starch content, while in cassava peels there is 45%, in addition the method of quantification of starch by iodometry may be susceptible to flaws, since titration experiments provide more qualitative results (Young & Volaric, 2020). In addition, the extraction of starch from sorghum grains proposed by Patrizi et al. (Patrizi et al., 2020), which consisted of submerged the biomass for 24 hours in NaOH 0.1% (w/v) solution at 4 °C, followed by mechanical reduction with water and left in decantation for 2 hours promoted a removal of 47.3%, such high yield might have occurred due to the longer exposure time of the biomass in solution, which facilitates the solubilization of starch in water over time.

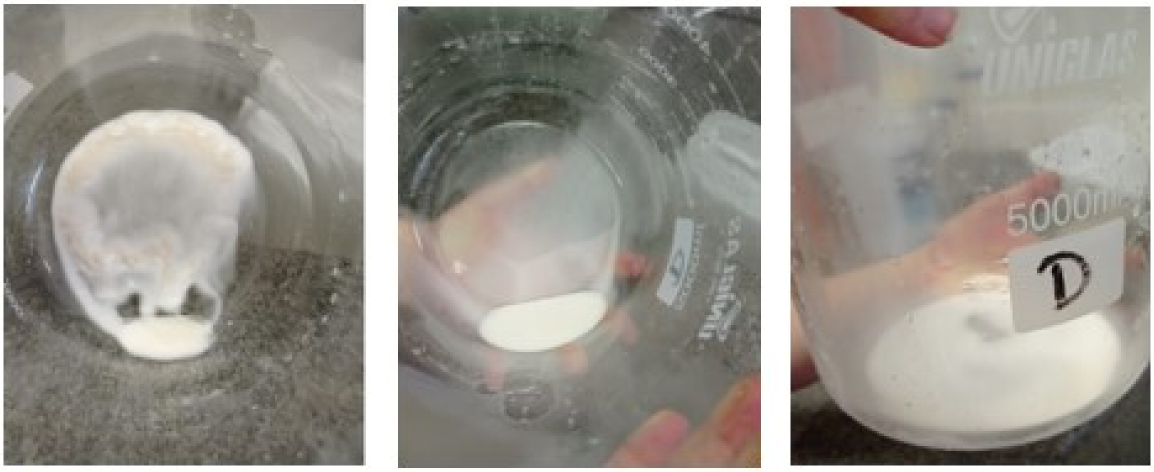

Figure 11. Starch retained in the bottom of the recipient after 15 cycles of washing and mechanical reduction.

Furthermore, the removal of starch from lignocellulosic waste by enzymatic hydrolysis is a more efficient strategy when it is desired to remove a more significant amount of starch present in agricultural waste, i.e., the process of enzymatic hydrolysis of cassava "*in natura*" using amylases for a reaction period of 55 hours presents a starch removal efficiency of 97.3%. The enzymatic removal of starch from barley bran dietary fiber was 87% (Karimi et al., 2018), which proves the efficiency of enzymes when used in hydrolytic processes due to their specificity to substrates. Thus, considering that 45% of the cassava peel is formed by starch, the removal achieved in this research was 16.3%, or 22.02 grams of starch. This removal was technically adequate for the next step of alkaline hydrolysis, because a sufficient amount of starch was removed to prevent its gelatinization during the pretreatment of biomass under high temperature conditions, facilitating the filtration step to separate the solid and liquid phases. However, new approaches can improve the removal of starch from lignocellulosic material, such as the application of amylolytic enzymes.

3.3.2 Composition of raw and pretreated cassava peel

The centesimal lignocellulosic compositions of cassava peels *in natura* and pretreated with sodium hydroxide 2% (w/v) are presented in (**Table 3**). The raw cassava peel showed 26.07% cellulose; 13.09% hemicellulose; 38.30% lignin, and 0.01% ash. In addition, other components were also determined such as water-soluble materials formed by inorganic compounds (aqueous extractives) and soluble materials in organic solvents consisting of terpenes, greases, waxes, and phenolic compounds (alcoholic extractives) (Castro, 2009) and correspond respectively to 9.40% and 4.78%. The contents of cellulose and hemicellulose were similar to (Awoyale et al., 2021) thar reported 25.8% and 11.6% respectively. Thus, the chemical composition (glucose, xylose, and arabinose) of the hemicellulosic of both cassava peels is similar.

Table 3. Lignocellulosic composition of raw and pretreated cassava peels.

