

Article

Study of Purified Cellulosic Pulp and Lignin Produced by Wheat Straw Biorefinery

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Abstract: With the world population rising, wheat straw production is expected to reach 687–740 million tons per year by 2050. Its frequent application as a fuel source leads to air, water, and soil pollution. Limited literature exists on methods for separating components of residual wheat straw. Optimal conditions for organosolv pulping of hydrolyzed wheat straw include 3% FeCl₃·6H₂O as a catalyst, a biomass-to-solvent ratio of 1:15 (*m/v*), and 50% ethanol:water as cooking liquor at 200 °C for 30 min. Desilication conditions involve extraction with 7.5% Na₂CO₃ at a biomass-to-solvent ratio of 1:20 (*m/v*) treated at 115 °C for 60 min. Lignin from hydrolyzed wheat straw showed similar properties to organosolv lignin from untreated straw, with minimal lignin alteration during hydrolysis. Hydrolysis significantly degraded cellulose. A 41% lignin recovery rate with 95% purity was achieved from pre-extracted hydrolyzed straw. Recovered cellulose after silica removal had 2% ash and 87% purity. The innovation of this process lies in the development of a comprehensive, sustainable, efficient, and economically viable biorefinery process that efficiently separates key components of wheat straw, i.e., xylose, lignin, cellulose, and silica, while addressing environmental pollution associated with its traditional use as fuel.

Keywords: wheat straw; lignin; cellulose; silica; hydrolysis; organosolv pulping



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1. Introduction

According to projections, the world's population is anticipated to increase by 23% to 9.71 billion by 2050 [1]. This growth will require a substantial increase in food production, estimated to be between 30% and 62% [2]. The resulting demand will create substantial pressure on resources, with agriculture being especially impacted, necessitating the implementation of innovative strategies.

Wheat, the world's most extensively cultivated cereal with an annual production of 529 million tons, is the second-most consumed food globally [3,4]. Rising food demand, especially in rapidly growing populations across Asia and Africa, will impact wheat production [1]. However, only the edible portion of the wheat plant is used for consumption, leaving behind significant residues, such as straw, husks, leaves, and stubble. These byproducts are commonly repurposed for applications such as animal bedding, feed, and as reinforcement, as well as being lightweight, and insulating materials in the construction industry [5].

But not all uses of these residues have a low environmental impact. The vast majority (66%) is left on the ground or burnt to regenerate soils. Decomposition produces methane, a greenhouse gas 72 times more harmful than CO₂, while burning releases large amounts of air pollutants, including particulate matter (PM₁₀), CO, and NO₂ [6,7]. These practices are banned in wheat-growing countries, as they are also a health hazard, causing lung cancer as well as cardiovascular and respiratory diseases [8].

Therefore, it is crucial to explore methods for adding value to these abundant residues. Wheat straw yields on average 1.3–1.4 kg of residues per kilogram of wheat grain [9], resulting in an estimated annual production of 687 to 740 million tons of wheat straw that could be sustainably used.

Petroleum remains widely used, despite being a limited resource with highly polluting extraction and use. In recent years, however, the development of biotechnologies has allowed for partial replacement of petroleum-based products with biobased alternatives. Due to their high oxygen content, lignocellulosic resources can be processed in a biorefinery to substitute petroleum products [10]. In this context, agricultural waste emerges as an ideal feedstock for biorefineries due to its high annual production, renewability, availability, low cost, and current underuse [6]. These lignocellulosic residues have already been used to produce different products such as biofuels, including bioethanol, paper, and bioplastics [8]. These innovations represent significant progress toward more sustainable and environmentally friendly alternatives. Nonetheless, additional advancements can be made by sequentially extracting individual components through a biorefinery approach, enabling the valorization of each biopolymer.

Considerable research efforts have been dedicated to the thorough examination of the chemical makeup of wheat straw, leading to the identification of noteworthy amounts of biopolymers, including xylan, cellulose, and lignin, along with minerals (ash) and organic extractives [6,9,11,12]. Previous investigations have mainly focused on the extraction and characterization of specific constituents found within lignocellulosic biomass, with an emphasis on silica, cellulose, and lignin [13–15], to foster the development of pioneering applications within the domains of biomaterials, biofuels, and bioactive compounds [6,15,16].

The present investigation used a sequential biorefinery approach to extract extractives, xylan/xylose, lignin, cellulose, and silica. This methodological choice facilitates the production of biobased materials with a diverse array of potential applications within the industry of food, packaging, construction, cosmetics, and pharmaceuticals [17,18].

This study is a component of a broader project starting with the extraction of extractives using a combination of organic solvent (ethanol) and water, and then an optimized hydrolysis process to enhance xylose yield, which can subsequently be converted into xylitol for further valorization [19–21].

Among the biopolymer extraction processes, the organosolv process, previously disregarded due to high solvent costs, is experiencing renewed interest [22]. Innovations in solvent recycling methods have the potential to make this extraction method industrially viable [23,24]. The use of iron chloride (III) as a catalyst has received high attention, given its abundance, low cost, and well-understood chemistry. Iron chloride has been suggested as a catalyst for its ability to degrade hemicellulose and lignin while preserving cellulose. Additionally, iron chloride is less corrosive compared to traditionally used mineral acids [25–27]. Ethanol stands out as an ideal solvent among the organosolv processes due to its low toxicity and potential for bio-sourcing [25–27]. Previous studies on catalytic organosolv pulping with FeCl_3 and ethanol:water were conclusive [20,21,28]. Due to its nature, the iron chloride catalyst is not consumed during pulping and is therefore recoverable, while sodium carbonate is recyclable through a simple treatment of sodium silicate with CO_2 . Thus, the reagents are recyclable and the process requires minimal reagent input, which enhances its economic viability. The economic feasibility of sequentially extracting all the components can be improved by the value-added potential of extracted compounds, thereby making the biorefinery concept based on the organosolv process viable.

This study aimed to isolate biopolymers from wheat straw that have undergone hydrolysis for xylose extraction and compare them with biopolymers isolated from non-hydrolyzed wheat straw. The aim was to explore various valorization possibilities based on the physicochemical characteristics of the products generated. While previous studies on wheat straw biorefinery have predominantly focused on extracting components for use as biopolymers or biofuels [13–15], this research emphasizes a comparison between pre-hydrolyzed and untreated wheat straw, with a particular emphasis on conversion via

the organosolv process into organosolv lignin and cellulosic pulp. The latter is further processed under mild desilication conditions to recover silica and purify cellulose. In this study, the physicochemical properties of organosolv lignin and cellulosic pulp derived from hydrolyzed wheat straw are compared to those obtained from untreated wheat straw, thereby underscoring the potential for full conversion of wheat straw into valuable products. We showed in our previous research that the proportion of cellulose is higher in wheat straw compared to rice husks [6,9,20]. Therefore, this study mainly focused on cellulose because there would be fewer problems with silica and delignification would be easier, as the silica and lignin contents of wheat straw are lower than those of rice husks [6,9,20].

2. Materials and Methods

2.1. Raw Materials

Wheat straw (WS) was purchased as animal bedding from HealthyStraw^{MD} (Blumenort, MB, Canada). To facilitate handling, remove contamination, and to obtain homogeneous size, the straw was washed, dried, ground by a laboratory mill (ULTRA CENTRIFUGAL MILL ZM 100, RETSCH GmbH & Co., Newtown, MA, USA), and sieved with an automatic mechanical sieve shaker (model 3990, C.E. Tyler Rotap Sieve, Painesville, OH, USA). Only the fraction within the 0.25 mm and 0.85 mm size range (60 to 20 mesh) was selected for further analysis. The sieved wheat straw (WS) was subjected to thorough rinsing with water and filtered using a vacuum until a clear filtrate was produced, ensuring the removal of any remaining starch and flour. The ground wheat straw fibers were then air-dried for 72 h at ambient temperature and stored in polyethylene bags. The moisture content was measured once the fibers were stabilized.

2.2. Wheat Straw Composition Determination

The biomass composition was analyzed during every stage of the biorefinery process. Extractives were measured using a two-step procedure based on ASTM D1107-96 [29], involving Soxhlet extraction using anhydrous toluene and ethanol (0.427:1, *v/v*), followed by hot water extraction to remove both hydrophilic and hydrophobic extractives.

The cellulose was quantified by converting lignin in water-soluble nitrophenolic compounds while hydrolyzing hemicelluloses and recovering solid cellulose using a nitric acid:ethanol solution (1:4, *v/v*) as per the Kürschner and Hoffner method. The lignin content was quantified following the method described in NREL/TP-510-42618 [30], which involves determining both Klason lignin and acid-soluble lignin after complete hydrolysis of polysaccharides with sulfuric acid. The ash content was measured based on ASTM D1102-84 [31] by calcination of the biomass in a muffle furnace at 600 °C for 6 h. The hemicelluloses were quantified as pentosans using the furfural index determination method, as outlined in CPPA-G-12 [32].

2.3. Pre Extraction Process

Extractives were removed using a pre-extraction step by refluxing the wheat straw (WS) with an ethanol:water mixture (1:1, *v/v*) in a 4 L glass reflux reactor at a 1:20 (*m/v*, 50 g of straw for 1 L of solvent) ratio for 6 h at 80 °C. The extractive-rich liquid phase was separated from the extractive-free wheat straw (WE) through vacuum filtration. The WE was subsequently air-dried at room temperature for 72 h and then stored in polyethylene bags. After stabilization, the moisture content was measured (Figure 1).

