



Eucalyptus grandis Forestry Residue Valorization: Distinct and Integrated Pretreatment Methods for Enhanced Xylooligosaccharide Production

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Abstract

Eucalyptus branches and bark represent highly abundant and available feedstocks with great potential for obtaining bio-based products. Distinct and integrated pretreatment fractionation strategies for eucalyptus branches and bark were performed for the efficient production of xylooligosaccharides (XOS). By combining pretreatments, a high yield of XOS was obtained from eucalyptus branches and bark. The branches and bark were presoaked in 8% (w/w) sodium hydroxide at 60 °C for 30 min to provide a deacetylation effect. The residues were then hydrothermally treated. The findings revealed that 4.64% of XOS originated from the bark and 6.19% from eucalyptus branches. It has been demonstrated that xylan may be selectively depolymerized during pretreatment by preventing excessive hydrolysis through the use of deacetylation in the first phase of the process. More XOS was produced using hydrothermal treatment, yielding 8.00% (w/w) in the branches and 5.12% in the bark. A significant amount of XOS with DP 2–5 might be obtained in certain experiments, up to 60%, but the most abundant XOS are usually those with DP > 5 (approximately 80% of all XOS). This work provides new insights into the effective generation of XOS under relatively mild conditions by overcoming the recalcitrant structure of eucalyptus branches and bark, representing a noteworthy advancement towards forestry leftover valorization.

Keywords Xylan · Oligosaccharides · Degree of polymerization · Eucalyptus residues · Branches · Barks

Highlights

- Eucalyptus branches and bark show potential are potential bioproduct sources.
- Hydrothermal (HP) and combined (CP) pretreatments demonstrated high selectivity for XOS production.
- Under ideal pretreatment conditions, high XOS recovery was achieved with low concentrations of monomers and degradation products.
- Using HP, approximately 39% of the xylan in the bark and approximately 50% of the branches can be hydrolyzed in XOS.
- The fraction of XOS varied according to the pretreatment method.

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Introduction

Eucalyptus plantations for industrial purposes, most of which go to the cellulose and paper industry, are fast-growing species that have been widely planted around the world, with 25 million hectare planted [1]. In Brazil, eucalyptus plantations are among the most productive forests in the world reporting 36.80 m³/ha/year. Most often, the forest industry generates a large amount of residue as a result of production processes, with approximately 33 million tons including bark, branches, and leaves [2], which are potential raw materials for bioproducts. Some of this forest debris remains in the field after harvest to protect and fertilize the soil; however, preparing the soil for future planting requires removing larger branches, smaller trees, remaining trunks, and leaves to reduce fire hazards because this debris is highly flammable [3]. According to IBÁ [2], 12.50% of forest residues remained in the field in 2020, whereas 57.20% of biogenic industrial and forestry residues were destined for energy cogeneration. The

latter are undervalued although they have the potential to be transformed into higher-value products. Eucalyptus (*E. grandis*) forestry residue contains hemicellulose, which is a valuable fraction that can be transformed into oligosaccharides.

Xylooligosaccharides (XOS) are non-digestible oligomers derived from the hydrolysis of xylan and are composed of xylose units connected by β -(1,4) glycosidic bonds [4]. These molecules reach the intestine intact, where they are broken down by intestinal microorganisms to form fermentation products, thereby providing benefits to the host. XOS are recognized as non-carcinogenic, hypocaloric, and anticariogenic and considered dietary fibers with prebiotic and biological properties that offer potential health benefits. Their resistance to acids and heat makes them useful in the food industry, and because of their prebiotic and biological properties, they also find applications in the agricultural, cosmetic, and pharmaceutical industries [5, 6]. The global XOS market is projected to reach USD 144.50 million by 2033 with a compound annual growth rate of 7.00% [7]. Additionally, although the commercial price is higher than that of other oligosaccharides, factors affecting the price of XOS range from 20.00 to USD 52.00/kg, depending on its purity [8].

Several agro-industrial residues have been investigated for XOS production, including sugarcane bagasse and straw [9, 10], corn cobs [11], and wheat straw [12]. Eucalyptus wood has also been used to obtain XOS. Nonetheless, research has predominantly focused on industrial wood residues and sawdust [8, 13–15], neglecting forestry sector by-products such as branches and bark, which have the potential to generate value-added products such as oligosaccharides.

Owing the recalcitrance of lignocellulosic biomass, minimization of fermentable sugar losses, as well as the minimal formation of inhibitory compounds, should be considered when choosing the pretreatment method and operating conditions. The methods for producing XOS from biomass include physical (grinding, ultrasonic, and microwave oven), chemical (use of bases, acids, oxidants, or organic solvents), physicochemical, biological (use of microorganisms or enzymes), and a combination of these pretreatment methods [16, 17]. Physical pretreatment increases the surface area and porosity of the material and reduces the crystallinity of cellulose, but requires a lot of energy. Biological pretreatment consumes less energy and allows the hydrolysis of lignin and hemicellulose; however, it is more time-consuming, requires strict control of operating conditions, and has lower hydrolysis rates than chemical and physicochemical methods [18]. After chemical or physicochemical pretreatment, biological methods are often used to improve the distribution of XOS fractions and obtain short-chain functional XOS [11]. Therefore, this study focused on the production of XOS using chemical and physicochemical methods.

Chemical pretreatments are generally more cost-effective than enzymatic treatments [16]. These methods use dilute alkaline solutions (NaOH at 0.1–8% w/w, between 40 and 80 °C, for 15 min to 1 h) [10, 19] or dilute mineral acids (H_2SO_4 at 0.1–5% v/v, between 121 and 170 °C for a few minutes to 1 h) [9, 20]. The use of alkaline agents allows for biomass deacetylation and lignin removal under severe conditions, rendering the xylan fraction more suitable for subsequent steps [11]. In contrast, xylan extraction with diluted acid can achieve similar levels of xylan solubilization into XOS in shorter reaction times than hydrothermal and enzymatic treatments. However, the formation of monomers and degradation compounds, such as furfural, during the reaction is challenging, although this can be controlled by adjusting the acid concentration and residence time [5, 17, 21]. Among physicochemical methods, hydrothermal pretreatment stands out because of its high biomass solubilization. A significant portion of xylan can be solubilized into oligomers in the hydrolysate depending on the severity of the process. Cellulose can be solubilized between 4 and 15%, whereas lignin undergoes partial depolymerization. This method is considered environmentally and economically favorable, as it does not require the addition of chemical agents, reducing the need for waste neutralization and minimizing corrosion issues, resulting in lower investment in high-cost equipment [4, 18]. Pretreatment is a critical step for efficient XOS production and accounts for approximately 40% of the total cost. The selection of the pretreatment method is crucial, considering the type of biomass, desired products, and economic and environmental factors [17].

In the conversion of lignocellulosic biomass, XOS has a higher value than cellulose, biofuels, and cogeneration energy, leading to significant research focus [8]. Capetti et al. [11] achieved a 22.80% conversion of xylan to XOS via alkaline pretreatment of corn cobs, using 1% (w/v) of NaOH at 121 °C for 40 min along with GH10 and GH11 xylanases. Carvalho et al. [9] reported a maximum xylan conversion of around 35% to XOS from sugarcane bagasse at 190 °C for 5 min with 0.5% of H_2SO_4 . Similarly, hydrothermal pretreatment of bamboo at 180 °C for 30 min yielded 34.35% conversion of xylan into XOS [22]. Poplar sawdust pretreated at 170 °C for 50 min achieved approximately 35% XOS conversion, which increased to 44.60% with subsequent endoxylanase treatment [23]. Additionally, optimal conditions (161 °C for 65 min) led to a 42.30% conversion of xylan to XOS from eucalyptus hybrid wood chips [15]. These conversions did not exceed the maximum obtained in this study using eucalyptus forestry residues under nonoptimized conditions.

Branches and bark, often seen as leftover materials in forestry, present a challenge owing to their limited reuse. This emphasizes the need to explore their potential to increase value and align themselves with circular economy

principles. This supports new waste management policies focusing on reusing biomass to obtain bio-based products and addressing sustainability concerns, efficient residue management, and competition for land for food production [4, 6, 16]. The objective of this study was to investigate distinct and integrated pretreatment methods for efficient XOS production from eucalyptus branches and bark. The degree of polymerization (DP) of the released XOS was analyzed. The efficiency of hydrothermal, dilute acid, and combined pretreatments was assessed under different reaction conditions to convert xylan into XOS from both the branches and bark. Additionally, the kinetic parameters and the impact of pretreatment on the chemical composition of the recovered solid residues were examined. Moreover, a comprehensive mass balance expanded the understanding of *E. grandis* forestry residue conversion and highlighted the potential for oligosaccharide production from highly available feedstocks.

Materials and Methods

Materials

The branches and bark, as eucalyptus forestry residues (EFR), belong to the *Eucalyptus grandis* (*E. grandis*) species. EFR were supplied by a local company in the bleached eucalyptus pulp sector, Suzano Papel e Celulose, a forestry sector located in São Miguel Arcanjo, São Paulo, Brazil. The residues were collected directly from the field after mechanical debarking of the logs. The material was air-dried until it reached a moisture content of approximately 10% (w/w). Subsequently, the residues were processed separately in an organic waste-processing machine (TR200; Trapp, Santa Catarina, Brazil). The branches were ground in a knife mill equipped (Disintegrator DM 540; IRBI- São Paulo, Brazil) with a trapezoidal sieve with a mesh opening of 1.50 mm in diameter. All the residues were stored in airtight plastic bags at room temperature until further use.

Chemical Characterization of EFR

Untreated and pretreated EFR were processed in a knife mill (Pulverisette 19; Fritsch GmbH, Idar, Oberstein, Germany) to obtain particle size less than <0.5 mm (35 mesh) for analysis of total ash and extractive contents. The total ash was determined gravimetrically by calcining the residues at 400 °C for 1 h, followed by calcination at 800 °C for 2 h (Muffle K48E, COEL, Brazil) [24]. The extractive content was determined only for untreated EFR, according to Rocha et al. [24], with modifications involving sequential extraction with cyclohexane-ethanol 1:1 (v/v) for 24 h in a Soxhlet apparatus, followed by water for 8 h.

Extractive-free samples were milled to a particle size of 0.08 mm (170 mesh) using an impact mill (Pulverisette 14; Fritsch GmbH, Idar, Oberstein, Germany), and the structural carbohydrates and lignin contents of were determined according to the NREL/TP-510–42618 procedure [25]. The concentrations of monosaccharides, organic acids, furfural, and hydroxymethyl-furfural (HMF) were determined using high-performance liquid chromatography (HPLC), as described in “Analytical Methods.” Chemical characterization was performed in triplicate.