Component/Composition (% w/w)	**In Natura**	**Pretreatment (NaOH 2% 30 min)**
Cellulose	26.07 ± 2.46	30.24 ± 0.44
Hemicellulose	13.09 ± 0.62	7.35 ± 0.19
D-Xylose	10.52 ± 0.53	6.12 ± 0.17
Arabinose	2.57 ± 0.10	1.25 ± 0.03
Total Lignin	38.30 ± 2.24	25.17 ± 1.82
ALS*	4.61 ± 0.24	4.77 ± 0.18
ALI*	33.69 ± 3.41	20.4 ± 2.76
Ashes	0.01 ± 0.01	0.01 ± 0.01
Extractives	14.17 ± 2.96	NA*
Total	**95.47**	**62.76**

**NA (Does not apply), ALS (Acid Soluble Lignin), and ALI (Acid Insoluble Lignin).*

On the other hand, the composition found for lignin is higher than reported by Ona et al. (Ona et al., 2019) - lignin content of 24.0%, this significant difference for the composition of lignin of cassava peels is, very likely, due to the result of different climatic and geomorphological conditions, also, lignin confers strength and protection to the crop, thus the higher the lignin content, the more adverse were the conditions of root development (Aruwajoye et al., 2017), i. e., the high content of lignin has the function of acting as a preventive barrier to possible enzymatic and microbial reactions of the hemicellulosic component (Moshi et al., 2015). In addition, high differences in lignin content can be related to different characterization methodologies, the methodology adopted in this work includes the determination of soluble and insoluble lignin that were respectively 4.61% and 33.69% (M. A. F. Pereira et al., 2021), some researches investigate only one fraction of lignin leading to lower results when compared to those determined in this investigation, (Moshi et al., 2015) for example, found 16% lignin for the same species of cassava. Regarding the mineral residue, the ash content found in cassava peels can be up to 5%, however there may be some variations that are justified by the composition of minerals present in the root and the type of soil in which it was grown (Ronko et al., 2020). The ash content found in this study was insignificant for the mass balance, additionally, the low ash content represents that the biomass is clear of possible inorganic inhibitors that negatively interferes in the chemical and enzymatic reactions (Ndongo et al., 2020).

Regarding the obtained data, the alkaline pretreatment (**Figure 12)** with sodium hydroxide 2% (w/v) for 30 minutes led to the highest removal of lignin from cassava peels compared with the other conditions tested, such removal of 34.20% obtained resulted in a final lignin content in biomass of 25.17%. In a study aiming at bioethanol production, cassava peels treated with NaOH 10% (v/v) for 30 minutes under heating and shaking resulted in a delignification of 46.82%, the efficiency of lignin removal by alkaline method is also perceptible from the high yield of reducing sugars obtained (0.79 g/g cassava peels) from cellulose and hemicellulose exposed to hydrolysis conditions (Amalia et al., 2021).