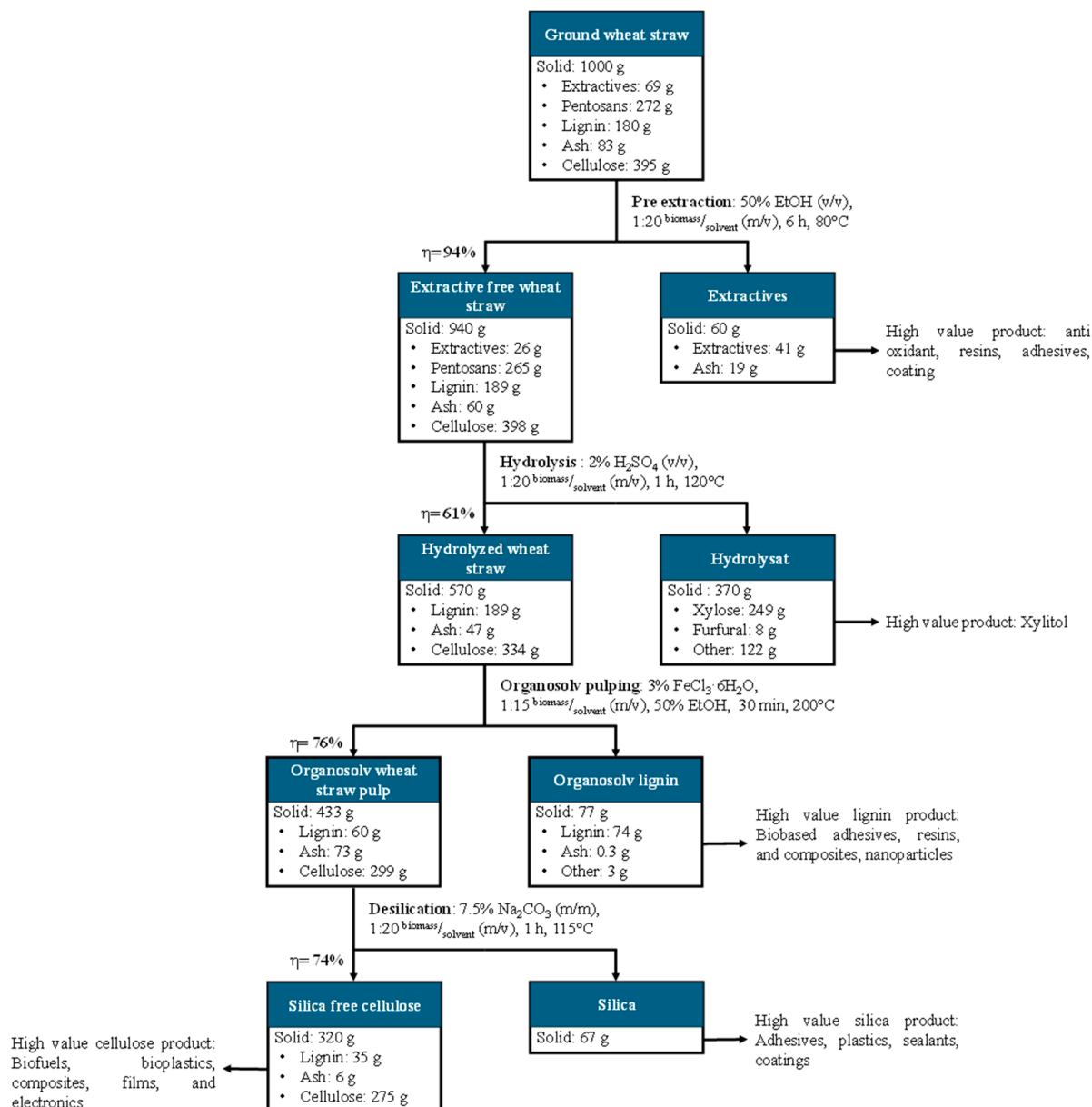


Figure 1. Mass balance of the process.

2.4. Dilute Acid Hydrolysis

The hydrolysis of WE was carried out according to the method described by Kasangana et al. [19]. WE was mixed with a 2% sulfuric acid aqueous solution (v/v) in a 2 L Parr reactor (series 4522, Parr, Moline, IL, USA) at a 1:20 (m/v, 50 g of WE for 1 L of solvent) ratio, before heating under pressure for 1 h at 120 °C. Then, the extractive-free hydrolyzed wheat straw (WEH) was separated via vacuum filtration and thoroughly washed with distilled water until neutral wash water was obtained. The WEH was then air-dried at room temperature for 72 h and stored in polyethylene bags. Once stabilized, the humidity was measured (Figure 1).

2.5. Isolation of Organosolv Lignin

Organosolv pulping was performed on pre-extracted and hydrolyzed wheat straw (WEH) and extracted wheat straw (WE) by applying a process developed in our laboratory [21]. Either WEH or WE was put into a 2 L Parr reactor (series 4522, Parr, Moline, IL, USA) with an ethanol:water mixture and iron chloride (FeCl₃·6H₂O) as the catalyst.

The reference pulping parameters used were taken from the studies by Durand et al. [20] and y Koumba Yoya et al. [21] on the pulping of rice husks and aspen wood. We have systematically explored the pulping parameters to maintain a 50:50 ethanol:water ratio, a catalyst concentration of 5% (of dry biomass), a pulping time of 90 min, and a temperature of 180 °C as pulping conditions for organosolv lignin isolation from WE for further studies of their purity and structure, as described below. The biomass:solvent ratio comes from the straw hydrolysis study of Kasangana et al. [19], in which a ratio of 1:20 (*m/v*) was used. The effect of these conditions on the lignin yield were determined by testing different ethanol:water mixtures (95:5, 70:30, 50:50, and 25:75), catalyst concentrations (0, 1, 3, 5, and 7% of dry biomass), pulping times (30, 60, 90, and 120 min), biomass:solvent ratios (1:5, 1:10, 1:15, and 1:20; *m/v*), and temperatures (160, 180, 200, and 220 °C). The effects of a selected parameter on the process were observed by varying the target parameter while keeping the other parameters fixed. The pulping process was monitored until the maximum temperature was attained, at which point the reaction was halted by immersing the reactor in an ice bath for 30 min. Following this, the contents of the reactor were subjected to vacuum filtration using Whatman number 1 filter paper. The resulting extractive-free hydrolyzed organosolv wheat straw pulp was then washed with 200 mL of ethanol. Consistently with the procedure described by Fellissia et al. [33], the spent liquor was diluted with water at a 1:4 ratio, and a small quantity of calcium chloride (CaCl₂) was introduced to promote lignin precipitation. The lignin was subsequently separated from the spent liquor via vacuum filtration on Whatman number 1 filter paper and was washed until the wash water reached a neutral pH. The organosolv lignin was then allowed to air-dry for 72 h at room temperature (Figure 1).

To further recover all the remaining lignin, a Soxhlet extraction with 250 mL of ethanol was performed for 6 h on the organosolv pulp. Following the procedure outlined by Parot et al. [28], after being washed with ethanol, the pulp was then allowed to air-dry for 72 h at room temperature. The ethanol solution, rich in lignin, was diluted with water at a 1:5 ratio, and a small amount of calcium chloride was added to promote lignin precipitation. The organosolv lignin was then separated by vacuum filtration using Whatman number 1 filter paper, with repeated washing until the water solution reached neutral pH. The collected organosolv lignin was subsequently dried at room temperature for 48 h. The resulting organosolv lignin samples were combined into a single sample for further analysis. The lignin recovery rates were calculated as reported previously [21]:

$$\text{Lignin recovery} = \frac{\text{Isolated lignin weight}}{\text{Biomass weight} \times \text{Klason lignin}} \quad (1)$$

2.6. Extraction of Silica from Wheat Straw Cellulosic Pulp

The silica extraction from wheat straw organosolv cellulosic pulp was performed following the procedure described by Pekarovic et al. [34]. WEH organosolv pulp or WE organosolv pulp was placed in a 500 mL reflux reactor in an oil bath with a sodium carbonate solution (Na₂CO₃). The reference desilication parameters used were taken from a study by Durand et al. [20] on silica extraction of rice husk. These are a sodium carbonate concentration of 10% (*m:m* of dry mass), a biomass solvent ratio of 1:25 (*m/v*), a time of 240 min, and a temperature of 115 °C. The influence of various conditions on the ash yield was assessed by experimenting with different sodium carbonate concentrations (1%, 2.5%, 5%, 7.5%, and 10% *w/w* of dry mass), biomass:solvent ratios (1:5, 1:10, 1:15, 1:20, and 1:25 *m/v*), extraction durations (30, 60, 120, 180, and 240 min), and temperatures (60 to 145 °C). The extraction time was measured from the moment the desired temperature was reached. The effects of a chosen condition on the process were observed by varying the target parameter while keeping the other parameters fixed. While the reactor content was still hot, it was filtered using Whatman number 4 filter paper and rinsed with hot water until the filtrate was clear. Silica-free, extractive-free organosolv cellulose from hydrolyzed wheat straw and non-hydrolyzed wheat straw were then air-dried at room temperature for

72 h and stored in polyethylene bags. After reaching a stable state, the moisture content was measured (Figure 1).

The process of producing silica from sodium silicate involved gradually acidifying the filtrate with sulfuric acid until neutral pH was attained.