Deacetylation

EFR deacetylation was performed using sodium hydroxide (NaOH) for alkaline fractionation. Process conditions, such as temperature, reaction time, and NaOH concentration, were defined based on previously reported results for the production of XOS from deacetylated sugarcane straw [10]. Approximately 20 g of residue samples was impregnated with an alkaline solution containing 80.00 mg NaOH/g_{EFR} in a solid-to-liquid ratio of 10% (w/w) for 40 min. Subsequently, the reaction was performed in a thermostatic bath (Q213-22; QUIMIS, São Paulo, Brazil) at 60 °C for 30 min. After the reaction, the samples were immediately cooled in an ice bath and the resulting fractions were separated via filtration using a muslin cloth. Deacetylation was performed in triplicate.

The alkaline liquid fractions were stored at –18 °C until further chemical composition analysis. The recovered solid fractions were washed to achieve neutral pH and then dried at 30 °C until they reached a moisture content of 10% (w/w). The deacetylated residues were stored in airtight plastic bags for subsequent chemical characterization and combined pretreatment.

Pretreatment

Three different methods were employed for XOS production: combined pretreatment (CP), dilute acid (DA), and hydrothermal pretreatment (HP). All pretreatments were conducted in stainless steel reactors with a 500-mL capacity, containing approximately 20 g of EFR, with a total solid loading of 10% (w/w). The reactions were performed in a glycerin thermostatic bath (Q213-22; QUIMIS, São Paulo, Brazil) at a specific temperature and reaction time for each assay (Table 1). CP was conducted with previously deacetylated EFR, with temperature reactions ranging from 175 to 190 °C and reaction times from 7.5 to 25 min. DA and HP methods were performed on untreated (non-deacetylated) EFR. In DA, 0.1% (v/v) H₂SO₄ was used at 170 °C, with reaction times varying between 5 and 15 min. For HP, the temperature ranged from 175 to 205 °C, with reaction times ranging from 5 to 25 min.

Table 1 Pretreatment methods and reaction conditions in branches and barks

Code	Method	Catalyst (% v/v)	Temperature (°C)	Time (min)
CP-01	Combined	Autohydrolysis	175	20*
CP-02				25*
CP-03				190
CP-04				7.5
DA-01	Diluted acid	H ₂ SO ₄ , 0.1%	170	15
DA-02				5
DA-03				10*
DA-04				15
HP-01	Hydrothermal	Autohydrolysis	175	10
HP-02				15
HP-03				20*
HP-04				25
HP-05			190	7.5
HP-06				15
HP-07				22.5
HP-08			205	5
HP-09				10
HP-10				15

*Experiments performed in triplicate

After the reaction, the reactors were immediately cooled in an ice bath and the fractions were separated by filtration using a muslin cloth. The slurry was washed until a neutral pH was reached and then dried at 30 °C to achieve a moisture content of 10% (w/w). The insoluble solid fractions were stored in airtight plastic bags for subsequent characterization. The liquid fractions (HH-hemicellulosic hydrolysates) were stored at − 18 °C for further compositional analysis.

The severity factor (SF) provides an approximate estimation of the combined effect of temperature and time used to convert lignocellulosic biomass. This was employed to evaluate the intensity of the pretreatment processes. For each EFR pretreatment, the SF was calculated following the methodology described by Overend et al. [26], as detailed in Eq. 1.

$$\log(\text{Ro}) = \log \left(t \cdot \exp \left(\frac{T - T_{\text{ref}}}{14.75} \right) \right) \quad (1)$$

where t is the residence time (min), T is the temperature (°C), T_{ref} is the reference temperature (100 °C), and 14.75 is an empirical value related to activation energy of the reaction.

In pretreatment processes using acid agents (diluted or concentrated), the SF must be adjusted using the pH value of liquid fraction by Eq. 2 [27].

$$\text{Ro}' = \log(\text{Ro}) - \text{pH} \quad (2)$$

where Ro' is the combined severity factor.

Reproducibility and reproducibility were assessed to measure the quality of the experiments. Previous studies by the research group (data not shown) were performed in triplicate in bench-scale process development (using 100-mL batch reactors). In this study, the experiments shown in Table 1 (marked with *) were performed in triplicate. Experiments were scaled up using 500-mL reactors and the reliability of the standard deviation and error show that the scientific findings are reliable and desirable, maintaining the lack of variability and correlation between the measurements.

Determination of Total Oligomers

HH samples resulting from CP, DA, and HP, along with the alkaline liquor obtained during deacetylation, were subjected to a post-hydrolysis process consisting of acidification with H₂SO₄ at a final concentration of 4% (w/w) at 121 °C for 1 h (Autoclave AV-100 Plus, Phoenix Luferco, Araraquara, São Paulo, Brazil) [28]. The samples were analyzed in triplicate. Subsequently, the samples were analyzed using HPLC to quantify carbohydrates, organic acids, HMF, and furfural, as described in “Analytical Methods.” The total oligomer content was calculated by subtracting the amount of monomers present in HH before and after the post-hydrolysis step.

Analytical Methods

Monomeric Sugars, Organic Acids, and Degradation Compounds

The concentrations of glucose, xylose, arabinose, and cellobiose were analyzed by HPLC using a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm) maintained at 55 °C with a refractive index detector Waters 410 at 40 °C. The eluent consisted of a 5 mM H₂SO₄ solution at a flow rate of 0.6 mL/min, with sample injections of 20-μL volume.

Acetic, formic, and levulinic acids were quantified using an ion exchange chromatography system (850 Professional IC, Metrohm, Switzerland). The system included a conductivity detector and MetroSep organic acid column (300 × 7.8 mm) set at 30 °C. A 0.5 mM H₂SO₄ solution was used as the eluent, with a 50 mM LiCl solution as the suppressor, at a flow rate of 0.5 mL/min, and sample injection volumes of 196 μL.

Prior to injection, diluted samples were filtered through a polyvinylidene fluoride (PVDF) membrane with a pore size of 0.45 μm (Millipore, MA, U.S). Glucose, xylose, arabinose, cellobiose, levulinic acid (Sigma-Aldrich), acetic acid, and formic acid (Synth) were used as the reference standards.

HMF and furfural was performed using HPLC (ACCELA 600, Thermo Scientific, San Jose, California, United States).

The system was equipped with UV detector (wavelength set at 280 nm) and a Thermo C18 column (50 × 2.1 mm) at 30 °C. The eluent, consisted of a 2.5% (v/v) C₂H₃N and 2 mM H₃PO₄ solution, injected at a flow rate of 100 µL/min, and sample injections of 20-µL volume. Prior to injection, diluted samples were filtered through a 0.22-µm PVDF membrane (Millipore, MA, U.S). HMF and furfural (Sigma-Aldrich) were used as reference standards.

Degree of Polymerization (DP) of XOS

Xylobiose (X2), xylotriose (X3), xylotetraose (X4), and xylopentaose (X5) in HH produced using different pretreatment methods were quantified using a modified methodology from Brenelli et al. [10]. High-performance anion exchange-pulsed amperometric detection (HPAE-PAD) chromatography was performed using an 850 Professional IC (Metrohm, Switzerland) chromatograph. The setup included a CarboPac PA100 column (4 mm × 250 mm) and CarboPac PA100 precolumn (4 mm × 50 mm).

The eluent composition involved a solvent gradient system containing solution A (water), solution B (NaOH 80 mM and NaOAc 500 mM), and solution C (NaOH 500 mM), at a flow rate of 1 mL/min. The gradient program consisted of: initially, from 0 to 10 min, 2% solution B, 15% solution C, and 83% solution A; then, from 10 to 20 min, varying from 2 to 20% solution B, from 15 to 50% solution C, and from 83 to 30% solution A; subsequently, from 20 to 28 min, 20% solution B, 50% solution C, and 30% solution A; and finally, from 28 to 30 min, changing from 20 to 2% solution B, from 50 to 15% solution C, and from 30 to 83% solution A. Before injection, the diluted samples were filtered through a 0.45-µm PVDF membrane (Millipore, MA, USA). Oligomer concentrations were determined using a calibration curve established using X2, X3, X4, and X5 standards (Megazyme International Ireland).

Mass Balances and Kinetic Analysis

The fractions resulting from pretreatment were analyzed for structural and non-structural compounds to perform mass balance calculations. The evaporation factor in the acid hydrolysis process was used to determine the chemical composition.

The concentrations of glucose, cellobiose, HMF, formic acid, and levulinic acid were adjusted using their respective stoichiometric factors. These components were used to determine cellulose concentration. For hemicellulose, the sum of the concentrations of xylose, arabinose, acetic acid, and furfural, individually adjusted using stoichiometric factors, was considered. The total lignin included the combined concentrations of soluble and insoluble lignin [25].

The conversion of arabinoxylan (C_{AX}), pretreatment yields (Y_{pr}), and XOS yields (Y_{XOS}) were calculated using Eqs. 3, 4, and 5:

$$C_{AX}(\%) = \frac{\text{solubilized compounds (g)}}{\text{arabinoxylan mass in the initial material (g)}} \times 100 \quad (3)$$

$$Y_{pr}(\%) = \frac{DM_{\text{pretreated material}}}{DM_{\text{initial biomass}}} \times 100 \quad (4)$$

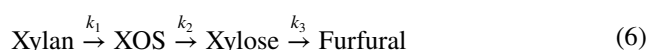
$$Y_{XOS}(\%) = \frac{XOS \text{ (g)}}{DM_{\text{initial biomass}}} \times 100 \quad (5)$$

where DM is the dry basis mass (g).

The experimental data generated from the hydrothermal pretreatment were used to develop a kinetic model for the conversion of lignocellulosic material into XOS. Since Sae-man [29] originally suggested a kinetic model for the two-step sequential acid hydrolysis of cellulose, several approaches have been developed for the conversion of biomass based on this model, including hemicellulose hydrolysis. Borrega et al. [30] and Dos Santos Rocha et al. [31] included the formation of oligomers and proposed the formation of more than one degradation compound during hemicellulose conversion.

Santucci et al. [32] proposed a simplified kinetic model for the conversion of hemicellulose during the hydrothermal pretreatment of sugarcane bagasse. In this model, all sugars, including oligomers and monomers present in the hydrolysate, as well as furfural and organic acids, were considered for the determination of the kinetic parameters.