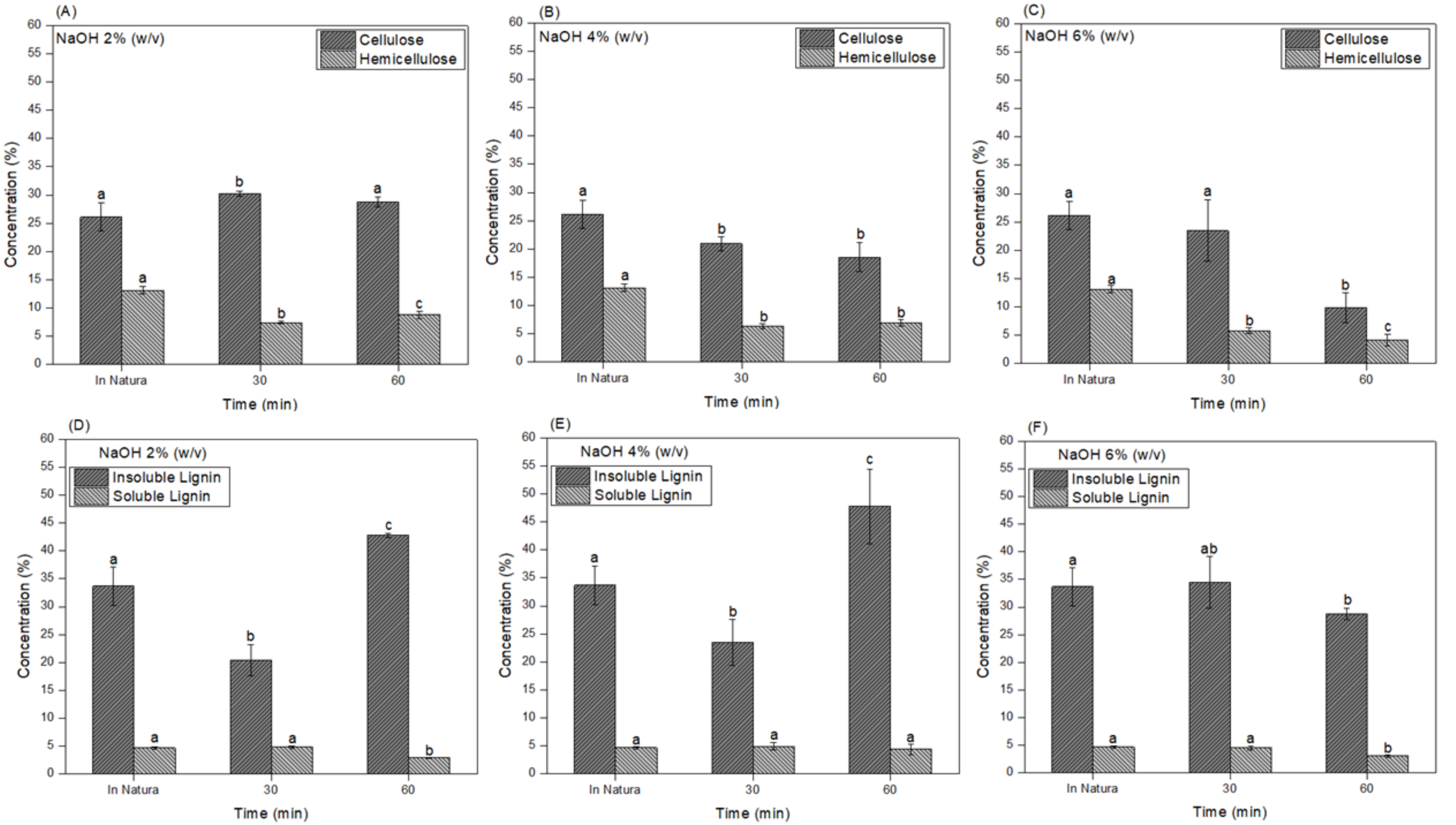

Figure 12. Results of the effect of alkaline pretreatment with sodium hydroxide (2%, 4% and 6%) on cassava peels (1:100) at 30 and 60 minutes. (A, B and C) composition of cellulose and hemicellulose and (D, E and F) composition of soluble and insoluble lignin.

Hence, alkaline reagents can effectively break the structures of lignin decreasing its recalcitrance and fiber distension, but can solubilize the hemicellulose causing significant losses (Sudha et al., 2018), the achieved composition of hemicellulose after alkaline hydrolysis with NaOH 2% was 7.35% by dry mass, which represents a loss of 43.86%, nevertheless it was the most mild condition tested for hemicellulose, for example its composition decreased to 4.05% when treated with NaOH 6% (w/v) for 60 minutes according to **Figure 1 - C**, i.e., practically 70% reduction of hemicellulose was evidenced when increasing the sodium hydroxide concentration and hydrolysis time, this occurs because the solubilization of hemicellulose is favored at high concentrations of alkaline compounds such as KOH and NaOH (Sudha et al., 2018). Hence, alkaline pretreatment facilitates lignin dissolution (Sindhu et al., 2014), due to saponification of the intermolecular bonds of hemicellulose and lignin causing changes in biomass chemistry such as, increased porosity, increased internal surface area, disruption of the carbohydrate-lignin complex, and swelling of cellulose (Nikzad et al., 2015). Regarding cellulose content, after alkaline hydrolysis with 2% NaOH for 30 and 60 minutes, there was an increase in composition to 30.24% and 28,70% by mass of cellulose respectively, this increment of 16.0% approximately occurred due to the enhancement of its crystallinity (Fang et al., 2022). Similar results were described for rice peels pretreated with 2% NaOH for 40 min, in which there was a concentration of cellulose of 17.12% and reduction of hemicellulose and lignin respectively of 23.84 and 25.40%, however these values can change depending on particle size (Shahabazuddin et al., 2018).

The removal of lignin was enhanced in the pretreatments with NaOH 2% and 4% for 30 minutes of hydrolysis in autoclave, the delignification achieved under these conditions were respectively 34.27 and 25.98%, being higher values in terms of insoluble lignin, This fact proves the efficiency of alkaline depolymerization that NaOH causes in the ester linkages between lignin and hemicellulose, hydrolyzing more lignin than the acid pretreatment, which degrade hemicellulose not being interesting for the production of XOS (Pereira et al., 2021; Rai et al., 2019). In addition, it was observed that the exposure of cassava biomass in NaOH 6% and hydrolyzed in times of 30 and 60 minutes did not show significant reductions in lignin content compared to the material pretreated under the above conditions. This fact is due to saturation in the ester bonds of the phenol group of lignin by increasing the sharp concentration of alkali and longer exposure time in extreme conditions of temperature and pressure (Wilkinson et al., 2014).

On the other hand, delignification with 6% NaOH was not efficient, probably a fraction of the lignin depolymerized during alkaline hydrolysis may have bound again to the fiber surface in the reaction medium, in this sense the values found in this study for total lignin after treatment with 6% NaOH for 30 and 60 minutes were 30.34% and 29.21% respectively (Li et al., 2014). In opposite, cassava peels treated with NaOH 2% and 4% for 60 minutes showed at the end a lignin composition of 45.64 and 42.91% respectively, this increase not evidenced in the literature must be related to the washes of the biomass after acid hydrolysis with H_2SO_4 72% (v/v) in the characterization step. The biomass ought be washed with abundant warm water during filtration to completely remove all vestiges of acid, since during the drying step the presence of acids results in the burning of the filter paper in the stove, leading to higher than expected insoluble lignin results due to the interaction of biomass fibers with paper fibers (A. Sluiter etal., 2008).