2.7. Bleaching of Cellulosic Pulp

Samples of bleached cellulosic pulp were prepared by consecutive delignification with sodium chlorite (NaClO_2) at 70 °C, using acetic acid/acetate as a buffer system with an approximate pH of 5, as described by Jayme and Wise [35].

2.8. Physicochemical and Spectroscopic Analysis of Lignin, Cellulose, and Silica

2.8.1. High-Performance Liquid Chromatography Analysis of Carbohydrates, Furfural, and 5-Hydroxymethyl Furfural

Carbohydrates, furfural, and 5-hydroxymethyl furfural (5-HMF) content of the residual liquor and filtrate from Klason lignin determination in WEH organosolv pulp and WE organosolv pulp were analyzed using high-performance liquid chromatography (HPLC) after neutralization using calcium carbonate (CaCO_3) and filtration at 0.45 μm .

According to the NREL [30] procedure, the carbohydrate content of neutralized samples was quantified using an Agilent 1200 series HPLC system (Agilent Technologies, Santa Clara, CA, USA) fitted with a Rezex RHM-Monosaccharide H^+ (8%) (300 mm $\times$ 7.8 mm) column and a refractive index detector (HPLC-RID). Nanopure water was used as a mobile phase under isocratic conditions with a flow rate of 0.5 mL/min and a temperature of 75 °C. The calibration curves were obtained using different standards for cellobiose, glucose, mannose, galactose, xylose, fructose, rhamnose, and arabinose (Sigma-Aldrich).

Furfural and 5-hydroxymethyl furfural (5-HMF) content of neutralized samples were quantified using an Agilent 1100 series HPLC system (Agilent Technologies, Santa Clara, CA, USA) fitted with a Zorbax SB-C18 (250 mm $\times$ 4.6 mm) column and a diode array detector (HPLC-DAD). Nanopure water and acetonitrile (95:5) were used as a mobile phase under gradient condition with a flow rate of 0.7 mL/min and a temperature of 30 °C. The calibration curves were obtained using standards for furfural and 5-HMF (Sigma-Aldrich). The monosaccharide content is presented as a percentage of total sugar in the hydrolysate.

2.8.2. Nuclear Magnetic Resonance Characterization of the Wheat Straw Organosolv Lignin and Cellulose

The quantification of free hydroxyl groups in lignin was performed using phosphorus nuclear magnetic resonance (^{31}P NMR), as described by Meng et al. [36]. This analysis was conducted on a Varian NMR 500 MHz spectrometer with 256 scans.

Organosolv lignin structural units and linkages were investigated by dissolving 90 mg of lignin in 0.7 mL of dimethylsulfoxide- d_6 . The lignin solution was then examined by heteronuclear single quantum coherence (HSQC) analysis using a Varian NMR spectrometer at 500 MHz.

The allomorph phases and crystallinity of the bleached cellulosic pulp obtained after silica extraction were analyzed using ^{13}C nuclear magnetic resonance (^{13}C NMR). This analysis was conducted with a Varian NMR spectrometer operating at 100 MHz and a 10 kHz MAS rate. The acquisition involved a 1 ms contact pulse with a 4 s delay between repetitions, using 4 mm tubes at ambient temperature. The total acquisition time was 4.5 h, during which 4096 scans were collected to accumulate the signal. The resulting signals were mathematically enhanced and subjected to Lorentzian/Gaussian deconvolution. The crystallinity of the bleached cellulosic samples was then calculated following the method described by Atalla et al. [37].

2.8.3. Fourier-Transform Infrared Spectroscopy

Lignin, silica, bleached cellulose, and unbleached cellulose underwent Fourier-transform infrared spectroscopy (FTIR) between 4000 cm^{-1} to 400 cm^{-1} using a Perkin Elmer Spectrum 400 with 64 scans.

Spectra of both bleached and unbleached cellulose samples were obtained and converted to absorbance. These spectra were then normalized to 1.0 at 1136 cm^{-1} , which corresponds to the C-OH stretching vibration. The ratio of the I α to I β allomorph phases was determined using the approach outlined by Imai et al. [38]:

$$f_{\alpha}^{IR} = \frac{A_{750}}{A_{750} + kA_{710}} \quad (2)$$

where A_x represents the integrated absorbance at the specified wavenumber and k is a constant with a value of 0.16.

2.8.4. Gel Permeation Chromatography of Organosolv Lignin Samples

The polymer properties of wheat straw organosolv lignin were evaluated using gel permeation chromatography. For this analysis, 20 mg of lignin were solubilized in 2 mL of tetrahydrofuran (THF) and then filtered through a $0.45\text{ }\mu\text{m}$ filter. Then, the samples were eluted using the Agilent 1200 series HPLC system (Agilent Technologies, Santa Clara, CA, USA) fitted with a PL gel $5\text{ }\mu\text{m}$ mixed-D ($300 \times 7.5\text{ mm}$) column and a diode array detector (GPC-DAD). Tetrahydrofuran (THF) was used as a mobile phase under isocratic conditions with a flow rate of 0.5 mL/min and a temperature of $50\text{ }^{\circ}\text{C}$. The calibration curve was obtained using polystyrene standards (580–28,770 Da).

2.8.5. X-ray Diffractometry

The bleached silica-free WEH organosolv cellulosic pulp from wheat straw, silica-free WE organosolv pulp, and reference cellulose samples (Avicel and FiloCell[®], Montréal, QC, Canada) were formed into tablets after being vacuum-dried at $45\text{ }^{\circ}\text{C}$ for 72 h. X-ray diffraction (XRD) analysis was conducted with a Malvern PANalytical AERIS powder X-ray diffractometer, equipped with a $\text{CuK}\alpha$ anode, operating at 40 kV and 8 mA. The instrument covered a 2θ range from 5° to 40° . The obtained diffractograms were examined using High Score (Plus) V3.0 software to assess the allomorphic phases, determine crystallite size via the Scherrer Equation (3) [39], and calculate crystallinity according to the Segal Equation (4) [40] as:

$$D = \frac{k\lambda}{\beta_{hkl} \times \cos\theta_{hkl}} \quad (3)$$

where k represents a correction factor (0.9), λ is the X-ray wavelength ($1.54060\text{ }\text{\AA}$), β_{hkl} is the full width at half maximum of the diffraction peak in radians, and θ_{hkl} is the diffraction angle of the peak.

$$CrI = \frac{(I_{200} - I_{am})}{I_{200}} \quad (4)$$

Here, I_{200} represents the maximum intensity of the (200) peak at 2θ within the range of 22° to 23° and I_{am} denotes the intensity attributed to the amorphous fraction at 2θ of 18° .

2.8.6. Viscometry Measurements of Polymer Properties of Bleached Cellulosic Pulp

Intrinsic viscosity measurements were used to determine the average degree of polymerization (DP) and the weight-average molar mass (M_w) for various samples, including bleached, silica-free WEH organosolv pulp, silica-free WE organosolv pulp, and reference cellulose materials such as Avicel, FiloCell[®], and cotton linter. These properties were determined by measuring the flow time of a cellulose solution in 0.5 M cupriethylene diamine using a CANNON Fenske 100 viscometer. Cellulose concentrations giving $DP \times C$ of 100, 120 and 140 corresponding to flow times of 135, 170 and 180 s were used. The mass-average molar mass was then estimated by multiplying the degree of polymerization (DP) by the molar mass of a single anhydroglucose unit, which is 162 g/mol .

2.8.7. X-ray Fluorescence (XRF) Analysis of Wheat Straw Ashes

The ash content of wheat straw and lignin samples was determined by combustion according to ASTM D1102-84 [31]. The ashes were then compressed into a holder for X-ray fluorescence (XRF) analyses performed on a Rigaku Primus II V2 apparatus, with a rhodium anode end-window X-ray tube at a voltage of 50 kV and current of 50 mA for 20 min. The data were acquired and analyzed using ZSX 7.66 software. Standardless independent parameters and a semiquantitative method were used.

2.9. Statistical Analysis

All statistical analyses were conducted using R software version 4.4.1 [41]. The analysis focused on two main parameters: lignin recovery rate and ash content of cellulosic pulp. For each parameter, an analysis of variance (ANOVA) was performed to assess the differences among the various experimental conditions using a linear model. The assumptions of ANOVA, including normality and homogeneity of variances, were verified. Due to violations of these assumptions, a logarithmic transformation was applied to the data, which subsequently met the ANOVA assumptions. Post hoc comparisons were carried out using the least significant difference (LSD) test at a 5% significance level to identify differences between groups. The findings are represented using bar plots, with error bars showing the standard error of the mean and letters indicating statistically distinct groups.

3. Results and Discussion

3.1. Evaluation of Biomass Composition throughout Different Stages of the Biorefinery Process

Figure 2 illustrates the composition of wheat straw at different stages of the biorefinery process. Analyzing the wheat straw structure after each step is critical to determine the potential of the biomass as a feedstock and the efficiency of the biorefinery process in terms of recovery and yield. Therefore, it is essential to quantify the extractives, hemicellulose, lignin, cellulose, and ash content of wheat straw at each step. Here, WS stands for neat wheat straw, WE for pre-extracted wheat straw, and WEH for pre-extracted and pre-hydrolyzed wheat straw composition (Figure 2).