The current work suggests a pseudo-homogeneous model of irreversible first-order processes for the conversion of hemicellulose from EFR following the mechanism reported by Santucci et al. [32]. However, in this study, the hydrolysis of xylan into total XOS was assumed and subsequently into xylose and furfural. The suggested response mechanism is as follows:



The following differential equations can be used to calculate the profile of each component over time for the suggested kinetic model (Eq. 6):

$$\frac{d[\text{Xylan}]}{dt} = -k_1 [\text{Xylan}] \quad (7)$$

$$\frac{d[\text{XOS}]}{dt} = k_1 [\text{Xylan}] - k_2 [\text{XOS}] \quad (8)$$

$$\frac{d[\text{Xylose}]}{dt} = k_2 [\text{XOS}] - k_3 [\text{Xylose}] \quad (9)$$

$$\frac{d[\text{Furfural}]}{dt} = k_3 [\text{Xylose}] \quad (10)$$

Kinetic rate (Eqs. 7, 8, 9, and 10) were used to fit the experimental data for each set of operating conditions.

The concentration of hemicellulose remaining in the pretreated EFR was used to determine the rate constant k_1 . The concentration of xylose oligomers and monomers and furfural content in the HH resulting from pretreatment were used to calculate k_2 and k_3 . The preexponential factor (A) and activation energy (E_a) were calculated using the Arrhenius equation (Eq. 11):

$$k = A \times \exp\left(-\frac{E_a}{RT}\right) \quad (11)$$

The rate constant k_i is determined by minimizing the sum of the squared errors between the experimental and theoretical concentration curves over time for a suitable set of conditions. The set of differential equations was solved using MATLAB® software (R2014b) to estimate the model parameters, together with the ODE4 solver (Runge–Kutta).

Results and Discussion

Chemical Composition of Branches and Barks

E. grandis EFR were analyzed to determine their chemical composition (Table 2), revealing notable variations in lignin and non-structural compounds, such as extractives and ashes. In branches, total lignin was more abundant ($27.20 \pm 0.39\%$) than in barks ($15.72 \pm 0.98\%$). These results agree with previously published work on *Eucalyptus nitens* (*E. nitens*) wood (23.72%) [14] and barks from different eucalyptus species with values between 14.40 and 16.80% for *Eucalyptus viminalis* (*E. viminalis*), *Eucalyptus botryoides* (*E. botryoides*), and *E. grandis* [33].

In this study, the extractives and ashes in the bark constituting $10.32 \pm 0.42\%$ and $8.75 \pm 0.04\%$, respectively. In the branches, these values were only 1.30 ± 0.21 and $0.63 \pm 0.01\%$, respectively. Bark has a higher content of extractives and ash than wood does. The content of

non-structural compounds in the bark is influenced by factors such as bark type (inner or outer and cortex content), mechanical size reduction, and source of the collected material, whether industrial or forest residue [34]. The extractives of eucalyptus residues are composed of fatty acids, terpenes, condensed tannins, resins, and flavonoids [35]. Notably, eucalyptus bark contains a higher amount of polar extractives than wood [34]. Neiva et al. [34] reported that industrial wood from *Eucalyptus globulus* (*E. globulus*) contained 4.45% and 0.75% extractives and ash, respectively, while bark contained 9.86% extractives and 5.37% ash. The difference in inorganic content between both residues was due to the higher content of calcium and iron in the bark samples than in wood. Eucalyptus bark ash principally consists of calcium oxalate crystals.

Regarding polysaccharide content, cellulose was predominant in both residues, comprising $45.09 \pm 0.28\%$ in branches and $50.31 \pm 0.34\%$ in bark. Meanwhile, the hemicellulose content (arabinoxylan + acetyl groups) ranged from $19.62 \pm 0.12\%$ in branches to $15.46 \pm 0.07\%$ in bark. Penín et al. [14] found 40.70% of cellulose and 24.86% of hemicellulose (including arabinoxylan, acetyl and uro-nyl groups) content for *E. nitens* industrial wood. Neto et al. [15] reported cellulose and hemicellulose contents of 43.90% and 17.40%, respectively, in sawdust derived from wood chips from *E. grandis* and *Eucalyptus urophylla* (*E. urophylla*). Lima et al. [33] showed a total content of polysaccharides (cellulose and hemicellulose) with values of 63.90% for *E. viminalis*, 65.20% for *E. grandis*, and 68.40% for *E. botryoides* barks. The chemical composition results showed slight variations compared to those in previous literature. However, these variances can be attributed to factors such as differences in species, cultivation conditions, and harvesting methods [21, 36].

An effective means of evaluating the changes in different constituents throughout the processes employed is to analyze the ratios between them. In the case of branches, the lignin-to-cellulose ratio was 0.60, while in barks, it was 0.31. Similarly, when assessing the hemicellulose-to-cellulose ratios were 0.44 for branches and 0.31 for barks, respectively. These variations highlight the differences between the materials, despite sharing the same plant origin.

Because of the higher hemicellulose content found in the branches, this material is more suitable to produce bio-compounds derived from the xylan fraction, such as XOS. However, bark residues also contain considerable amounts of polysaccharides, which can be explored for this purpose. Additionally, the high content of extractive components in bark residues can be used for the co-production of phenolic compounds with different pharmacological applications such as antioxidant and antimicrobial agents [34, 37]. Therefore, considering the chemical composition of eucalyptus branch

Table 2 Chemical composition of un-treated eucalyptus branches and barks

Components (% m/m)	Branches	Barks
Cellulose	45.09 ± 0.28	50.31 ± 0.34
Hemicellulose	19.62 ± 0.12	15.46 ± 0.07
Lignin	27.20 ± 0.39	15.72 ± 0.98
Extractives	1.30 ± 0.21	10.32 ± 0.42
Ashes	0.63 ± 0.01	8.75 ± 0.04

and bark residues, these materials are interesting biomass resources for value-added production in biorefineries.

Combined Pretreatment (CP)

Although naturally present in plant tissues, XOS are less abundant than the main structural polysaccharides such as cellulose and hemicellulose. In lignocellulosic biomass, pretreatment converts insoluble hemicellulose into several soluble oligosaccharides; however, the concentration and structure of these compounds depend on the method and severity of the treatment [4]. The eucalyptus branches and bark were subjected to alkaline treatment under mild reaction conditions to hydrolyze the ester groups, aiming to partially remove the acetyl content in the residues. Residual amounts can assist in hemicellulose hydrolysis. The assay was conducted as a preliminary step before hydrothermal pretreatment to assess the effect of deacetylation of EFR on XOS production.

During the alkaline treatment, a pronounced difference in the process yield (Y_{pt}) was observed between the two residues; the branches achieved a yield of $91.95 \pm 0.18\%$, whereas the barks had $79.79 \pm 0.18\%$. This suggests higher solubilization of the bark components in the alkaline liquor.

By comparing the chemical composition of the investigated EFR (Fig. 1), it was observed that the hemicellulose fraction was the primary component solubilized in both residues after alkaline treatment. This finding is consistent with previous literature, which suggests that alkaline pretreatment enables the solubilization of one or more biomass fractions, with solubilization being more pronounced in the hemicellulosic fraction owing to the crystalline nature of cellulose [38]. The deacetylated branches exhibited a hemicellulose solubilization of 10.77%, with only a 2.14% reduction in cellulose content. In the barks, both hemicellulose and cellulose were more affected by the alkaline pretreatment, demonstrating solubilization of 16.51% and 15.86%, respectively.

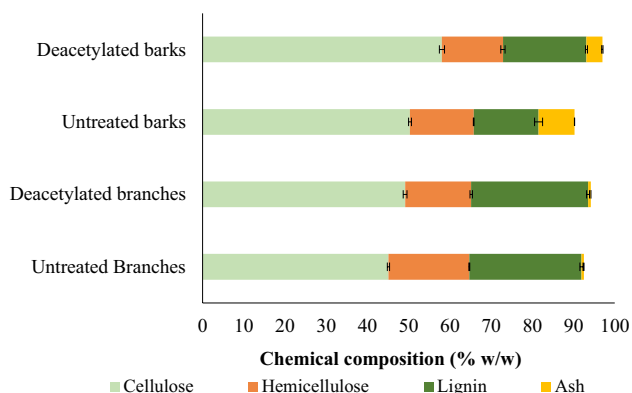


Fig. 1 Chemical composition of untreated and deacetylated branches and barks

Hemicellulose solubilization in eucalyptus branches was similar to that reported by Jayapal et al. [39] in alkaline pretreatment of sugarcane bagasse. The hemicellulose removal ranged from 9.15 to 11.91% for reactions with 8% and 12% NaOH, respectively.

Although the barks exhibited a higher fraction solubilization, the removal of acetyl groups was lower than that of the branches. While the branches achieved a removal percentage of 83.55%, the barks only achieved a removal of 64.54%. These results suggested that the reaction conditions selected for the deacetylation of EFR performed better in the branches. This was evidenced by the high percentage of acetyl group removal from the hemicellulose fraction of the branches along with less degradation of the xylan chain into monomers. The alkaline liquors contained acetic acid at 4.60 g/L (branches) and 2.50 g/L (barks), with xylose at 0.06 g/L (branches) and 1.57 g/L (barks). In the deacetylated solid fractions, organic acids were below the detection limit.

On the other hand, lignin solubilization was low in both residues, approximately 6.02% in the bark and 6.31% in the branches. These results are consistent with the literature, indicating that under low-severity conditions, the removal of most acetyl groups occurs along with a partially fraction of lignin [10, 40]. The decrease in lignin content in the residues during the deacetylation stage may enhance the digestibility of eucalyptus branches and bark in the subsequent conversion process. Additionally, the partial removal of lignin may decrease the formation of degradation compounds in hemicellulosic hydrolysates derived from the partial degradation of lignin. These compounds are highly toxic to microorganisms and can affect downstream processes [10, 17].

The differences found in the solubilization of hemicellulose and cellulose may be due to the barks having a larger geometrical surface area, which could have facilitated the impregnation of the alkaline solution during the deacetylation step, resulting in a greater solubilization of these structural components. Additionally, branches have a higher lignin content in their chemical composition, which may have made the biomass matrix more resistant, rendering the lignocellulose complex less accessible for solubilization under the reaction conditions during deacetylation.

Hemicellulose from hardwoods, such as eucalyptus, is often found in an acetylated form, similar to other biomass sources [41]. These acetyl groups can dissociate into acetate ions, which can catalyze the solubilization of polysaccharides into degradation products depending on their concentration [42]. Alkaline pretreatment alters the material structure by cleaving the ether bonds between lignin and hemicellulose as well as the ester bonds between lignin, hemicellulose, ferulic, and p-coumaric acids. Additionally, it reduces the content of acetyl groups and uronic acids in the xylan fraction, thus reducing the possible inhibitory effects. Therefore, deacetylation of lignocellulosic biomass

may mitigate the necessity for subsequent detoxification of the hydrolysate containing XOS, which is required for its utilization in later biological stages [10, 17].

Eucalyptus bark demonstrated a lower removal of acetyl groups compared with the results achieved by Chen et al. [19] for the deacetylation of corn stover (73.00%), which was treated at 60 °C with a residence time of 20 min and NaOH concentration (0.4% w/w).