The cellulose content was reduced in the pretreatment conditions with 4% and 6% NaOH for 30 and 60 minutes. The biomass pretreated with 4% NaOH in both times showed a cellulose content of 20.91% and 18.53% respectively. This more subtle reduction was also observed in the treatment performed with 6% NaOH for 30 minutes, in which the cellulose content reached 23.45%. These insignificant removals in cellulose composition are due to the inherent crystalline nature of this polymer, being more resistant to chemical stresses (Pereira et al., 2016). Pretreatment in the presence of 6% NaOH for 60 minutes, on the other hand, was more aggressive in terms of cellulose and hemicellulose reduction, these reductions of 62.29% and 69.06% by mass respectively, indicate that hydroxyl (OH -) groups in high concentrations during longer exposure times of the biomass make the medium more reactive, facilitating the degradation of the hemicellulosic content (Wilkinson et al., 2014). Hemicellulose was reduced under all the conditions tested and cited above. After hydrolysis of the biomass this average reduction of 51.96% of hemicellulose is justified by the fact that hemicellulose presents an amorphous structure that can suffer degradation more easily in the presence of highly reactive chemical species such as acids, alkalis and oxides (Pereira et al., 2016, Chundawat et al., 2010).

Therefore, it is evident that the optimum pretreatment condition was: NaOH 2% (w/v) for 30 minutes at 121 °C and 1.1 bar. This resulted high delignification content (34.20%) and low hemicellulose degradation (43.86%), which would be ideal to produce XOS by enzymatic route. This fact was also proven by the studies of Khalid et al. (Khalid et al., 2019) applying alkaline pretreatment with 2% NaOH in rice hulls, in which there was a reduction of lignin and hemicellulose fractions and concentration of cellulose contents. The increase in cellulose represents greater solubilization of lignin and hemicellulose and conversion of amorphous cellulose to crystalline cellulose (Remli et al., 2014). Alkaline pretreatment with 2% NaOH was also more effective when applied to cassava roots for the recovery of reducing and total sugars, in which the same effect was verified in the concentration of cellulose, one of increase from 36.27% to 52.34% in mass, in addition the contents of hemicellulose and lignin were reduced to 27.15% and 14.59% (Kamalini et al., 2018). Finally, using alkaline pretreatment to solubilize the glucose, xylose and arabinose fractions of rice straw, the maximum yield obtained was for glucose (254.6 g/kg biomass) using 1.33 M NaOH for 60 min at 80 °C. Thus, it is proven that among the chemical pretreatment, the alkaline one with NaOH at lower concentrations and high temperatures is more efficient in terms of delignification and less solubilization of hemicellulose, which for obtaining XOS becomes a classic advantageous method compared to acid and oxidative treatments (Kim et al., 2016).

3.3.3 Xylooligosaccharides production

Enzymatic hydrolysis experiments were kinetically evaluated until 96 hours using enzyme concentrations of 0.5, 1.5 and 3.0% (v/v) in 20 mg of substrate. A study using endo-xylanases, also of fungal origin, for XOS production from different agricultural wastes showed that the degree of XOS polymerization and yield relative to xylan depend mainly on enzyme concentration and reaction time (Szczerbowski et al., 2014).

3.3.3.1 Yield of xylooligosaccharides

The appropriate reaction time for production of XOS from cassava peels by enzymatic route was 48 hours, as it resulted in a total amount of XOS of 396.5 mg of XOS/g of xylan. To ensure that the enzyme reaction had been stabilized within 48 hours in all three enzyme concentration conditions, the Tukey test was applied to the yield values for the 48 and 96 hours and it was confirmed that there was not significant difference within a probability of less than 5% between the values, confirming the stability of the enzyme reaction. As presented in (**Table 04**) the conversion of xylan into XOS (X2 - X6) progressed with longer hydrolysis time at higher enzyme concentration, starting with 142.12 mg of XOS/g of xylan reaching approximately 396.5

mg of XOS/g of xylan. This mechanism was also verified in the yields of XOS illustrated in (**Figure 13**).

Table 4. Conversion of xylooligosaccharides to xylan from hemicellulose of cassava peels.

Time (Hours)	**XOS/Xylan (% (w/w))**		
	0.5% enzyme	**1.5% enzyme**	**3.0% enzyme**
6	39.05 ± 0.08	91.79 ± 0.50	142.12 ± 4.69
12	62.48 ± 3.94	185.09 ± 1.77	224.07 ± 3.53
24	115.64 ± 0.99	268.95 ± 4.46	291.79 ± 1.88
48	147.53 ± 5.48	326.12 ± 5.06	396.50 ± 11.44
96	131.42 ± 2.59	326.21 ± 3.39	410.23 ± 5.93

The xylan extracted from cassava peels was used to obtain XOS using xylanase from *T. longibrachiatum*, however, new researches with xylanases from different microbial origins are being developed in order to maximize the obtainment of high purity XOS. The method of obtaining XOS via enzymatic hydrolysis is greatly preferred because it prevents the formation of unwanted compounds such as furfural, acetic acid, and formic acid. In addition, the xylanase used should present some characteristics such as low β-xylosidase activity to form low polymerization XOS and high substrate specificity (Gupta et al., 2022).