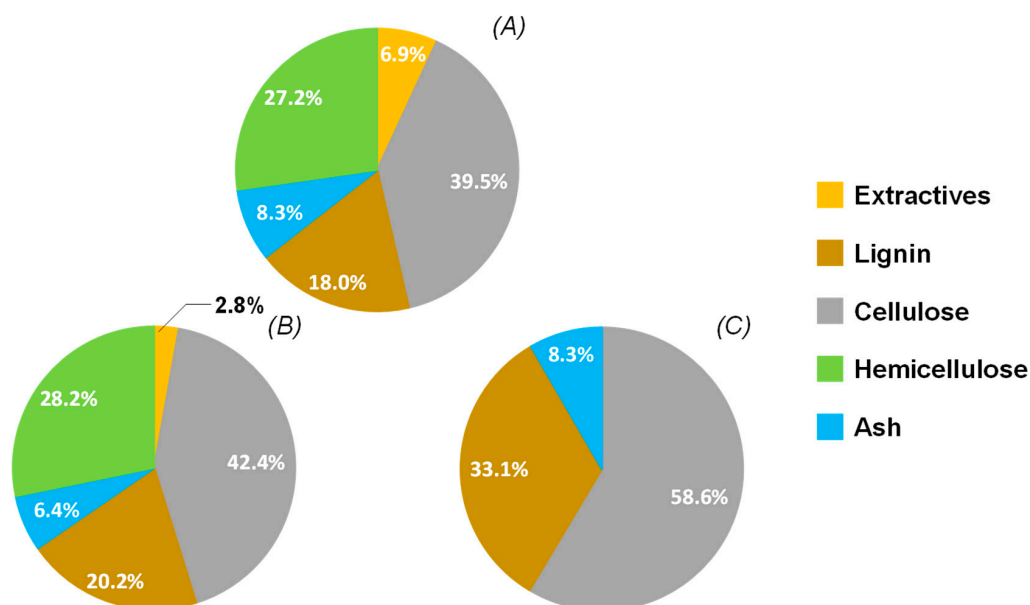


Figure 2. Average composition of: (A) WS, (B) WE, and (C) WEH (% of dry mass).

3.1.1. Composition of the Original Raw Wheat Straw

The proportions of biomass constituents determined in WS were in agreement with published data on wheat straw. The original (neat) WS studied here was determined to consist of $6.9 \pm 0.3\%$ extractives, $39.5 \pm 0.7\%$ cellulose, $27 \pm 1\%$ hemicelluloses, $18 \pm 1\%$ lignin,

and $8.3 \pm 0.2\%$ ash. The wheat straw used in previous studies was reported to consist of 3–19% extractives, 32–47% cellulose, 19–35% hemicelluloses, 5–24% lignin, and 4.8–12.8% ash [6,9,11,42]. These proportions of biopolymers are similar to those reported for straw varieties from the Gramineae family, such as rice straw (30–38% cellulose, 7–13% lignin, and 19–32% hemicellulose), barley straw (31–34% cellulose, 14–15% lignin, and 24–29% hemicellulose), oats (31–37% cellulose, 16–19% lignin, and 27–38% hemicellulose) and rye (33–35% cellulose, 16–19% lignin, and 27–30% hemicellulose), and are attributed to its function as support—rigid, but flexible enough to sustain its own weight—while resisting external forces [43]. The significant amount of silica in wheat straw determined in our study is consistent with results reported in the literature [42,44]. Stem is built of vascular tissues providing for water, nutrients and sugars transport throughout the plant and in the case of Gramineae for the transport of silicic acid, which is then incorporated as silica during the plant growth [45,46]. Despite presenting difficulties for its application as a fuel or in alkali-based processes, these issues are not relevant for the acid-based method employed in this study [47]. Various techniques have been investigated for extracting and purifying silica from wheat straw to harness its distinctive properties [42]. Silica-derived materials, recognized for their outstanding mechanical strength, thermal stability, biocompatibility, and adsorption capabilities, have been reported for use in diverse sectors, such as agriculture, construction, electronics, and pharmaceuticals [48–50]. The use of amorphous and fine silica particles with a large surface area has been explored in applications such as adhesives, plastics, sealants, coatings, inks, toner, cosmetics, food additives, and defoamers [51]. Notably, the extraction of silica from wheat straw yielded significant advancements in the development of environmentally friendly alternatives to traditional sources of silica, thereby reducing reliance on environmentally harmful extraction methods such as silica sand quarries and open-pit mines [52,53]. While wheat straw has been extensively used in material-based lignocellulosics and minerals, there is potential for improved use of its separated constituents. As wheat straw is a representative member of Gramineae, study of it provides data for improved exploitation of other members of this family.

3.1.2. Pre-Extraction Step with Ethanol:Water (1:1, *v/v*) of Wheat Straw

The pre-extraction step was determined to reduce both the extractives and ash content in WS. Extractive-free wheat straw (WE) was determined to contain $2.8 \pm 0.4\%$ extractives, $6.4 \pm 0.7\%$ ash, $28.2 \pm 0.2\%$ hemicellulose, $42.4 \pm 0.6\%$ cellulose, and $20.2 \pm 0.3\%$ lignin. The ethanol:water pre-extraction reduced the extractives (determined by standard protocol) from $6.9 \pm 0.3\%$ to $2.8 \pm 0.4\%$. The reduction from $8.3 \pm 0.2\%$ to $6.4 \pm 0.7\%$ in ash content indicates the presence of water-soluble minerals in wheat straw [54]. Pre-extraction, as highlighted by Koumba-Yoya et al. [21], is essential to achieve successful organosolv pulping, as it improves both the hydrolysis and pulping efficiency by preventing parasitic reactions between the catalyst and extractives.

3.1.3. Wheat Straw Hydrolysis

After pre-hydrolysis, no hemicellulose-related sugars were detectable by the CPPA-G-12 protocol in WEH, thus confirming their effective removal by pre-hydrolysis. Nevertheless, it is important to note that the complete extraction of hemicelluloses is impossible. WEH was determined to contain $8.3 \pm 0.6\%$ ash, $58.6 \pm 0.3\%$ cellulose, and $33.1 \pm 0.7\%$ lignin, while the hemicellulose (pentosan content) was impossible to determine as not enough furfural was released to be detected, raising questions on the suitability of this method to measure small amounts of hemicelluloses in biomass. This result can actually be taken as a fair confirmation of the successful removal of xylan during pre-hydrolysis. This outcome can be considered a clear indication of the effective removal of xylan during pre-hydrolysis. In line with the findings of Durand et al. [20], a decrease in acid-soluble lignin content in wheat straw is observed after hydrolysis, from $0.75 \pm 0.03\%$ to $0.56 \pm 0.05\%$. The acidic conditions of hydrolysis not only partially extract lignin from the biomass but may also induce condensation reactions, which are detrimental to pulping and create challenges

for subsequent processing [55,56]. Therefore, it is a real challenge for organosolv pulping to generate both organosolv lignin and pulp from pre-hydrolyzed wheat straw. When compared to other straws such as rice straw (30–38% cellulose and 7–13% lignin), barley straw (31–34% cellulose and 14–15% lignin), oats (31–37% cellulose and 16–19% lignin) and rye (33–35% cellulose and 16–19% lignin), WEH is highly concentrated in lignin and cellulose. The abundance of cellulose in wheat straw is attributed to its role in providing structural support to the plant. The renewable and biodegradable nature of cellulose has received widespread recognition and several studies focused on the extraction of cellulose from wheat straw to produce nanocellulose fiber, cellulose nanocrystals, and cellulose hydrogel [15,57,58]. The high tensile strength of cellulose fibers makes them interesting for composite material applications. Furthermore, cellulose can be chemically, microbially, or enzymatically converted into glucose. This glucose serves as an appropriate substrate for microbial fermentation or chemical processes, leading to the production of biofuels, alcohols, and monomers such as 5-HMF, lactic acid, and gluconic acid [59]. The versatility of cellulose as a sustainable resource has spurred research efforts aimed at fully harnessing its potential in the production of biofuels, bioplastics, composite materials, films, and electronic components [59,60]. The high lignin concentration, which is the most abundant aromatic polymer on Earth, in wheat straw can be attributed to its role in facilitating nutrient and mineral exchange between the roots, leaves, and spike via specialized hydrophobic structures. Historically regarded as a byproduct of the paper industry and used primarily as a low-cost fuel, lignin has recently attracted attention as a renewable resource with potential applications in adhesives, carbon-based materials, and composites [61–63]. Lignin's complex structure, which includes functional groups like methoxyl, carbonyl, carboxyl, and hydroxyl, along with three aromatic phenylpropane units (p-hydroxyphenyl, syringyl and guaiacyl), positions it as a significant source of aromatic compounds with the potential to replace petroleum-based products. [64,65]. The extracted lignin can be subsequently transformed into biobased composites, resins and adhesives supporting the development of sustainable materials with a reduced environmental footprint. [61–63]. Thus, organosolv pulping offers considerable potential for generating both organosolv pulp and lignin.

3.2. Adapting the Organosolv Process to Wheat Straw for High Lignin Recovery

This study aimed to modify a pulping process, previously tailored for hardwood, to suit agro-waste. It also aimed to compare the results with those previously obtained for rice husk by Durand et al. [20], who showed that the proportion of cellulose is higher in wheat straw compared to rice husks. Consequently, the pulping conditions previously defined in our laboratory [20], i.e., the biomass:solvent ratio, the ethanol:water ratio, temperature, time, and amount of catalyst, were investigated. The effect of a chosen parameter on the process was examined by varying the target parameter while holding the other parameters constant.