Brenelli et al. [10] investigated the deacetylation of sugarcane straw. They observed that the pretreatment increased the removal of acetyl groups with increasing time and temperature, reaching 75.00% deacetylation at 80 °C in 60 min. Increasing the NaOH concentration from 6 to 8% reduced the time and temperature required, resulting in 80.00% deacetylation in 30 min at 60 °C. These results are similar to those achieved with eucalyptus branches but lower than those achieved with barks. Overall, the results obtained from the branches and bark indicate that alkaline pretreatment was effective in acetyl group removal, with pronounced differences between the branches and barks in terms of solubilization and the composition of the liquors produced. This confirms that the efficiency of deacetylation may also depend on the type of biomass in addition to the reaction parameters.

In this study, XOS production derived from EFR was conducted at the laboratory scale in the batch mode. This method facilitates the identification of ideal conditions for obtaining desired products and provides insights into the complex mechanisms involved in the degradation of lignocellulosic materials. Given the complexity involved in scaling up pretreatment procedures [43], initially conducting experiments at the laboratory scale is considered cost-effective for analyzing the parameters influencing the pretreatment step. This is primarily because the process is performed in small-scale reactors, which have lower cost requirements, easier control operations, and minimal demands for input materials and energy consumption compared to pilot-scale operations [10, 18].

Subsequently, the deacetylated EFR were subjected to CP pretreatment and four reaction conditions were evaluated (Table 1). The implementation of CP on deacetylated branches and bark provides valuable insights into the biochemical transformations occurring in these residues (Table 3). Differential hemicellulose recovery in the liquor was evident, with the branches showing a more pronounced solubilization (47.20 to 64.08%) than the barks (36.21% and 55.00%).

When analyzing the mechanisms of arabinoxylan conversion, CP directed most of the reaction towards the formation of XOS, with branches showing more notable conversions. Variations in temperature and time conditions revealed that at 190 °C and 7.5 min of reaction, the highest XOS conversions were achieved: 38.67% in branches and 31.29% in barks. However, a further increase in time led to a higher

pretreatment severity. Conducting pretreatments at high SF causes a higher formation of H^+ because of water autoionization and removal of acetyl groups from the hemicellulose fraction [23]. In the CP of EFR the SF increased from 3.52 at 7.5 min to 3.83 at 15 min. Consequently, this resulted in a decrease in pH in HH, ranging from 5.44 to 4.66 in branches and from 4.90 to 4.25 in barks. This change can be attributed to the increase in the acetic acid content in the HH caused by the increased SF, leading to the degradation of XOS into xylose and increased furfural formation (Table S1-Supplementary Information).

Analysis of the hydrolysis products revealed distinct profiles for the formation of xylose, arabinose, and their oligomers. In the branches, xylose was the main component present in HH, increasing from 1.14 to 4.60% with respect to temperature and/or time. In barks, xylose formation ranged from 0.55 to 1.81% under the most severe conditions. In contrast, the predominant formation of arabinose and its oligomers occurred in the barks, with values of approximately 6.07% at 175 °C for 20 and 25 min, which decreased to 3.69% at 190 °C for 15 min. In the branches, the maximum formation of arabinose and its oligomers was 1.08% at 175 °C for 25 min, while the furfural showed similar behavior and close conversions between both residues with values ranging between 0.16 and 1.32%.

Regarding the selectivity of XOS over xylose, the CP results indicated high selectivity in both the branches (96.64% and 89.07%) and barks (98.06 to 93.95%). The results showed that the best conditions for XOS production by CP were 190 °C for 7.5 min for both EFR. This resulted in a yield of 6.19% XOS and 0.53% xylose in the branches (Fig. 2a) and 4.64% XOS and 0.21% xylose in the barks (Fig. 2b).

Under the best treatment conditions (CP-03), a detailed analysis of HH revealed a distinct composition. In branches, there were 0.03 g/L of glucose, 0.68 g/L of xylose, 0.10 g/L of arabinose, and its oligomers, along with a notable concentration of 7.06 g/L of XOS. Degradation compounds included 0.04 g/L of HMF, 0.12 g/L of furfural, 0.05 g/L of formic acid, and 0.48 g/L of acetic acid (Table S1-Supplementary Information).

In the case of barks, despite the absence of sugars from the cellulose fraction, 0.27 g/L of xylose, 0.19 g/L of arabinose and its oligomers, and a total of 5.27 g/L of XOS were produced. Degradation compounds were found at 0.06 g/L of HMF, 0.11 g/L of furfural, 0.03 g/L of formic acid, and considerable concentration of 1.27 g/L of acetic acid (Table S2-Supplementary Information).

The composition of the feedstock material affects the biomass conversion process. In some cases, removing xylan fractions can be difficult due to the linkages between lignin and hemicellulose, as well as strong hydrogen bonding interactions among polysaccharides. This interaction among

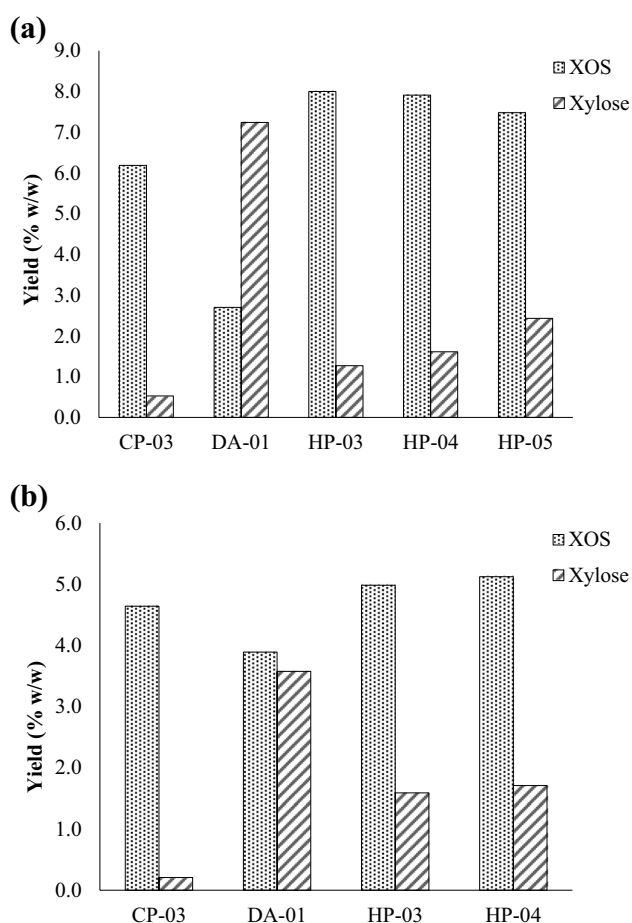


Fig. 2 Yield of XOS and xylose (% w/w, based on input material) achieved under the best pretreatment conditions: **a** branches and **b** barks

biomass cell wall components makes the xylan solubilization or extraction processes more complex [39]. Therefore, differences in xylan solubilization patterns, as well as in the conversion to oligomers and monosaccharides in the hydrolysates obtained by the different pretreatment methods investigated in this study, may be attributed, in part, to the higher arabinoxylan content and greater proportion of acetyl groups in branches, facilitating the attainment of higher XOS concentrations compared to barks. Additionally, the branches exhibited a lower content of extractive compounds than the barks. These extractive compounds contribute to material recalcitrance, making delignification and subsequent conversion steps more difficult [35].

Diluted Acid Pretreatment (DA)

The use of diluted acid in the solubilization of arabinoxylan into XOS was evaluated at a fixed acid concentration and temperature, while varying the reaction time (Table 1). In branches, SF ranged from 1.05 to 1.49, whereas in barks, it

ranged from 0.04 to 0.45. This discrepancy in SF under the same pretreatment conditions is due to the distinct pH of HH resulting from the process. The liquors from branches exhibited lower pH values (1.75–1.71) than those from the barks (2.79–2.72), which influenced the results obtained for the evaluated residues.

The use of 0.1% (v/v) H_2SO_4 in DA pretreatment resulted in remarkable hemicellulose solubilization. For the branches, the ranges were 74.00%, 81.44%, and 86.97% at 5, 10, and 15 min, respectively. For the barks, the values were 75.82%, 79.21%, and 89.31% at the same reaction intervals (Table 3). Comparatively, the use of H_2SO_4 as a pretreatment agent led to higher hemicellulose solubilization compared to the results obtained in CP for both residues and using temperatures and/or reaction times lower than those of the HP method (HP: 01–06 for branches and HP: 01–04 for barks). For XOS production, the DA method allows relatively short reaction times, during which the glycosidic bonds of xylose are broken at various points along the chain, resulting in different fractions of XOS [5], which can be advantageous on an industrial scale because of its ability to produce XOS with different applications.

The results obtained with EFR demonstrated that the DA method was more efficient in converting bark residues into XOS (Fig. 3). In only 5 min of reaction, 30.08% of the arabinoxylan was converted into XOS, which dominated the profile of the compounds obtained. With increasing time, the conversion slightly decreased to 28.77% at 10 min and 28.15% at 15 min. Xylose showed a more pronounced variation with increasing reaction time, ranging from approximately 28.00% at 5 min to 41.00% at 15 min. Regarding the formation of arabinose and its oligomers as well as furfural, there were small variations in the evaluated times. These results suggest that increasing the time from 5 to 15 min may have promoted greater direct solubilization of arabinoxylan chains into xylose, as the fraction of solubilized hemicellulose increased with time (Table 3). However, increasing the time did not promote greater degradation of higher-DP XOS into smaller molecules, as XOS conversions showed minimal changes in the three analyzed time intervals.

The results from the branches exhibited different behavior, demonstrating a significant conversion of arabinoxylan into xylose (Fig. 3). In a short 5-min reaction period, there was a conversion of 44.98%, which increased to 61.41% and 72.91% at 10 and 15 min, respectively. With extended reaction time, there was an increase in the production of arabinose and its oligomers (from 1.57 to 2.02%), as well as furfural (0.64 to 2.50%). However, the conversion of xylan into XOS was low: 16.78% at 5 min, which decreased to 10.30% and 6.11% at 10 and 15 min, respectively.