The maximum yield of XOS obtained from different lignocellulosic waste necessarily depends on the type of pretreatment applied to the biomass because the extraction of xylan is related to this process and the exposure time of the substrate to the enzyme complex. Different studies indicate that the maximum activity of xylanases occurs between 24 and 48 hours depending on the type of waste used, being possible to obtain maximum yields of XOS as, 443 mg of XOS/g of xylan from sugarcane bagasse treated with NaOH 2% (m/v) (Aachary & Prapulla, 2009), 257 mg XOS/g xylan from pineapple peels treated with 15% (w/v) NaOH (Banerjee et al., 2019), 347 mg XOS/g xylan from rice husks treated with 18% (w/v) NaOH (Khat-udomkiri et al., 2018) and 242 mg XOS/g xylan from coconut waste treated with NaOH 20% (w/v) (Jnawali et al., 2018). With this, the yield of XOS relative to xylan found in this research (396.5 mg XOS/g xylan) is 80% lower when compared to the average yields presented above. However, it is a very promising result when compared with the studies of Agung et al. who first produced XOS from cassava peels with alkaline extraction of xylan, and obtained 10.55 mg/mL of reducing sugars in 1% substrate hydrolyzed with endoxylanase from *Bacillus subtilis* from soil termite abdomen and yield of 6.04

mg of XOS/g of xylan, so the conversion found in this research represents 87.7% more. So, cassava peels are waste that can be increasingly exploited to obtain hemicellulosic-based bioproducts, such as XOS, simply by testing and exploring different pretreatments for the peels which can facilitate the hydrolysis of hemicellulose by the action of xylanases.

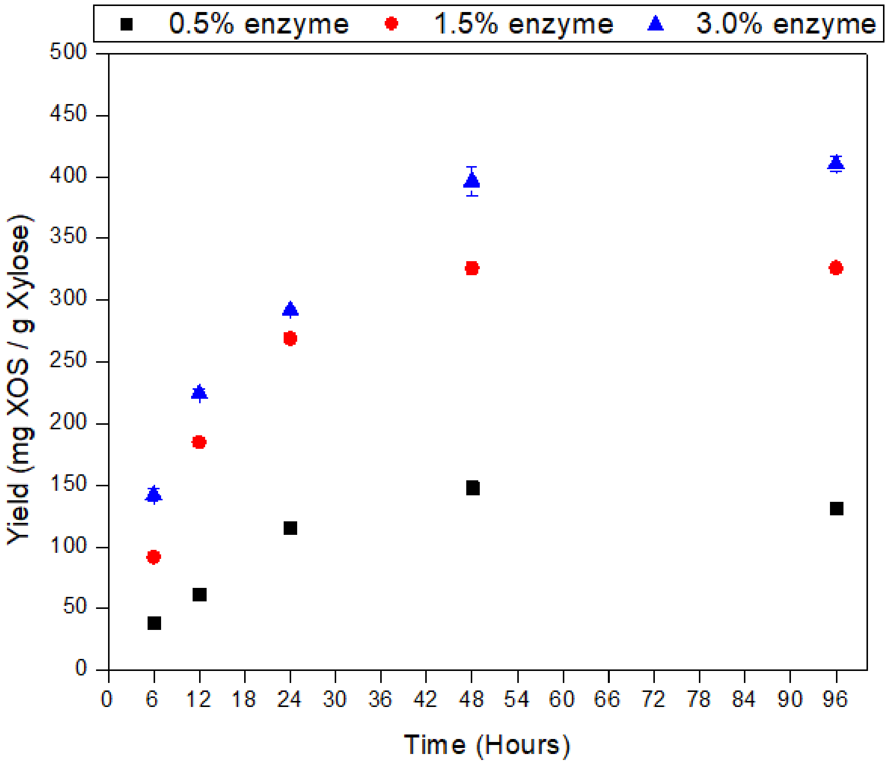

Figure 13. Kinetic profile of obtaining xylooligosaccharides in relation to xylose from cassava peels in enzymatic concentrations of 0.5%, 1.5% and 3.0% (v/v).

The curves of 1.5% and 3.0% (v/v) enzyme showed a very close conversion trend, a fact perceptible from the yields of XOS in 24 hours for these enzyme concentrations, which were respectively 268.95 mg of XOS/g of xylan and 291.79 mg of XOS/g of xylan, these close values imply that in the following hours the production will reach its maximum value and the yield will be constant. Finally, as the enzymatic hydrolysis progressed with each enzyme concentration the XOS yields increased, which corroborates with the studies proposed by Cebin et al. (Cebin et al., 2021) that obtained the same trend in their XOS obtainment profile. Finally, the process of XOS production from commercial xylan from different biomass sources such as, sugarcane bagasse; rice straw; corn cob; fruit residues and others has been widely explored. However only one study has been performed on the production of XOS using fungal xylanase and cassava peels. Thus, it is clear that cassava peels have great potential for bioconversion into XOS when used fungal xylanases reaching yields of up to 400 mg XOS/g of xylan with enzyme doses of 3.0% (v/v) to 3.2U/mL in 1% substrate (20 mg). This fact corroborates the results proposed by Gupta et al. that obtained approximately 430 mg XOS/g of sugarcane bagasse xylan in up to 48 hours of reaction.