The process modification was performed on hydrolyzed wheat straw (WEH). The lignin recovery and the pulp yield were taken as determining the efficiency of the process. Figure 3 shows the lignin recovery rate under different pulping conditions.

The composition of the pulp was determined by measuring its residual lignin and ash content, following the standardized quantification methods as described previously. After the pre-hydrolysis process, the biomass consists of lignin, cellulose, and silica, as most of the hemicelluloses were removed by hydrolysis. As described by Durand et al. [20], the Klason lignin extracted from the cellulose pulp was also analyzed for ash content. The best lignin recovery achieved under the conditions studied did not go above 50%.

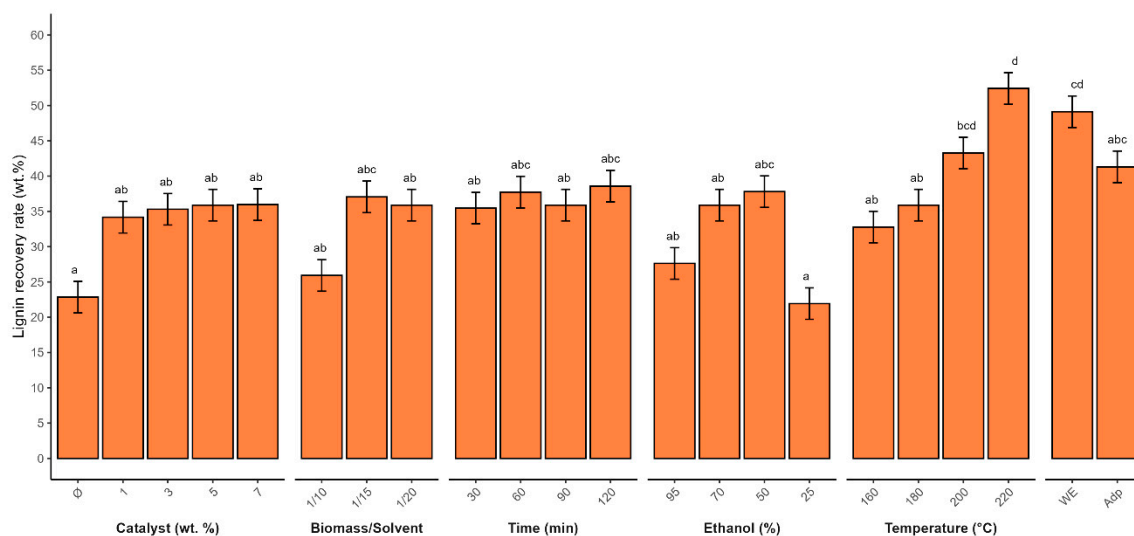


Figure 3. Lignin and recovery rate (% of content in original samples) for all the pulping conditions used on WEH: organosolv lignin recovery rate from extractive-free wheat straw and adapted parameters (Adp) indicating organosolv lignin recovery rate from a process performed with the best conditions. Bars with the same letter are not significantly different, as determined by LSD tests at 5% significance.

3.2.1. Effect of the Catalyst Concentration

To evaluate how the concentration of catalyst affected the lignin recovery, the quantity of catalyst was varied from 0 to 7%. Increasing the catalytic load led to an increase in lignin from $22 \pm 8\%$ to $36 \pm 1\%$, reaching a plateau at 3% with an average recovery rate of $35.3 \pm 0.3\%$. The pulping of black spruce showed a comparable pattern at 5%, as did the delignification of aspen wood at 1.5% and the delignification of rice husk at 5%, all using a similar organosolv process [20,21,28]. Ruiz et al. [66] reported a pH of 4 as optimal for the pulping of wheat straw. However, this study did not consider the effect of temperature on hydrolysis. As severity increases (pressure and temperature increase) in an acidic medium, lignin condensation reactions compete with the cleavage of α -ether and β -ether bonds, leading to simultaneous solvolysis and acidolysis of lignin. While low acidity levels promote lignin solvolysis and acidolysis, a balance between these reactions is obtained with increasing acid concentration [55,56]. As temperature and acidity increase, cellulose hydrolysis is promoted resulting in lower pulp yield with higher cellobiose and glucose concentration in the residual liquor. As previously observed with rice husk [20], a significant lignin recovery rate ($22 \pm 7\%$) was achieved even in the absence of a catalyst (0%). Therefore, this study used a catalyst concentration of 3% (Figure 3).

3.2.2. Effect of Wheat Straw:Solvent Ratio on the Organosolv Lignin Recovery from Pre-Hydrolyzed Wheat Straw

To evaluate how the wheat straw:solvent ratio affected the rate of recovery, this parameter was changed from 1:5 to 1:20. Due to its composition and high porosity, wheat straw absorbs solvent up to four times its weight [67]. Below a 1:5 ratio, the solvent volume is insufficient to enable the reaction. The other ratios (1:10 to 1:20) led to minor differences in lignin and cellulose recovery, with values fluctuating between $25 \pm 10\%$ and $37 \pm 2\%$ for lignin and cellulose recovery, respectively. Previous studies on organosolv pulping of wheat straw reported a preference for biomass:solvent ratios between 1:10 and 1:20, with a notable consensus about 1:10 [58,66,68,69]. As reported in Durand et al. [20], who studied the organosolv delignification of rice hulls, this parameter had a minimal effect on the rate of recovery. However, while the duration of the washing step following delignification was reduced with an increased biomass ratio for rice hulls, wheat straw did not follow a similar pattern. Considering solvent cost, low return, and the considerable time required

for processing large volumes of solvent, using a fixed biomass:solvent of 1:15 was selected (Figure 3).

3.2.3. Effect of Pulping Time on the Organosolv Lignin Recovery

To determine the effect of pulping time on recovery, the duration was varied between 30 and 120 min. Increasing the time from 30 to 120 min resulted in a modest rise in lignin rate of recovery, from $35 \pm 1\%$ to $39 \pm 1\%$, combined with a slight reduction in pulp yield. Wildshut et al. [58] observed a similar trend by extending the pulping time from 60 to 120 min, leading to a slight increase in lignin rate of recovery from 39% to 49% and a small decrease in pulp yield from 65% to 62%. The delignification of rice hull showed a comparable trend, as an extension from 30 to 120 min increased the lignin rate of recovery from $35.5 \pm 0.5\%$ to $42 \pm 2\%$ along with a decrease in pulp recovery rate from $83.0 \pm 0.1\%$ to $73 \pm 2\%$. While increasing the duration from 30 to 120 min led to a 3% increase in lignin recovery for rice husk, the organosolv lignin yield from wheat straw increased by only 0.8%. Given the factors of cellulose degradation, low yield, energy costs, and time consumption, extending the pulping time was not efficient. As a result, the pulping time was reduced from 90 min to 30 min (Figure 3).

3.2.4. Effect of Pulping Temperature on the Wheat Straw Organosolv Lignin Recovery

To evaluate how the pulping temperature affects biopolymer recovery, the maximal temperature of organosolv pulping was changed from 160 to 220 °C. This parameter significantly affected the yields of lignin and cellulose. Raising the temperature to 220 °C led to a substantial increase in lignin yield from $33 \pm 1\%$ to $52 \pm 2\%$ combined with a significant decrease in pulp yield from $71 \pm 1\%$ to $23 \pm 4\%$. This trend does not diverge from the outcomes of Durand et al. [20] study on rice husks [68]. An earlier study on organosolv pulping of wheat straw used temperatures between 85 °C and 210 °C with a preference for 180 °C and reaching lignin recovery rate of 84% and pulp yield of 48% [58,66,68,69]. Wildshut et al. [58] varied temperatures from 160 to 210 °C with a similar result. It is known that increasing the temperature induces a decrease in pH, which could be favorable for higher lignin recovery, but with a trade-off of lower pulp yield. The median temperature of 200 °C was selected to achieve a balance between an increased lignin recovery rate and a reduced pulp yield, as the ultimate goal was to improve the delignification to obtain higher-purity cellulose (Figure 3).

3.2.5. Effect of Ethanol:Water Ratio on the Organosolv Lignin Recovery from Pre-Hydrolyzed Wheat Straw

To evaluate how the ethanol:water ratio affects the lignin rate of recovery, the EtOH concentration was changed from 25% to 95%. Regarding its impact on the procedure, this parameter is the second most significant. A considerable rise in lignin recovery rate from $22 \pm 2\%$ to $37.8 \pm 0.3\%$ was observed when the ethanol content was raised to 50%. Exceeding this limit resulted in a decrease in lignin recovery to $27 \pm 2\%$. Similar trends were observed in previous studies [20,58,66,68,69]. This trend has been previously attributed to the Hildebrand parameter of both lignin and the solvent, which provides insight into the extent of interaction among these materials. However Wildshut et al. [58] related an increase in ethanol content to an increase in pH preventing acidolysis reaction and reducing lignin solubility. Consequently, it can be concluded that the identified pattern results from both lignin affinity for the solvent and delignification reaction. The 50:50 ethanol:water ratio led to the best result, and it was selected for further studies (Figure 3).