In DA, there was no increase in the concentration of XOS compared to the CP and HP methods (Fig. 2a, b). Furthermore, DA was more severe in the branches,

Table 3 Effect of different branches and barks pretreatment conditions on hemicelluloses, arabinoxylans, and XOS

Residue	Code	SF	Hemicellulose solubilization (% w/w)	C _{AX} (% w/w)**				Y _{XOS} (% w/w)***	XOS selectivity (% w/w)
				Xylose	XOS	Arabinose oligomers + arabinose	Furfural		
Branches	CP-01*	3.51	47.20	1.14	32.88	0.97	0.16	5.26	96.64
	CP-02	3.61	50.31	1.36	33.89	1.08	0.21	5.42	96.15
	CP-03	3.52	56.85	3.29	38.67	0.97	0.88	6.19	92.15
	CP-04	3.83	64.08	4.36	35.51	0.97	1.32	5.68	89.07
	DA-01	1.05	74.00	44.98	16.78	1.57	0.64	2.70	27.17
	DA-02*	1.35	81.44	61.41	10.30	1.70	1.47	1.66	14.36
	DA-03	1.49	86.97	72.91	6.11	2.02	2.50	0.98	7.73
	HP-01	3.21	46.94	3.12	34.07	1.44	0.26	5.49	91.62
	HP-02	3.38	64.79	4.24	39.23	1.49	0.40	6.32	90.25
	HP-03	3.51	65.33	7.89	49.70	1.77	0.69	8.00	86.30
	HP-04	3.61	71.98	10.00	49.14	1.92	0.88	7.91	83.09
	HP-05	3.52	80.57	15.10	46.47	1.49	2.16	7.48	75.47
	HP-06*	3.83	86.63	28.67	41.78	1.22	6.09	6.73	59.31
	HP-07	4.00	89.61	37.99	25.42	1.04	9.41	4.09	40.09
	HP-08	3.79	99.10	39.53	12.37	1.12	14.32	1.99	23.83
	HP-09	4.09	99.30	37.14	6.02	0.91	17.45	0.97	13.95
	HP-10	4.27	99.52	30.38	4.24	0.42	19.29	0.68	12.26
Barks	CP-01	3.51	36.21	0.55	27.68	6.07	0.16	4.10	98.06
	CP-02*	3.61	39.51	0.59	28.14	6.07	0.20	4.17	97.93
	CP-03	3.52	51.81	1.39	31.29	4.69	0.86	4.64	95.75
	CP-04	3.83	55.00	1.81	28.17	3.69	1.13	4.17	93.95
	DA-01	0.04	75.82	27.61	30.08	9.28	1.39	3.89	52.14
	DA-02*	0.41	79.21	34.46	28.77	10.16	2.27	3.72	45.51
	DA-03	0.45	89.31	40.94	28.15	10.92	3.27	3.64	40.75
	HP-01	3.21	67.91	13.14	30.80	6.47	0.96	3.99	70.01
	HP-02	3.38	65.28	13.02	24.89	4.77	1.39	3.22	65.65
	HP-03	3.51	72.95	12.28	38.51	5.67	1.78	4.98	75.82
	HP-04	3.61	76.79	13.21	39.59	5.03	2.43	5.12	74.98
	HP-05	3.52	89.83	18.51	22.28	2.59	8.98	2.88	54.62
	HP-06	3.83	88.37	16.26	17.78	1.86	11.84	2.30	52.23
	HP-07	4.00	98.50	16.77	10.51	1.08	15.83	1.36	38.53
	HP-08	3.79	99.31	15.09	6.66	0.70	16.99	0.86	30.62
	HP-09	4.09	99.13	12.58	2.61	0.36	17.02	0.34	17.16
	HP-10	4.27	99.16	7.77	1.24	0.00	18.24	0.16	13.75

*Experiments conducted in triplicate with a standard deviation < 0.40

**Conversion relative to the amount of arabinoxylan present in the initial material

***Y_{XOS}: relative to the DM of input material

resulting in a low XOS yield (2.70% in 5 min and 0.98% after 15 min). In the barks, XOS production remained constant (3.89 to 3.64%). The XOS selectivities over xylose were 27.17% and 7.73% for branches and 52.14 to 40.75% for barks (Table 3). This difference in the conversion behavior of EFR during DA in XOS formation may be related to the intrinsic characteristics of the material, such as acetyl groups, which, together with H₂SO₄, also act as catalysts for hemicellulose solubilization. This interaction

can influence the conversion of xylan to different products over time.

Compared to other studies, the conversion of arabinoxylan into XOS through DA pretreatment in barks exceeded the results for sugarcane bagasse (~25%) at 132 °C and 170 °C for 15–60 min with 0.1% and 1% H₂SO₄ [9]. However, in EFR, the conversions were lower than those achieved in the treatment of sugarcane bagasse at 150 °C and 170 °C for 5.5 and 15 min with 1 to 5% H₂SO₄, which reached 36.76% and

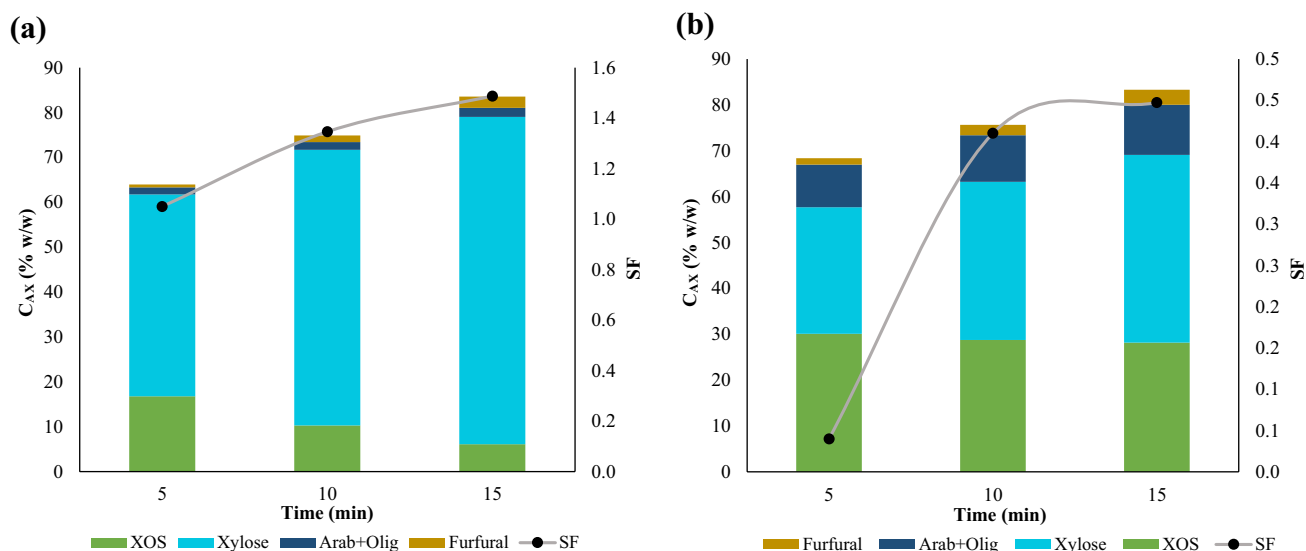


Fig. 3 Arabinoxylan conversion (C_{AX}) during DA pretreatment of **a** branches and **b** barks

40.21% conversion into XOS [20]. These discrepancies can be attributed to variations in the raw material composition and experimental conditions between studies.

The resulting HH from the best DA (01) for eucalyptus residues showed in branches concentrations of 0.47 g/L of glucose, 0.21 g/L of cellobiose, 9.24 g/L of xylose, 3.03 g/L of XOS, and 0.32 g/L of arabinose and its oligomers. HMF, furfural and formic acid were found at low concentrations (0.03 g/L, 0.08 g/L, and 0.04 g/L, respectively), while acetic acid was solubilized at a concentration of 2.67 g/L (Table S1-Supplementary Information).

In barks, values of 1.62 g/L for glucose, 4.56 g/L for xylose, 4.37 g/L for XOS, and 1.53 g/L for arabinose and its oligomers were found, with cellobiose not being detected. The formation of degradation compounds included 0.33 g/L of HMF, 0.15 g/L of furfural, 0.06 g/L of formic acid, and only 0.44 g/L of acetic acid (Table S2-Supplementary Information).

Hydrothermal Pretreatment (HP)

Ten different conditions were evaluated, varying the temperature and time (Table 1), resulting in SF ranging from 3.21 and 4.27. The variations in temperature and time demonstrated efficiency in hemicellulose solubilization, with the initial condition HP-01 achieving 46.94% solubilization in branches and 67.91% in barks. As the severity increased, the recovery of hemicellulose in HH also increased. Notably, hemicellulose was solubilized in approximately 99% of both branches and barks in the temperature range of 205 °C (Table 3).

In the HP method, water hydronium ions act as catalysts, promoting the fragmentation of biomass cell wall

components. This primarily results in the breakdown of xylan into XOS and xylose as well as the cleavage of acetyl group bonds in the hemicellulose fraction. These reactions alter the pH of the obtained hydrolysates owing to the formation of acetic acid, which also catalyzes the depolymerization reactions of the arabinoxylan chain [18]. In the HP of EFR, the pH values of the hydrolysates ranged from 3.46 to 3.00 in branches and from 3.30 to 3 in bark.

In the initial trials of the HP method (01–06), the conversion of arabinoxylan was primarily directed towards the formation of XOS in both residues. In the branches, the temperature of 175 °C allowed an increase in XOS as the SF increased, reaching 49.70% conversion at SF 3.51. However, this conversion slightly decreased at SFs 3.61 and 3.52, resulting in 49.14% and 46.47% XOS conversions, respectively. On the other hand, the production of XOS suffered a significant reduction when temperature and time combinations resulted in SFs of 4, reaching only 4.24% conversion under the highest severity condition (SF 4.27).

Regarding the recovery of XOS in the barks, it was observed that at a temperature of 175 °C, increasing the time to 15 min (SF 3.38) resulted in a slight decrease in the conversion of arabinoxylan into XOS. However, at 20 min (SF 3.51) and 25 min (SF 3.61), it was possible to increase the recovery of XOS in HH, resulting in XOS conversions of 38.51% and 39.59%, respectively, which were the highest XOS conversions for the trials conducted in bark residues.

In contrast to branches, at higher temperatures (190 °C and 205 °C), the barks experienced more significant degradation of XOS, resulting in a substantial decrease in conversion to 22.28% and 1.24% at the highest severity (4.27). This suggests that the bark residues are more temperature-sensitive, with increased severity leading to more intense

degradation of XOS, possibly due to the formation of unwanted by-products and the greater susceptibility of the xylan structure to high temperatures.

In this sense, the maximum conversion to xylose in the branches (39.53%) was achieved at a temperature of 205 °C, in trial HP-08. In contrast, in the barks, the maximum conversion was 18.51% at 190 °C, in the trial HP-05. The formation of arabinose and its oligomers showed conversions of up to 1.92% in the branches (HP-04) and 6.47% in the barks (HP-01). The formation of furfural increased progressively with increasing temperature and/or time, resulting in values close to each other in both residues at the highest severity, 19.29% in branches and 18.24% in barks. However, it was observed that the removal of approximately 90% of the hemicellulose fraction from the barks had a greater impact on the degradation of sugars, both in their oligomeric and monomeric forms, converting them into furfural.