3.3.3.2 Profile of obtained oligomers

The production of XOS that present low degree of polymerization (DP) are more attractive for prebiotic applications, this occurs because XOS with low DP are easily degraded by specific probiotic strains due to their smaller molecular structure, facilitating their uptake by microorganisms. Different pretreatments used on lignocellulosic biomass can generate XOS with varieties of DPs (Ho et al., 2018; Monteiro et al., 2021).

In this research, xylopentaose (X5) was identified at higher concentrations reaching 119.76 mg/L during 48 hours of exposure of 1% substrate in 3.0% enzyme. This fact of higher concentration of X5 was also verified by Agung et al. (2016) who obtained 5.96 mg of X5/g of xylan, this confirms that endo-xylanases show high activities in hydrolyzing xylan from hemicellulose into DP 5 XOS. Xylobiose (X2), which shows more prebiotic properties due to its lower degree of polymerization, showed a significant increase from 12 hours at enzyme concentrations of 1.5% and 3.0%, being interesting for food and pharmaceutical applications. The concentrations of XOS with DP varying between (X2 and X6) are represented in (**Figure 14**) at different enzyme concentrations.

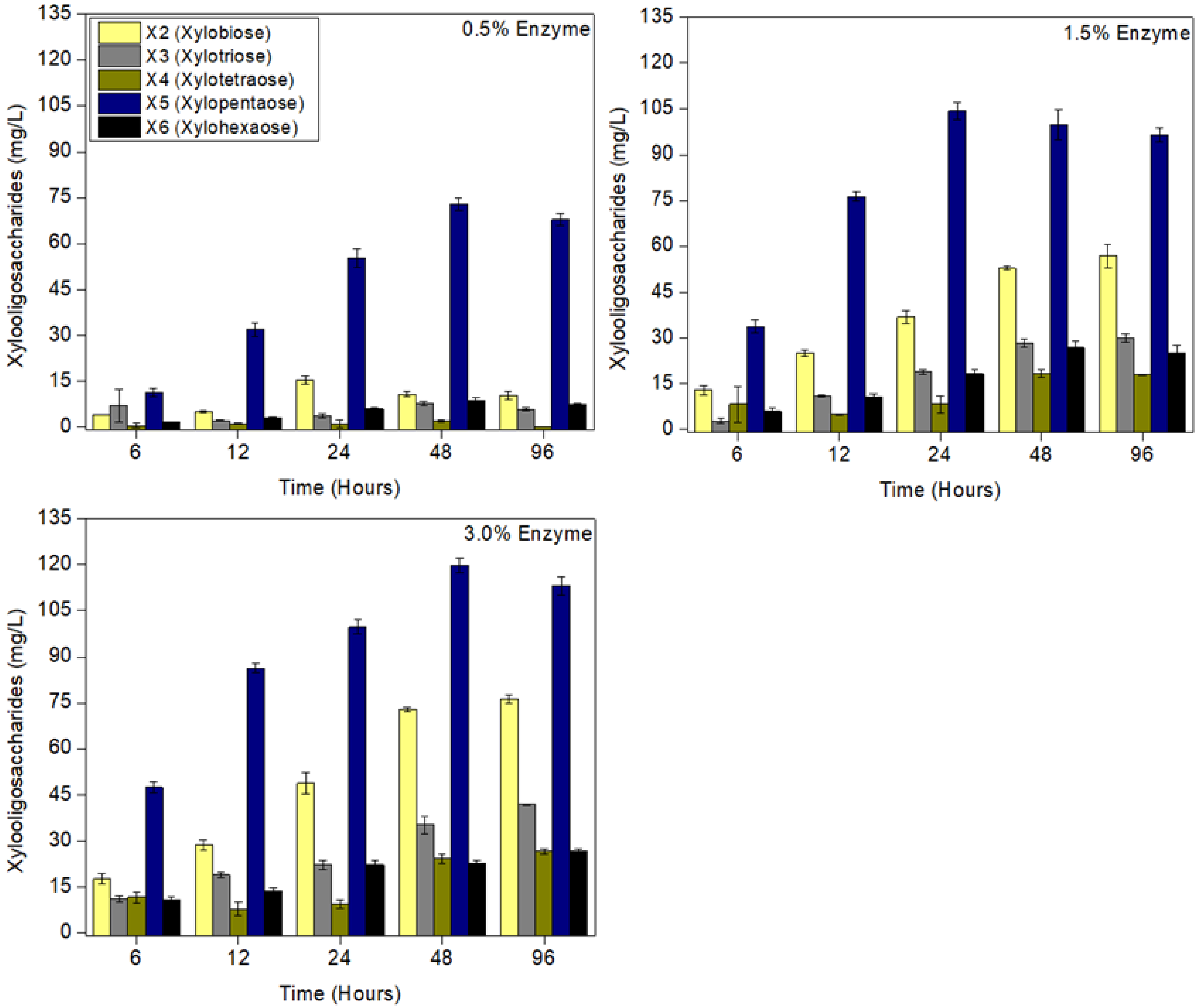

Figure 14. Profile of xylooligosaccharides of different polymerization degrees along time in enzymatic concentrations of 0.5%, 1.5%, and 3.0%.