3.2.6. Effect of Pre-Hydrolysis on the Organosolv Lignin Recovery Rate from Wheat Straw

To evaluate how pre-hydrolysis affected the lignin recovery rate, pulping was performed on the WE sample in comparison with the WEH sample with reference parameters (90 min, 180 °C, and 5% FeCl₃). The lignin recovery increased from $35.3 \pm 0.2\%$ to $49 \pm 2\%$. A comparable pattern was noted in the pulping of rice husk to a higher extent, with an

almost twofold increase in lignin recovery without a pre-hydrolysis step. Earlier studies addressed this observation, noting structural lignin alterations at temperatures above 180 °C, including cleavage of the ether bonds in β -O-4 and resinol substructures, shifts in the ratio of three monolignols, and demethoxylation reaction. Acid pre-treatment contributes to the cleavage of β -O-4 and the production of reactive intermediates with free phenolic groups and carbonyl groups. Demethoxylation further increases the number of reactive phenolic intermediates. The free phenolic groups and carbonyl groups contribute to condensation reactions, forming new C-C bonds [70]. Organosolv pulping using sulfuric acid as a catalyst is also known to cause lignin condensation. The negative effect of acid hydrolysis on lignin recovery stems from lignin condensation reactions, resulting in reduced recovery rates. This hypothesis is corroborated by a significant rise in recovery rates as temperature increases, which facilitates the cleavage of more resistant bonds. The pulp yield followed a similar trend, increasing from $71 \pm 1\%$ to $83.58 \pm 0.02\%$. This can be explained by a preferential hemicellulose hydrolysis reaction competing with cellulose hydrolysis during pulping. Hemicelluloses are branched polysaccharides exhibiting no tendency to formation of ordered crystalline structure, and are therefore more susceptible to acid hydrolysis reactions [25]. Additionally, FeCl_3 , used in the present organosolv pulping procedure as a catalyst, was shown to increase the enzyme's access to cellulose by selectively targeting hemicelluloses for removal [26,71]. In the absence of hemicellulose, the hydrolysis reactions between both polysaccharides no longer compete, leading to deterioration of cellulose. The extraction of hemicellulose during wheat straw hydrolysis therefore leads to a reduction in cellulose yield during the subsequent pulping stage.

As in our previous study [20], hydrolysis had a negative effect on both lignin recovery and pulp yield.

In summary, the optimal conditions for delignification WEH to maximize the rate of recovery organosolv lignin are: 3% of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, a biomass solvent ratio of 1:15, an ethanol:water ratio of 50:50, 30 min of pulping time, and a maximum temperature of 200 °C (Figure 3).

When performed on WEH, the best organosolv process parameters provide a lignin recovery rate of $41 \pm 2\%$ and a pulp recovery of $76 \pm 1\%$. The organosolv process provided increased lignin recovery and pulp yield when carried out on WE, suggesting that the hydrolysis step leads to decreasing results. Although the hydrolysis step facilitates the extraction of xylose from the biomass, it negatively impacts the pulping process by reducing both the lignin recovery rate and cellulose yield compared to those obtained from non-hydrolyzed biomass. However, it is important to note that the recovery rate and yield resulting from organosolv pulping on both WEH and WE are still below those reported in previous studies [58,66,68,69].

The cellulosic pulp produced under the optimal organosolv conditions applied to WEH consists of 14% lignin, 17% ash, and 69% cellulose. The sample underwent a step for silica extraction developed by Durand et al. [20] to produce ash-free cellulose from this pulp.

3.3. Wheat Straw Organosolv Lignin Characterization

Table 1 displays the characteristics of the lignins extracted through the organosolv process from WE and WEH.

The purity of the lignins was assessed using Klason lignin determination, along with acid-soluble lignin. In addition, the filtrate from Klason lignin determination, containing HMF, monosaccharides, and furfural, categorized as "other" in Table 1, was conducted using HPLC. The ashes were examined using X-ray fluorescence (XRF) spectroscopy. The organosolv lignins were determined to be of high purity ($95.9 \pm 0.1\%$), and low ash content (WEH $0.39 \pm 0.02\%$ vs. WE $0.50 \pm 0.03\%$), making these lignins suitable for structural analysis and high-value applications. It is not surprising that most minerals remained associated with cellulose and not with organic solvent soluble lignin, in which they were determined to be lower than 1%. XRF analysis of the ashes from the cellulose samples revealed a silica content of 67.6%, a common issue in studies involving grass samples [72].

The acidic pulping conditions used did not allow for the solubilization of silica, which explains the high ash content in cellulosic pulp samples. However, previous studies showed that silica in monocotyledons is closely associated with lignin, hemicellulose, and cellulose [46], thereby posing challenges in their segregation. It is important to observe that only a minimal amount of iron (26.3%) was identified in the ashes of the WEH lignin sample, while a significant amount was found in the ashes of the WE lignin sample (72.6%). This finding aligns with Durand et al. [20]. The residual content of organosolv lignin from WEH was 3.8%, while from WE it was 5.3%, and this consisted of residual sugars and related substances. Given the comparable ash and sugar levels in both samples, it can be inferred that hydrolysis does not significantly affect the purity of the isolated organosolv lignin. High-performance liquid chromatography (HPLC) analysis of the hydrolysates, derived from Klason lignin determination, indicates that the residual sugars primarily include 5-hydroxymethylfurfural (5-HMF) and furfural, which are acid-catalyzed byproducts of hexose and pentose conversions. Consistently with previous studies, the reduction in hemicellulose content in WEH lignin led to a lower furfural concentration of $45 \pm 2\%$ and a higher 5-HMF concentration of $37 \pm 2\%$ compared to those determined in the hydrolysates obtained from Klason lignin determination in organosolv lignin isolated from non-hydrolyzed wheat straw with a higher furfural concentration ($71 \pm 3\%$) and a lower 5-HMF concentration ($20 \pm 1\%$). These findings further validate the specificity of the pre-hydrolysis process, which initially targets hemicellulose (xylan) prior to degrading cellulose. The pre-hydrolysis seems to make the cellulosic residue remaining associated with organosolv lignin prone to acid transformation to 5-HMF.

Table 1. Characteristics of the organosolv lignins isolated from WE and WEH.

Characterization		Organosolv Lignin from Extractive-Free Hydrolyzed Wheat Straw (WEH)	Organosolv Lignin from Extractive-Free Wheat Straw (WE)
Klason lignin (insoluble)		$95.9 \pm 0.1\%$	$94.4 \pm 0.6\%$
Acid-soluble lignin		$0.266 \pm 0.004\%$	$0.260 \pm 0.001\%$
Hydrolysate composition		3.8%	5.3%
	Furfural	$45 \pm 2\%$	$71 \pm 3\%$
	5-HMF	$37 \pm 2\%$	$20 \pm 1\%$
	Other	$17 \pm 2\%$	$9 \pm 2\%$
Ash content		$0.39 \pm 0.02\%$	$0.53 \pm 0.03\%$
Ash composition			
	Fe ₂ O ₃	26.3%	72.6%
	SiO ₂	67.6%	14.7%
	Other (CaO, P ₂ O ₅ , SO ₃ , K ₂ O, ...)	6.1%	12.7%
Lignin polymer properties			
Molecular weight (Mn)		470 ± 5 Da	538 ± 12 Da
Molecular weight (Mw)		1033 ± 43 Da	1297 ± 139 Da
Free hydroxyl groups in organosolv lignins (³¹ P NMR)	S	0.70 mmol/g	0.73 mmol/g
	G	1.13 mmol/g	1.17 mmol/g
	H	0.44 mmol/g	0.44 mmol/g
	Aliphatic	0.70 mmol/g	0.64 mmol/g
	Carboxylic	0.173 mmol/g	0.170 mmol/g
	S/G	0.62	0.62
Lignin aromatic units (HSQC NMR)	S	23.8%	25.5%
	G	53.1%	51.8%
	H	23.1%	22.8%
	S/G	44.8%	49.2%
Lignin interunit linkages (HSQC NMR)	β-O-4'	33.8%	50.5%
	β-5'	17.6%	10.4%
	β-β	20.0%	8.3%

3.3.1. Fourier-Transform Infrared Spectroscopy of Lignin Samples

In this work, the band assignments in the lignin FTIR spectra were derived from earlier studies on organosolv wheat straw lignin [68]. The FTIR spectra of the lignin samples, as illustrated in Figure S1 in the Supplementary Material, reveal characteristic bands that are typically observed in Gramineae species. Notably, the characteristic lignin bands of SGH lignin are present in spectra for both the WE and WEH lignin samples, specifically at 1264 cm^{-1} , 1213 cm^{-1} , and 1121 cm^{-1} , respectively. Additionally, the stretching bands of O-H and C=O at 3400 cm^{-1} and 1703 cm^{-1} , respectively, indicate the presence of aliphatic hydroxyl and carbonyl groups, likely originating from residual carbohydrates.

3.3.2. ^{31}P Nuclear Magnetic Resonance Analysis of Organosolv Lignins

The ^{31}P NMR spectra of the lignins were carefully examined and quantitatively analyzed using similar methods to previous studies [36] to gain more accurate information about the free hydroxyl groups present (Figure 4). Both lignins are classified as SGH lignin types and display phenolic hydroxyl signal associated with p-hydroxyphenyl, syringyl, and guaiacyl groups. Quantitative assessment indicated a significant guaiacyl free hydroxyl concentration in WEH and WE lignins, as well as a similar S/G ratio of 0.62. Despite undergoing different treatments during the extraction process, the unit distribution within both WEH and WE samples exhibited a consistent pattern. However, a variation in the overall content of free hydroxyl groups was evident, with the WE lignin displaying a comparable concentration to the WEH lignin produced under standard conditions (3.12 mmol/g and 3.10 mmol/g, respectively). Conversely, the WEH lignin showed a slightly higher concentration of free hydroxyl groups at 3.80 mmol/g, indicating its capability for enhanced chemical reactivity.