The selectivity of XOS in the branches showed considerable variation owing to the formation of xylose. The trial with the lowest severity (3.21, HP-01) had the highest selectivity for XOS over xylose (91.62%), although with a low XOS yield (5.49%). These results are consistent with those of previous studies by Carvalho et al. [9], who also found higher selectivity under conditions of lower XOS yield during steam explosion pretreatment of sugarcane bagasse.

Comparing the EFR, the total XOS production in branches was higher than that obtained in the barks, with maximum yields of XOS of 8.00% in the HP-03 condition for branches and 5.12% in the HP-04 condition for barks, with low yields of xylose of approximately 1.27% for branches and 1.71% for barks (Fig. 2a, b).

Following the assessment of the three pretreatment methods, it was observed that CP in the barks did not result in a significant difference in maximum XOS conversion compared to HP. In HP, a conversion of up to 39.59% of the arabinoxylan present in the input material was achieved at 175 °C for 25 min (Table 3). This may be attributed to the fact that the bark residues, during the alkaline pretreatment stage, underwent less removal of acetyl groups; therefore, the remaining acetyl groups in the barks served as a catalyst to hydrolyze hemicellulose into XOS, resulting in conversions comparable to those obtained in untreated barks subjected to HP.

However, the limited availability of acetyl groups in the branches proved to be ineffective in promoting greater hemicellulose solubilization into XOS. The partial removal of acetyl groups from both biomasses led to an increase in the reaction temperature during CP, as both materials achieved the highest XOS yields at 190 °C compared to the HP method, which resulted in a higher XOS content and greater hemicellulose solubilization at 175 °C. These results align with previous research [44], which observed that in sequential pretreatments, partial deacetylation of

biomass during the first pretreatment leads to slower kinetics in the subsequent pretreatment due to the limited catalyst availability.

The three methods employed to produce XOS from EFR demonstrated that HP was the most efficient in achieving higher total XOS production in the HH (Fig. 2a, b). For the branches, the best condition was HP-03 (175 °C/ 20 min), resulting in 9.05 g/L of XOS, 1.63 g/L of xylose, 0.37 g/L of arabinose and its oligomers, 0.12 g/L of glucose, 0.09 g/L furfural, 0.03 g/L HMF, 0.16 g/L formic acid, and 0.61 g/L acetic acid (Table S1-Supplementary Information).

In the barks, the best conditions were HP-03 (175 °C for 20 min) and HP-04 (175 °C for 25 min), with XOS concentrations of 5.58 g/L and 5.68 g/L, respectively. However, increasing the time to 25 min led to a higher formation of by-product, as shown in Table S2-Supplementary Information. Therefore, to produce XOS from barks, the most efficient condition was HP-03. In addition to XOS, the HH had concentrations of 2.02 g/L of xylose, 1.06 g/L of glucose, 0.74 g/L of arabinose and its oligomers, 0.18 g/L of HMF, 0.19 g/L of furfural, 0.34 g/L of formic acid, and 0.79 g/L of acetic acid.

The HP method is advantageous for XOS production because it requires short residence times, yields low concentrations of degradation compounds, and is considered eco-friendly owing to the absence of additional chemicals in the process. Furthermore, the content of high-DP XOS in HH can be subsequently reduced using endoxylanases to convert larger XOS into smaller DP XOS, which are of greater importance in the industrial sector [10, 11].

In comparison to some literature results, the best HP condition for the branches resulted in higher XOS concentrations and lower by-products formation compared to the values obtained for sugarcane bagasse hydrolysate produced at 180 °C for 20 min, which had 7.50 g/L of XOS [45]. Moreover, the maximum yield achieved in wheat straw treatment (4.90 ± 0.2 g of XOS per 100 g of biomass) at 180 °C for 40 min reported by Huang et al. [12] was similar to that achieved by the barks (0.05 g/g), but lower than the maximum reported for eucalyptus branches (0.08 g/g).

The barks achieved a XOS yield similar to that reported by Neto et al. [15] (0.061 g/g of biomass) in the pretreatment of a mixture of wood chips from *E. grandis* and *Eucalyptus urophylla* (*E. urophylla*) (1:1) under the optimized conditions of 161 °C for 65 min. However, the branches obtained a higher yield, even under non-optimized conditions and with a shorter reaction time during pretreatment (only 20 min), resulting in lower concentrations of undesirable products, such as xylose and furfural.

A previous study by Penín et al. [14], *E. nitens* wood pretreated between 190 and 195 °C, with severities of 3.47 and 3.62, produced XOS yields of approximately 8 and 9 g per 100 g of treated wood, respectively. These results are

comparable to the maximum yield achieved in this study for branches, which was 8.00% at 175 °C and a severity of 3.51.

However, the XOS production obtained from EFR pretreatment was lower than that reported for sugarcane straw (9.80% w/w) at 190 °C for 20 min [10] and that reported by Romaní et al. [13] in the autohydrolysis treatment at 195 °C, which achieved an 10.02 g of XOS per 100 g of initial of *E. globulus* wood chips. It is important to highlight that the EFR (branches and barks) used in this study belong to the *E. grandis* species and are from forestry and non-industrial sources, suggesting that forestry sector residues can also be a viable source for XOS production.

The results obtained from the pretreatment of eucalyptus branches and barks highlighted the critical influence of temperature and reaction time on XOS production from lignocellulosic residues. The source of the raw material plays a pivotal role, as evidenced by comparisons with previous studies, suggesting that the pretreatment conditions and the raw material composition affect the characteristics of the hydrolysates, including the XOS concentration and the formation of other products. The variation in the experimental conditions with respect to selectivity, XOS yield, and subproduct formation emphasizes the importance of adjusting the pretreatment conditions to maximize the desired products.

The utilization of lignocellulosic residues as precursors for bioproducts contributes to the valorization of these resources through the implementation of integrated processes under the biorefinery concept. In this context, the use of EFR for XOS production expands the possibilities of utilizing these materials, offering several advantages such as obtaining higher value-added subproducts (xylose, arabinose, xylitol, and furfural) from abundant and low-cost resources [6, 17]. This approach not only enables more efficient waste management but also promotes the maximization

of resource utilization in accordance with sustainability policies. Thus, it provides an alternative to incineration and contributes to reducing waste accumulation in the forestry sector [16].

XOS DP Distribution

Pretreatment with EFR revealed that the conditions that showed the highest XOS production were CP-03, DA-01, and HP: 03–05 for branches and CP-03, DA-01, and HP: 03–04 for barks. HH exhibited variations in the molecular size of the XOS produced by the different pretreatment methods evaluated (Fig. 4a, b), emphasizing that the process severity influences the molecular characteristics of the XOS resulting from the pretreatment. According to de Freitas et al. [5], the structure of XOS may vary also according to the biomass source used to produce HH. These oligosaccharides can have different DP, varying between 2–12 units, and may contain side chain branches of functional groups such as arabinofuranosyl, uronic acids, and acetyl groups. These structural variations have the potential to influence the physicochemical and biological properties of XOS.

Xylan depolymerization is a complex process that involves fast and slow reactions. Initially, a high-molecular-mass XOS is formed, which degrades into a low-molecular-mass XOS. These, in turn, can be fractionated into xylose, which can be converted into furfural and other by-products [32].

In the analysis of the branches, the best conditions for XOS production through the CP and HP methods mainly resulted in fractions with DP > 5, ranging from 41.54 to 78.86%. In CP-03, fractions X3–X5 showed similar values (ranging from 5.41 to 6.96%), whereas X2 accounted for only 3.86% of the total. On the other hand, the HP method indicated that an increase in temperature and/or time led

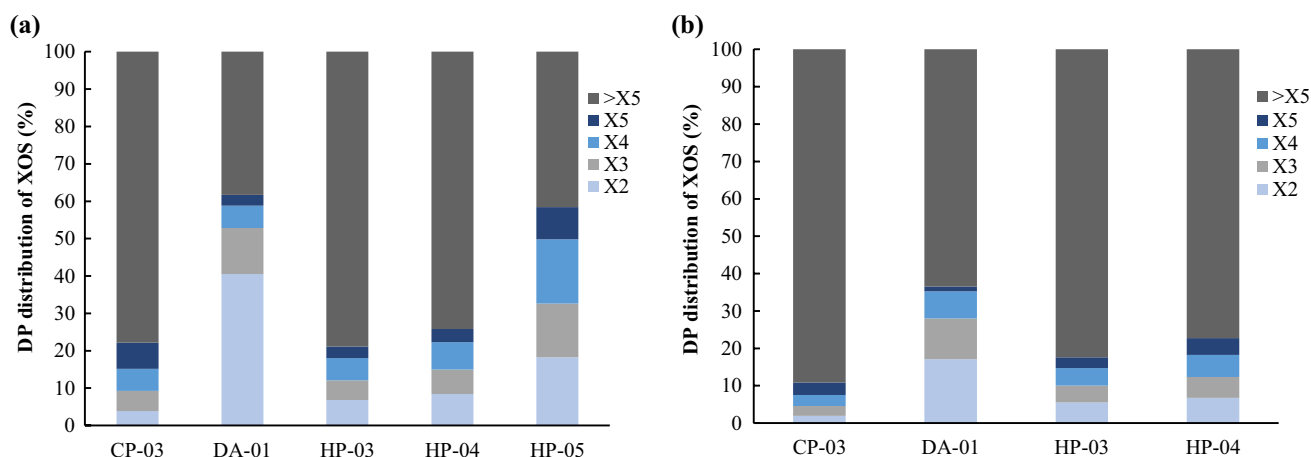


Fig. 4 Distribution of DP of XOS under the best pretreatment conditions for **a** branches and **b** barks

to an increase in XOS with a lower DP. X2 increased from 6.82 to 18.22%, X3 from 5.25 to 14.43%, X4 from 6.00 to 17.17%, and X5 from 3.08 to 8.64%. In contrast, the use of diluted acid in the pretreatment (DA-01) resulted in a different DP distribution for XOS, with greater degradation into lower DP molecules. In this case, 40.51% of the total XOS were X2, a value higher than those observed in the other HH, 38.30% of XOS with DP > 5, 12.34% of X3, and the remaining 8.86% of X4 + X5 (Fig. 4a).