The kinetic study is presented up to 96 hours and was carried out under constant stirring at 180 rpm, 50 °C and pH 6. The hydrothermal pretreatment of the biomass with dilute alkali mainly favored the release of X5 and X2 at the evaluated times of the kinetics, the highest concentrations achieved of X5 and X2 were 119.76 mg/L and 72.82 mg/L, respectively. These results are aligned to Cao et al. that reported higher yield of X5 after hydrothermal pretreatment of sugarcane bagasse at 160 °C for 70 minutes and when modifying the thermodynamic conditions to 180 °C and 40 minutes the highest yield was of X2. According to literature, the time and temperature of biomass exposure are important parameters that directly influence the profile of obtaining XOS, higher temperatures of the reaction system facilitates in the wetting of the matrix and the efficiency of extraction and conversion of XOS (Plaza & Turner, 2015). The concentrations of xylotriose (X3) obtained in 0.5% of enzymes were extremely low and varied little throughout the kinetics only with the increase of the enzyme concentration to 3.0% the production of X3 began to increase reaching approximately 40 mg/L, which confers prebiotic properties because they have polymerization degree 3 being easily metabolized by probiotic bacteria, in this sense the increase in enzyme concentration causes a gradual increase in the amount of X3. Finally, the concentrations of X4 and X6 remained almost constant over time, the concentrations of xylotetraose and xylohexaose varied respectively from 11.63 to 26.27 mg/L and 10.83 to 26.71 mg/L from 6 to 96 hours. The results found here show that alkaline pretreatment on cassava peels followed by enzymatic hydrolysis with endo-1-4-β-xylanase promotes the production of X5 in larger quantities, this fact can be optimized with the addition of β-xylanases that break long chains into short chains, making it feasible for prebiotic applications. X2 was also produced in significant quantities, which can be purified and applied in food, beverages, and pharmaceuticals. XOS with a degree of polymerization between 2 - 4 may be more desirable because they have a lower molecular mass, which facilitates their interaction with probiotics, provides thermal stability, is sweeter, and has a high marker value (Forsan et al., 2021).

3.3.4 Mass balance

Figure 15 illustrates the mass balance of the lignocellulosic components of cassava peels after pretreatment for removal of water-soluble starch, alkaline hydrolysis with NaOH 2% (w/v) for 30 minutes and enzymatic hydrolysis of hemicellulose to obtain xylooligosaccharides (X2 - X6), calculated for 300 g of dry mass from the principle of the law of conservation of mass without the presence of chemical reactions.

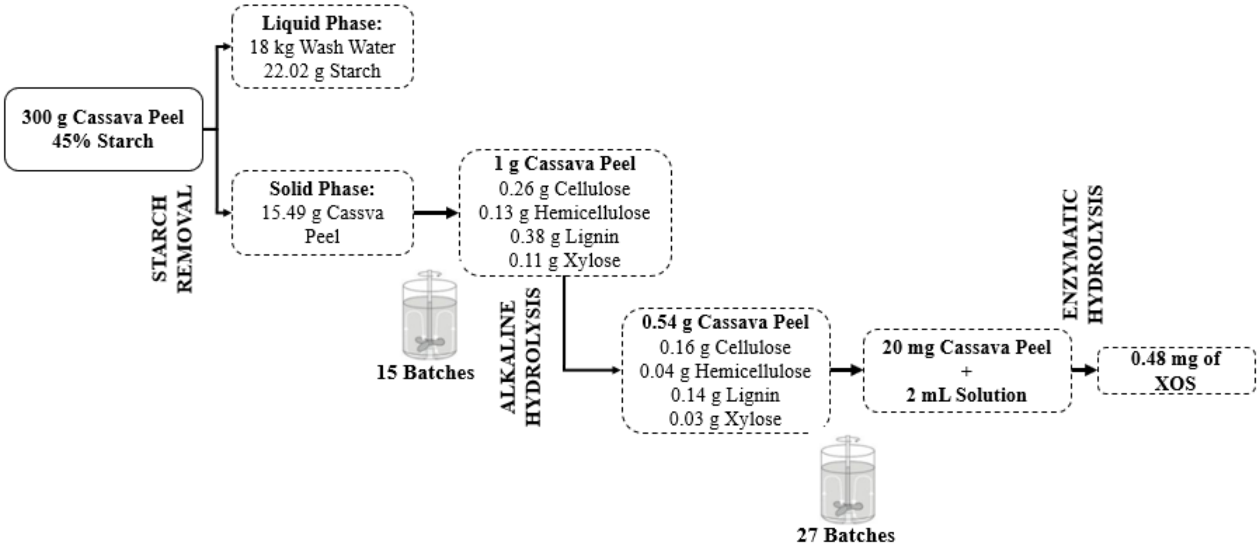

Figure 15. Mass balance to produce xylooligosaccharides from cassava peels pretreated with 2% NaOH for 30 minutes followed by enzymatic hydrolysis