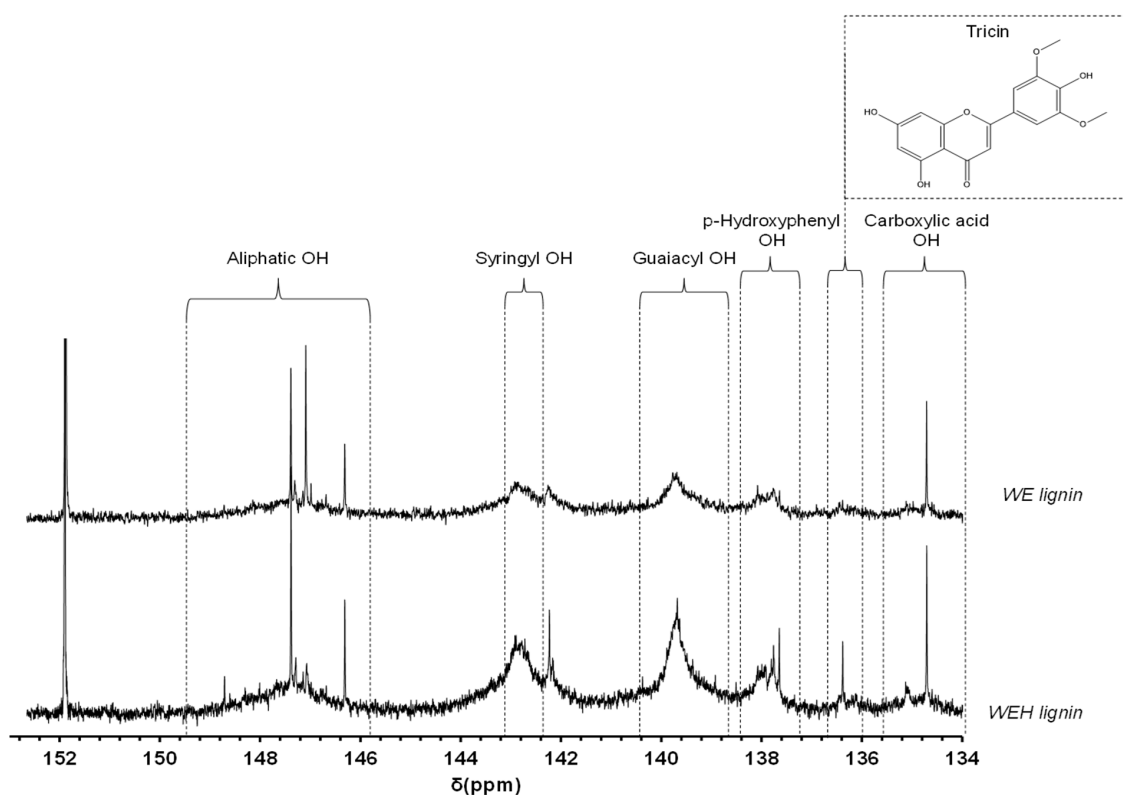


Figure 4. Quantitative ^{31}P NMR spectra of WEH and WE lignin: aliphatic hydroxyl (149.4–145.8 ppm), syringyl hydroxyl (143.2–142.4 ppm), guaiacyl hydroxyl (140.4–138.7 ppm), p-hydroxyphenyl hydroxyl (138.5–137.3 ppm), tricin hydroxyl (136.7–136.0 ppm), and carboxylic acid hydroxyl (136.6–134.0 ppm).

Increasing the temperature results in two significant outcomes in terms of lignin recovery. Firstly, it promotes the disruption of bonds, thereby improving the overall recovery process. Secondly, it facilitates the liberation of a higher number of free hydroxyl groups. This is evidenced by the similar hydroxyl content observed in both lignin samples isolated following the process with standard parameters at 200 °C (3.10 mmol/g and 3.91 mmol/g). Notably, the levels of aliphatic OH (0.70 mmol/g and 0.64 mmol/g) and carboxylic acid OH (0.173 mmol/g and 0.170 mmol/g) are also similar in both lignin samples.

Tricin, a flavonoid commonly reported in Gramineae lignins [73,74], was detected in both lignin samples (0.08 mmol/g in WEH and 0.12 mmol/g in WE). The fact that triclin remained intact in the WEH lignin sample even after undergoing acid hydrolysis provides evidence of its substantial incorporation in lignin. This suggests that triclin behaves as a lignin monomer, participating in its biosynthesis, and is therefore integrated into its structures [75]. Recent findings showed that non-conventional monomers from the phenylpropanoid biosynthetic pathway (including monolignol ester conjugates, monolignol acetates, and monolignol p-hydroxybenzoates), as well as compounds originating from outside the traditional monolignol biosynthetic pathway (such as flavonoids, hydroxystilbenes, and hydroxycinnamates), can act as genuine lignin monomers. These compounds undergo radical coupling reactions with monolignols and lignin oligomers to become incorporated into the lignin polymer [76]. Wheat straw lignin belongs therefore to a separate class of “non-canonic” or “atypical” lignins built from moieties other than only three standard monolignols, as it includes triclin in its structure.

3.3.3. Heteronuclear Single Quantum Coherence Spectra of Wheat Straw Lignin Samples

The HSQC analysis of both lignin samples provides detailed information on the distribution of lignin subunits, the types of interunit linkages, the presence of cinnamic acid derivatives, and the levels of impurities. Some of the impurities are found at the anomeric carbon region of the polysaccharide region ($\delta\text{C}/\delta\text{H}$ 110–60 ppm/6.0–3.0 ppm), and interunit linkages (β -O-4, β -5, β - β') are found in the oxygenated aliphatic area ($\delta\text{C}/\delta\text{H}$ 90–50 ppm/6.0–2.0 ppm), along with impurities. In the unsaturated aromatic region ($\delta\text{C}/\delta\text{H}$ 160–90 ppm/8.0–6.0 ppm), lignin subunits (guaiacyl, syringyl and p-hydroxyphenyl), as well as flavonoid and cinnamic acid derivatives (triclin, p-coumarate and ferulate), are observed. Correlations were made according to previous studies on wheat straw lignins [13,77,78], and are detailed in Table S1, and Figures S2 and S3 in the supporting data file.

Correlation peaks from β -O-4 interunit linkage (A_γ , $\delta\text{C}/\delta\text{H}$ 60.31/3.58 ppm), found in both lignin samples, are characteristic of native lignins. A signal belonging to the γ -acylated β -O-4 substructure was observed in spectra of both lignins (A , $\delta\text{C}/\delta\text{H}$ 60.12/4.05 ppm), structures commonly found in grass lignin [13,77,78]. Wheat straw lignin is acylated by both acetate and p-coumarate acid substructure at the γ -position of the lignin side chain, making it an acylated lignin [13,77,78]. The cross peak assigned to β - β' resinols interunit linkage (C_α $\delta\text{C}/\delta\text{H}$ 85.80/4.67 ppm, C_β $\delta\text{C}/\delta\text{H}$ 54.35/3.10 ppm, C_γ $\delta\text{C}/\delta\text{H}$ 71.54/4.22 ppm) is the most prominent in the WEH organosolv lignin sample and the correlation peak assigned to β -5' phenylcoumaran interunit bonds (B_β $\delta\text{C}/\delta\text{H}$ 52.30/3.76 ppm) is weak in WE organosolv lignin spectra. The semiquantitative analysis, achieved through volume integration of the signal contours, reveals that the predominant lignin substructure is β -O-4, (33.8% in lignin from WEH and 50.5% in lignin from WE) followed by varying amounts of condensed β -5' phenylcoumaran (17.6% in lignin from WEH and 10.4% in lignin from WE) and condensed β - β' resinol (20.0% in lignin from WEH and 8.3% in lignin from WE) substructure. β -O-4 presence as the predominant interunit bond suggests that the native lignin structures have been well preserved. Although β -O-4 bonds are the predominant linkages in both lignins, their proportion is lower in WEH compared to WE. This reduction is related to the susceptibility of β -O-4 bonds to acid hydrolysis. Both quantitative and qualitative information indicates the presence of additional condensed C-C bonds in WEH organosolv lignin and a condensed lignin, as previously theorized. The presence of β -D-xylopyranoside correlation peaks in both lignin is consistent with the trace amounts

of polysaccharides, as confirmed by the hydrolysis products found in filtrate of Klason determination and analyzed by HPLC. The area correlated with the methoxy group is prominent in spectra of both lignin samples, which is characteristic of lignins with a high S/G ratio, as syringyl units have two methoxy groups.