In the barks, most of the XOS in the HH had a DP > 5, as shown in Fig. 4b, but CP generated the highest proportion of these molecules (89.14%). On the other hand, the DA-01 method produced higher percentages of X2, X3, and X4 (17.08%, 10.93%, and 7.24%, respectively) than the other tests. However, XOS with DP > 5 still accounted for 63.47% of the total, a higher percentage than that obtained in the branches with the same pretreatment method. In contrast, the HP method led to a decrease from 82.36 to 77.26% in the fractions with DP > 5 in the barks due to an increase in reaction time, resulting in the formation of smaller chain-size XOS. The final proportions were 6.73% for X2, 5.58% for X3, 5.93% for X4, and 4.50% for X5, with the X2 to X4 fractions having lower proportions than the HH of branches obtained in HP-03 and HP-04 (Fig. 4a).

Determining the DP of XOS is essential because of its variability and different applications. These include their utilization in food formulations to enhance organoleptic properties and as prebiotic fibers in human supplements. Additionally, XOS have applications in animal nutrition and agriculture, serving as an additive for animal feed and agents to increase nutrient absorption in crops. In the pharmaceutical and medicinal contexts, XOS is used in the preparation of hydrogels for drug administration and for the prevention and treatment of gastrointestinal diseases, diabetes, arteriosclerosis, colon cancer, and obesity control [6]. Moreover, XOS with ramifications of uronic, ferulic, and methyl glucuronic acids may exhibit antioxidant and anti-allergic properties associated with the phenolic substituents present in its structure. The antioxidant, moisturizing, and emollient capacities of XOS make it suitable for use in the formulation of cosmetic products [17].

XOS with a DP of X2 and X3 are noteworthy in the industry because of their prebiotic properties. They are used to stimulate selective growth of the intestinal microbiome, such as *Bifidobacterium* and *Lactobacillus*, which produce short-chain fatty acids that inhibit the growth of pathogenic microorganisms. However, oligosaccharides with high DP also show prebiotic potential, serving as a substrate to promote the selective growth of *Bacteroides*. X2 has gained prominence as an active ingredient in food and pharmaceutical products because of its high sweetness (equivalent to 30.00% of sucrose) and its faster fermentation kinetics by the healthy intestinal microbiome [4–6].

Therefore, HH produced by EFR pretreatment presents opportunities to explore these different XOS fractions. In branches, XOS with low DP, such as X2, can be produced through DA (DA-01), achieving a near concentration of this compound using a short residence time (5 min) and lower temperature compared to HP-05 (175 °C/25 min). The X2 content obtained using the DA-01 and HP-05 was 1.23 g/L and 1.53 g/L, respectively. For X3–X5, HP-05 showed the highest concentration of these compounds and presented a better distribution of different XOS fractions. However, HP-03 produced the highest total XOS content with a high DP (> X5).

In the case of barks, the production of XOS with a low DP can be achieved in a single step using DA-01. This resulted in concentration in the HH of approximately 0.80 g/L, 0.48 g/L, and 0.32 g/L of X2, X3, and X4, respectively. Nevertheless, considering the higher total XOS concentration obtained through HP for branches and barks, especially HP 03–04, which exhibited a higher content of fractions > X5, this method was more suitable for producing these high-value compounds. Subsequently, they can be converted into low-DP XOS via enzymatic hydrolysis.

Pretreatment Effect on the Composition of the Pretreated Solid Fraction

Chemical characterization of biomass after pretreatment is essential to assess the impact of process conditions and provide data for kinetic models, enabling a deeper understanding of the dissolution mechanisms of components and the production of derivatives during pretreatment.

In all the three pretreatment methods evaluated, changes in the composition of the recovered solids were observed (Table 4). Specifically, CP, DA, and HP had a greater impact on the removal of cellulose from the barks, reflecting the concentrations of compounds derived from cellulose in the HH (Tables S1 and S2-Supplementary Information). Regarding hemicellulose, CP had a smaller impact due to the lower availability of acetyl groups in the previously deacetylated biomass. As for non-structural components such as ash, the barks showed higher contents after pretreatment because of the higher total ash concentration in the feedstock.

In terms of lignin, some removal was observed because using only water at high temperatures was not sufficient for delignification, and low-severity conditions were applied in the DA method. The HP and DA methods were more efficient in lignin removal from the branches, reaching up to 58.50% solubilization (HP-10) and 33.32% (DA-03), respectively. In the barks, the highest lignin removal (35.32%) was achieved with CP (CP-03).

In the CP-02 test for both EFR, DA: 02–03 in the barks, HP: 03 and 06 in the branches, and HP: 02, 06, and 10 in the barks showed higher contents of insoluble lignin (Klason)

Table 4 Yield of branches and barks pretreatments under different conditions and chemical composition of the cellulignin

Residue	Code	Y_{pr} (% w/w)**	Components solubilization (% w/w)			Chemical composition (% w/w)			
			Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin	Ash
Branches	Untreated branches					45.09 ± 0.28	19.62 ± 0.12	27.20 ± 0.39	0.63 ± 0.01
	Deacetylated branches					49.15 ± 0.46	16.00 ± 0.30	28.38 ± 0.34	0.68 ± 0.00
	CP-01*	85.39	1.33	47.20	10.85	58.54 ± 0.25	10.20 ± 0.94	30.54 ± 0.67	0.47 ± 0.02
	CP-02	84.93	1.89	50.31	9.03	58.52 ± 0.59	9.65 ± 0.54	31.34 ± 1.45	0.48 ± 0.00
	CP-03	79.74	3.44	56.85	19.08	61.40 ± 0.79	8.94 ± 0.03	29.72 ± 0.13	0.33 ± 0.02
	CP-04	78.72	3.90	64.08	19.41	61.68 ± 0.77	7.51 ± 0.49	29.87 ± 1.02	0.31 ± 0.01
	DA-01	71.63	2.23	74.00	21.24	62.93 ± 0.87	5.98 ± 0.24	30.58 ± 0.29	0.16 ± 0.00
	DA-02*	65.79	3.63	81.44	29.16	67.45 ± 0.53	4.64 ± 0.28	29.91 ± 0.28	0.12 ± 0.00
	DA-03	59.64	11.76	86.97	33.32	68.48 ± 1.68	3.61 ± 0.18	31.21 ± 0.24	0.11 ± 0.01
	HP-01	81.02	3.86	46.94	25.02	60.00 ± 0.22	11.83 ± 0.03	28.23 ± 0.62	0.43 ± 0.01
	HP-02	81.46	18.77	64.79	30.82	60.32 ± 1.32	9.34 ± 0.41	30.99 ± 1.55	0.28 ± 0.00
	HP-03	74.65	8.08	65.33	26.31	61.66 ± 1.18	8.30 ± 0.17	29.81 ± 0.31	0.23 ± 0.01
	HP-04	72.06	21.95	71.98	38.72	62.78 ± 0.19	7.76 ± 0.11	29.24 ± 0.23	0.23 ± 0.01
	HP-05	69.26	1.76	80.57	31.46	67.00 ± 0.29	4.73 ± 0.21	28,20 ± 0.66	0.07 ± 0.00
	HP-06*	64.98	5.94	86.63	30.55	66.78 ± 0.48	3.39 ± 0.22	29.74 ± 0.51	0.09 ± 0.02
	HP-07	61.82	9.92	89.61	34.02	67.40 ± 1.25	2.78 ± 0.01	29.78 ± 0.60	0.04 ± 0.01
	HP-08	62.86	11.95	99.10	40.79	70.84 ± 0.28	0.26 ± 0.00	28.74 ± 0.27	0.17 ± 0.00
	HP-09	49.21	35.87	99.30	51.81	71.75 ± 1.70	0.27 ± 0.01	27.84 ± 1.17	0.13 ± 0.01
	HP-10	45.33	38.67	99.52	58.50	71.16 ± 0.99	0.20 ± 0.02	29.05 ± 0.55	0.15 ± 0.01
Barks	Untreated barks					50.31 ± 0.34	15.46 ± 0.07	15.72 ± 0.98	8.75 ± 0.04
	Deacetylated barks					58.05 ± 0.62	14.82 ± 0.51	20.26 ± 0.23	3.86 ± 0.16
	CP-01	82.58	5.89	36.21	31.25	68.70 ± 0.84	11.89 ± 0.07	17.52 ± 0.41	2.28 ± 0.09
	CP-02*	81.82	9.02	39.51	25.12	67.17 ± 0.79	11.40 ± 0.20	19.29 ± 1.34	2.46 ± 0.07
	CP-03	76.41	10.79	51.81	35.32	70.13 ± 1.08	9.69 ± 0.16	17.79 ± 0.85	2.46 ± 0.13
	CP-04	76.46	11.27	55.00	33.88	69.98 ± 1.30	9.06 ± 0.17	18.20 ± 0.07	2.76 ± 0.13
	DA-01	58.63	22.87	75.82	14.47	70.02 ± 0.80	5.65 ± 0.18	24.26 ± 0.12	2.76 ± 0.00
	DA-02*	58.35	24.33	79.21	11.24	68.68 ± 0.48	4.85 ± 0.30	25.17 ± 0.99	2.87 ± 0.06
	DA-03	58.14	25.63	89.31	9.06	65.52 ± 0.92	2.42 ± 0.04	25.03 ± 0.36	2.82 ± 0.09
	HP-01	66.53	28.65	67.91	13.37	65.28 ± 1.54	7.55 ± 0.11	24.76 ± 0.10	2.41 ± 0.00
	HP-02	67.30	19.03	65.28	4.33	64.98 ± 0.10	7.17 ± 0.19	23.99 ± 0.06	3.87 ± 0.00
	HP-03	62.00	30.96	72.95	5.64	63.14 ± 0.43	6.36 ± 0.06	26.96 ± 0.42	3.53 ± 0.01
	HP-04	63.20	31.79	76.79	4.04	59.62 ± 1.47	5.22 ± 0.11	26.21 ± 0.66	3.43 ± 0.14
	HP-05	62.31	32.87	89.83	3.16	66.60 ± 0.56	2.59 ± 0.14	30.02 ± 0.38	2.69 ± 0.05
	HP-06	59.58	34.95	88.37	3.66	61.89 ± 0.42	2.85 ± 0.06	28.64 ± 0.26	4.89 ± 0.57
	HP-07	51.42	35.68	98.50	27.44	71.18 ± 0.44	0.43 ± 0.02	25.09 ± 0.10	3.30 ± 0.01
	HP-08	58.53	38.78	99.31	25.21	69.67 ± 1.36	0.20 ± 0.01	26.59 ± 0.55	2.81 ± 0.06
	HP-09	52.62	39.34	99.13	27.05	67.61 ± 1.04	0.25 ± 0.04	25.41 ± 0.78	2.90 ± 0.02
	HP-10	50.56	34.12	99.16	10.61	66.70 ± 0.55	0.22 ± 0.00	28.28 ± 0.02	4.21 ± 0.07

*Experiments conducted in triplicate with a standard deviation < 0.40

** Y_{pr} : relative to the DM of input material

compared to pretreatments at lower SF. This can be attributed to the formation of pseudo-lignin deposited on the fibers of the pretreated material as a result of condensation reactions between the solubilized lignin fragments and the carbohydrate degradation products (HMF and furfural). This process redistributes a material with characteristics similar to lignin on the biomass surface, thus increasing

the insoluble lignin content in the pretreated solid residue, which can affect cellulose digestibility. The formation of pseudo-lignin has been observed in pretreatments involving acidic reactions, but it can occur in any type of pretreatment under low-pH conditions [46].