The system was fed 300 g of cassava peels of this value 45% by mass corresponds to the starch content. The yield of starch removal from cassava peels from 15 cycles of washing with water and mechanical reduction was 16.31%. Thus, in the liquid stream 22.02 g of water-soluble starch and 18 L of washing water were obtained. The solid stream after drying at 70 °C for 24 hours showed a mass yield of 5.16%, so the amount of biomass obtained was 15.49 grams. For the next step of alkaline hydrolysis, it was used 1 g of this material (26.07% cellulose; 13.09% hemicellulose; 38.30% lignin and 10.52% xylose) in 100 mL of 2% NaOH solution, so it is possible to produce up to 15 batches of alkaline hydrolysis. After treatment with alkali, the material was filtered to separate the solid and liquid phase, the solid material after drying for 24 hours at 100 °C showed a yield of 53.88%, thus 0.54 grams of hydrolyzed material was obtained consisting of 30.24% cellulose; 7.35% hemicellulose; 25.17% lignin and 6.12% xylose. In the enzymatic hydrolysis a fixed value of substrate was set at 1% for a working volume of 2 mL, thus 20 mg of treated cassava peels were used in the alkaline step, and it was possible to produce up to 27 batches of enzymatic hydrolysis. Finally, from the xylose content (6.12%) obtained in the biomass characterization and the best yield in the enzymatic hydrolysis step (396.5 mg XOS/g of xylose) it was possible to obtain 24.21 mg of XOS/g of treated residue. Finally, considering the other batches mentioned above, it is possible to produce 9.44 g of XOS from 300 grams of cassava peels, i.e., a conversion of 3.27%.

Prospecting an industrial scenario, a local company producing cassava flour operates at 14 tons of root per hour. It can carry out 8 batches of cassava per day. After 1 month, 3,360 tons of processed cassava, of which 10% of the material is eliminated in the form of peels. Thus, 336 tons of cassava peels are generated that by the mass balance can be converted into 10.98 tons of XOS contributing to the increase of biorefineries. At the cost level, taking into consideration only the primordial feedstock for the production of XOS, represented by **Table 05** and by the mass balance presented, to produce 1 gram of XOS the average cost necessary only with feedstock would be US$ 2.24, at industrial scale the production of XOS from cassava peels by could be economically viable since it could reuse the enzyme to operate multiple batches, since enzymes are the most expensive raw material.

Table 5. Cost of main feedstocks for XOS production from cassava peels by alkaline and enzymatic hydrolysis.

Feedstock	Supplier / Quantity	Cost (US$*)
Sodium Hydroxide	Nox Lab. Solution / (500 g)	5.33
Phosphate Buffer Solution pH = 6	Neon Chemistry / (500 mL)	4.30
Enzyme Endo-1,4-β-Xylanase	Megazyme / (20.000 U/mL)	235.76

* *US Dollar*

3.3.5 Conclusion

Depolymerization of hemicellulose from cassava peels has shown to be a promising strategy for obtaining XOS. The classical model of pretreatment of biomass with sodium hydroxide at high temperature has proved efficient in removing lignin but can be optimized to modern pretreatments which avoid the formation of unwanted by-products. In addition, cassava-based biomasses are rich in starch that can be exploited for other bioconversions. The combination of the enzyme complex with the biomass showed great specificity, resulting in a maximum conversion of XOS to xylan of approximately 50%, which in mass terms means 24.21 mg of XOS produced from 1 gram of pretreated residue. The concentration profiles of XOS with different degrees of polymerization indicated that the xylan from cassava peels result in X2 and X5 in higher concentrations being interesting for food and pharmaceutical applications. Finally, the valorization of agricultural waste to produce bioactive compounds has raised interest in the biorefinery chain such as the use of cassava peels to obtain XOS which is a promising strategy when innovative pretreatment techniques are applied.

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4 CHAPTER 4 – CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

In this work the use of cassava peels was evaluated as an alternative source for the production of XOS, contributing to the valorization of agroindustrial waste. The cassava peels for being rich in starch require an initial step for its removal, since during the alkaline treatment for removal of lignin the high content of starch results in gelatinization of biomass at high temperatures.

The alkaline treatment with NaOH 2% for the delignification of biomass proved to be more efficient compared to the treatments with NaOH 4 and 6%, the high concentration of alkali implies a greater solubilization of hemicellulose not being interesting for obtaining XOS. In the enzymatic hydrolysis step, by means of kinetics, results of 396.5 mg of XOS/g of xylose were obtained, working with a fixed mass of 1% substrate in relation to a reaction volume of 2 mL, in 3.0% of enzyme (3.2 U/mL) and 48 hours of reaction under constant temperature and agitation.

The results obtained in this work demonstrate cassava peel as a potential material, but lower explored, for obtaining bioactive compounds such as XOS, that appreciation a prebiotic effect. However, for future work, it is indicated:

- Evaluate other methods of starch removal, such as the use of amylolytic enzymes.
- Verify other pre-treatment techniques for lignin removal.
- Characterization the liquor from the liquid phase of alkaline hydrolysis, for possible recovery of reducing sugars.
- Verify the reuse of the enzyme making the process viable on an industrial scale.
- Test the biological activity of XOS by microbial activity.