On the other hand, in batch pretreatment systems, lignin-carbohydrate droplets may deposit on the surface of the

pretreated material, hindering the subsequent conversion of solids. However, this phenomenon can be reduced in continuous and semi-continuous systems, where these particles can be continuously removed by the liquid stream from the system, preventing them from accumulating on the surface of the pretreated material and increasing the digestibility of cellulignin [42]. This results in an overall increase in the process efficiency.

Mass Balance and Kinetics Modeling

Mass balance during the pretreatment of EFR for XOS production is crucial for assessing the components of the hydrolysate and residual solid fraction. This treated biomass can be subsequently hydrolyzed to produce valuable by-products, such as cello-oligosaccharides (COS), fermentable sugars, lignin, phenolic compounds, and organic acids [17, 47], offering opportunities for the co-production of value-added products and bioenergy.

In this study, HP-03 was selected for XOS production from eucalyptus branches and barks. This condition revealed a high concentration of total XOS in HH with a low content of degradation compounds, as shown in Tables S1 and S2 (Supplementary Information). The mass balance was then detailed for this assay.

The eucalyptus branches pretreated at 175 °C/ 20 min resulted in a solid fraction with a high residual cellulose content of 91.92%, along with significant amounts of lignin (73.69%), xylan (34.67%), and ash (23.98%). Solids recovery after HP resulted in 13.45 g, containing 8.29 g of cellulose, 4.01 g of lignin, 1.12 g of xylan, and 0.03 g of total ash. In the liquid fraction, it was possible to recover glucose and xylose monomers at 0.02 g and 0.25 g, respectively. Additionally, hemicellulose degradation compounds, such as furfural and acetyl groups, amounted to a content of 0.10 g. Other components solubilized in the liquor, such as lignin, ash, and extractives, among others, accounted for 3.10 g. Furthermore, 1.60 g of total XOS were recovered in the liquor, of which 0.11 g corresponded to X2, 0.08 g to X3, 0.10 g to X4, 0.05 g to X5, and 1.26 g to the XOS fractions with higher GP (> X5). From the eucalyptus branches, XOS yields of 8.00% were obtained in relation to the input residue quantity, 49.70% in relation to the xylan content in the material, and 77.13% in relation to the xylan solubilized in the hydrolysate from the post-hydrolysis process.

After the HP of the barks, 11.00 g was recovered in the pretreated solid residue. This residue is composed of 6.95 g of cellulose, 2.97 g of lignin, 0.70 g of xylan, and 0.39 g of ash, representing remaining percentages of 69.04%, 94.36%, 27.05%, and 22.17%, respectively. In the liquor, 0.32 g of xylose, 0.17 g of glucose, 0.10 g of acetyl groups, and 0.05 g of furfural were recovered. Additionally, other structural and non-structural components of the barks were solubilized,

totaling 5.96 g. Concerning XOS production, 1.00 g of total XOS was recovered in the HH, with 0.06 g of X2, 0.04 g of X3, 0.05 g of X4, 0.03 g of X5, and 0.82 g of XOS > X5. The XOS yields obtained from the barks were 4.98% in relation to the input material, 38.51% in relation to the xylan present in the residue, and 63.00% in relation to the xylan quantified in the hydrolysate obtained after post-hydrolysis.

Thus, from 1 kg of pretreated EFR, it was possible to obtain 80.00 g of total XOS from the branches and 50.00 g from the barks. The results highlight the remarkable difference in the compositions of the hydrolysates obtained from the branches and barks subjected to the same method and pretreatment conditions. This revealed significant variations in the products resulting from the pretreatment process between these two parts of the tree, highlighting the unique characteristics of each biomass component.

Compared with the literature, eucalyptus branches outperform other biomass sources, such as poplar sawdust [23], poplar *Populus* spp. [48], and sugarcane bagasse [9, 45] in the production of XOS. Although eucalyptus barks produced less XOS than branches, their results are comparable to those of previous studies, such as those on bamboo [22] and eucalyptus hybrid wood chips [15].

Branches release higher amounts of XOS, which can be converted into a growing range of bioproducts. For the branches under study, kinetic data were examined. The data obtained from the characterization of the resulting fractions during HP were used to develop a kinetic model for the conversion of hemicellulose present in eucalyptus branches. The experimental data and the values predicted by the kinetic model indicate that the conditions evaluated in the pretreatment were effective in xylan removal, which increased solubilization with increasing reaction temperature (Figures S1–S3, Supplementary Information).

Table 5 presents the rate constants related to xylan degradation. Constants k_1 , k_2 , and k_3 increased as the reaction temperature was raised. Temperatures of 175 °C and 190 °C appear to be more suitable for the formation of XOS during HP, as the constant k_1 is higher than the constants k_2 and k_3 . However, a shift in the predominant reactions occurred at 205 °C. This temperature appears to be too high for XOS formation, favoring the degradation of XOS into xylose instead, with constant k_2 surpassing constant k_1 at this temperature.

Increasing the reaction temperature from 175 to 205 °C resulted in a 4.42-fold increase in the rate constant related to the transformation of xylan into XOS (k_1) and a 67.91-fold increase in the formation of xylose from XOS (k_2).

Furthermore, in all temperature ranges, the degradation of xylose into degradation products such as furfural was the slowest step, as indicated by the lower rate constants k_3 . This implies that the formation of furfural from xylose is slower than that of the other chemical transformations involved in xylan degradation. When the pretreatment

Table 5 Rate constants of xylan hydrolysis during branches HP

Temperature (°C)	k_1 (min ⁻¹)	k_2 (min ⁻¹)	k_3 (min ⁻¹)	Model R^2		
				XOS	Xylose	Furfural
175	0.0430	0.0138	0.0109	0.982	0.972	0.848
190	0.1099	0.0653	0.0255	0.972	0.937	0.767
205	0.1900	0.9372	0.0475	0.916	0.883	0.741

Table 6 Estimation of activation energy and preexponential factor for xylan hydrolysis during branches HP

Rate constant	E_a (kJ/mol)	$\ln(A)$	Arrhenius R^2
k_1	88.45	20.65	0.983
k_2	236.62	59.06	0.979
k_3	87.53	19.00	0.995

temperature was increased from 175 to 205 °C, a 4.36-fold increase in the rate constant related to the degradation of xylose to furfural (k_3) was observed. From the data obtained from the rate constants, the kinetic parameters were calculated according to the Arrhenius equation (Table 6).

Activation energy is a crucial parameter in the assessment of kinetic studies because it influences the kinetic behavior of the reaction step. The results obtained for the HP of EFR showed that the formation of xylose may require a subsequent hydrolysis step, as the activation energy for the degradation of XOS into monomers had a value of 236.62 kJ/mol. This value is higher than that obtained for the conversion of xylan into XOS and the formation of furfural from xylose. Because xylan is easily hydrolyzed at high temperatures and excessive temperatures lead to higher dehydration activation energy, that is, the dehydration of XOS to furfural is easily accelerated, excessive temperature usually results in the formation of large by-products (xylose and furfural). The activation energy results showed that low temperatures slowed up the hydrolysis of xylan, whereas higher temperatures significantly accelerated the degradation of xylan to XOS. This phenomenon was also observed in previous studies [31, 32]. Therefore, 175 °C was considered to be the optimal temperature for XOS formation.

The results indicate good agreement between the proposed model and experimental data. The R^2 values ranged from 0.979 to 0.995 for the kinetic parameters, suggesting that the model is reliable and can be applied to the optimization and scale-up processes for XOS production. Scale-up responses should be considered in pilot and escalation approaches. Biomass conversion and XOS production reactions should be evaluated before scale-up (laboratory scale) to determine if there is a risk of uncontrolled events, as discussed in this paper. For instance, hazardous reactions (at high temperatures) can raise the scale by up to 3× on each

scale; therefore, it is important to carefully monitor the reaction rates each time.

On the other hand, the results are consistent with previous studies reported in the literature, confirming the influence of temperature on the conversion of xylan into various intermediate products, such as oligosaccharides, monosaccharides, and degradation products. The values found in step 1 (k_1) for E_a and $\ln A$ were close to the values obtained for the autohydrolysis of xylan from agave bagasse, with values of 75.02 kJ/mol and 16.88, respectively [49]. Dos Santos Rocha et al. [31] reported values of 220.23 kJ/mol for E_a and 53.66 for $\ln A$ during stage 2 (k_2) in the HP of sugarcane straw. In contrast, Borrega et al. [30], and Santucci et al. [32] reported higher values for kinetic parameters, such as the activation energy and preexponential factor, for the conversion of xylan into XOS during the pretreatment of different types of biomass.

The results obtained in this study highlight the potential use of EFR for XOS production through HP, which presents economic and environmental benefits [10]. Furthermore, the subsequent enzymatic hydrolysis of the hydrolysates can increase the recovery of smaller-sized XOS, which are highly valued in the industry owing to their diverse range of applications.

Conclusion

Hydrothermal pretreatment was the most effective method for producing XOS from eucalyptus branches and bark. The behavior of these raw materials during pretreatment can be influenced by their inherent properties, which affect xylan degradation and dissolution reactions. This study focuses on the potential of EFR to obtain high-value bioproducts, highlighting the complexity of converting xylan into XOS. This research presents unique challenges in achieving maximum efficiency in producing XOS from underutilized residues. Therefore, this study contributes to the understanding of the complexity of xylan-to-XOS conversion and underscores the need for further research to optimize these processes for lignocellulosic biomass valorization.

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Author Contribution Jenniffer Andrea Tamayo-Peña: conceptualization, experimental results, development of experimental methodologies, investigation, formal analysis, data curation, writing—original draft, writing—reviewing and editing, and visualization; Laura Plazas Tovar: methodology (kinetics models), formal analysis, validation, writing—original draft, and writing—reviewing and editing; Lívia Caldas Alencar Pacheco: validation and writing—reviewing and editing; Adilson Roberto Gonçalves: conceptualization, validation, writing—reviewing and editing, and co-advisor; Telma Texeira Franco: conceptualization, validation, resources, writing—reviewing and editing, advisor, project administration, and funding acquisition.

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Data Availability All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics Approval Neither humans nor animals were used to perform the study reported by this manuscript.

Conflict of Interest The authors declare no competing interests.

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