The unsaturated aromatic region is similar in both samples. Signals belonging to syringyl units ($S_{2,6}$ $\delta C/\delta H$ 104.16/6.68 ppm, $S'_{2,6}$ $\delta C/\delta H$ 107.48/7.24 ppm), guaiacyl units (G_2 $\delta C/\delta H$ 112.17/6.72 ppm, G_5 $\delta C/\delta H$ 115.68/6.73 ppm, G_6 $\delta C/\delta H$ 120.48/6.55 ppm), and *p*-hydroxyphenyl units ($H_{2,6}$ $\delta C/\delta H$ 128.48/7.08 ppm) are found, thus confirming previous structural analysis of wheat straw lignin and in agreement with previous studies on other grass lignins [13,77,78]. The semiquantitative evaluation, performed through volume integration of the signal contours, suggests that the primary lignin units are guaiacyl (53.1% in lignin from WEH and 51.8% in lignin from WE) followed by syringyl (23.8% in lignin from WEH and 25.5% in lignin from WE), and a slightly lower amount of *p*-hydroxyphenyl (23.1% in lignin from WEH and 22.8% in lignin from WE) units. Although different from those calculated by ^{31}P NMR, these proportions follow a similar trend and confirm the SGH nature of wheat straw lignin. Multiple cross peaks correlated with tricin (T_6 $\delta C/\delta H$ 99.37/6.24 ppm, T_8 $\delta C/\delta H$ 94.78/6.60 ppm, T_3 $\delta C/\delta H$ 104.16/7.01 ppm, $T_{2',6'}$ $\delta C/\delta H$ 104.75/7.35 ppm), indicating its richness in the analyzed lignin samples. This observation supports the previous theory, which confirms the substantial incorporation of tricin into the lignin structure. This additional analysis reinforces the idea that these compounds participate in the biosynthesis of lignin, undergoing radical coupling reactions with monolignols and lignin oligomers to become part of non-canonic lignin polymers [76]. The detection of *p*-coumarate signals ($pCA_{3,5}$ $\delta C/\delta H$ 119.69/6.71 ppm, pCA_{β} $\delta C/\delta H$ 120.08/6.97 ppm, $pCA_{2,6}$ $\delta C/\delta H$ 130.82/7.56 ppm, pCA_{α} $\delta C/\delta H$ 145.18/7.56 ppm) in this study corroborates previous findings regarding the presence of cinnamic acid derivatives in lignin and ferulate subunits (FA_{β} $\delta C/\delta H$ 120.08/6.97 ppm, FA_6 $\delta C/\delta H$ 120.47/7.26 ppm, FA_2 $\delta C/\delta H$ 145.18/7.56 ppm, FA_{α} $\delta C/\delta H$ 145.18/7.56 ppm), peaks that are less prominent in the WEH lignin samples confirmed by the semiquantitative analysis (16.7% and 18.9% in lignin from WEH vs. 18.5% and 32.0% in lignin from WE). This phenomenon can be attributed to the hydrolytic cleavage of ester bonds associated with ferulic acid residues. These are proposed to serve as bridges between lignin and polysaccharides [79,80]. The results obtained from ^{31}P and HSQC NMR, are complementary and they complete the structural information about the studied lignin samples.

3.3.4. Polymer Properties of Wheat Straw Organosolv Lignins

The analysis of gel permeation (GPC) chromatograms provides the data for molecular weight characteristics of the hydrolyzed wheat straw organosolv lignin samples. Lower average molecular weight (470 g/mol) and average molecular weight (1033 g/mol) were determined for the organosolv lignin isolated from the WEH compared to WE sample (538 g/mol and 1297 g/mol). The polydispersity of the WEH and WE samples are determined to be 2.3 and 2.4, respectively. These reduced values of M_n and M_w of lignin from WEH compared to lignin from WE suggest a degradation (depolymerization) of lignin during pre-hydrolysis.

To investigate the cause of these lower values, gel permeation chromatography (GPC) analyses were performed on WEH lignin, which was processed according to standard parameters (5% $FeCl_3$, 180 °C and 90 min) reported in the literature for wheat straw organosolv pulping [21]. Factors such as the hydrolysis stage and elevated pulping temperatures were evaluated as potential contributors. The M_n and M_w values obtained for the WEH lignin sample produced with reference parameters were found to be higher compared to WE and WEH lignin samples (690 g/mol and 1908 g/mol). This indicates that the high pulping temperature had a negative effect on the polymer properties of lignin.

While the M_n and M_w values for WEH lignin samples generated under standard conditions were higher relative to those produced under optimized conditions, the polydispersity index was notably lower in the latter. This implies that lignin extracted under the

optimized organosolv parameters tends to consist of smaller, more uniformly distributed particles. These findings differ from the results presented in earlier literature reporting molar masses from 860 g/mol to 2800 g/mol for the number-average molecular weights and from 1647 g/mol to 5100 g/mol for the average molecular weights of wheat straw organosolv lignins, obtained by using H_2SO_4 , FeCl_2 , CuCl_2 , $\text{Ga}(\text{OTf})_3$, ZrOCl_2 , and $\text{Sc}(\text{OTf})_3$ as catalysts at temperatures ranging from 105 °C to 160 °C and times ranging from 0 to 210 min using acetic acid, formic acid, and ethanol as solvents [81–83]. Numerous investigations into the organosolv pulping of wheat straw have employed conditions similar to those utilized in the present study, including the use of an ethanol mixture, temperatures of 150–180 °C for durations of 60–120 min, and an acidic catalyst. Nonetheless, our research incorporated distinct pre-treatment techniques, such as pre-extraction and hydrolysis, as well as varying temperature ranges and catalysts. Although all utilized catalysts are categorized as acids, their mechanisms of action exhibit considerable differences. The specific catalytic processes involved in organosolv pulping remain incompletely elucidated, yet it is clear that these processes vary significantly between different methods [27,56].

Despite originating from the same biomass source but exposed to different steps of pre-treatments, the lignins obtained from WEH and WE exhibit similarities in terms of purity, interunit bonding, and SGH unit ratio. This structural similarity makes WEH lignin a viable alternative to wheat straw lignin obtained from original untreated wheat straw. Furthermore, its lower molecular weights (M_w and M_n) and increased reactivity make it an excellent precursor for the production of valuable and reactive lignin-based products [61–63].

3.4. Improvement of the Silica Extraction Process from Wheat Straw Cellulosic Pulp

Another objective of this study was to adapt the silica extraction method developed by Durand et al. [20] study on rice husk to a silica rich cellulosic pulp produced via WEH organosolv pulping. A parallel objective was established to optimize silica extraction while retaining cellulose integrity. Using a weak base proved to be both cost-effective and readily accessible, eliminating the need for specialized equipment. This method not only reduces equipment wear but also lowers the environmental footprint by optimizing the waste management procedure. Contrary to the study by Durand et al. [20], the extraction of silica using Na_2CO_3 has already been conducted [34,84,85]. However, it was performed on raw wheat straw as a pre-treatment, but not applied to wheat straw cellulosic pulp. The extraction of silica from cellulosic pulp issued from organosolv pulping is where the novelty of the process lies here.

Previous studies on wheat straw desilication, despite being scarce, still provide interesting data for comparison with our results [34]. Even if silica solubilization kinetics are known to have a heterogeneous reaction of order 0.5, it is complex to apply to a substrate such as cellulosic pulp, since reactions of the base with cellulose containing residual lignin need to take into account specific surface area and cellulose crystallinity [86].

To reduce the ash content in the organosolv pulp extracted from WEH, the extraction parameters [20], i.e., time, base amount, biomass:solvent ratio, and temperature, were investigated. The effect of a chosen parameter on the process was examined by varying the target parameter while holding the other parameters constant.

The treated cellulosic pulp's ash contents are presented based on the oven dry-cellulose. The impact of varying parameters on the cellulosic pulp ash content is detailed in Figure 5.

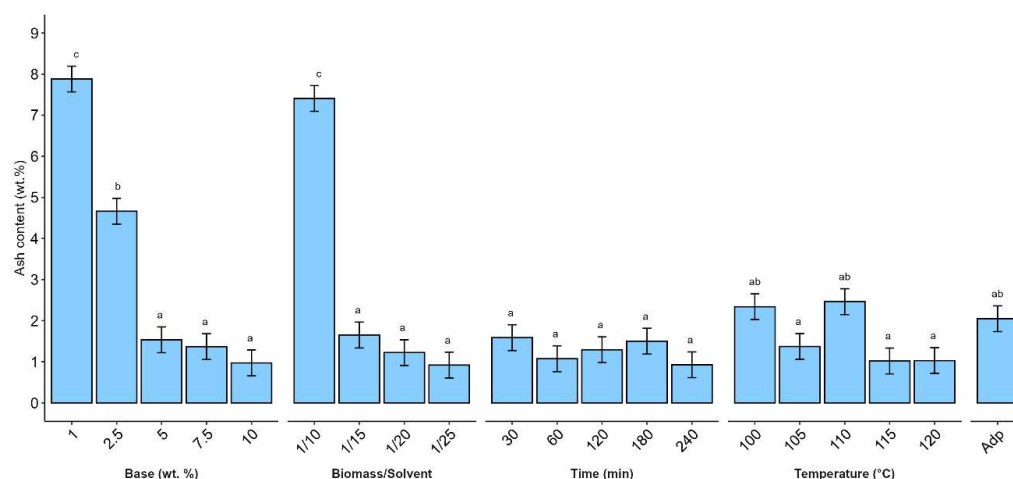
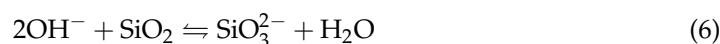


Figure 5. Ash content of the cellulosic pulp as a function of the silica extraction conditions adapted parameters (Adp) depicting ash content from best parameters. Bars with the same letter are not significantly different based on LSD tests at 5% significance level.

3.4.1. Effect of Na_2CO_3 Concentration on the Silica Removal Efficiency from Cellulosic Pulp Obtained from Hydrolyzed and Extractive-Free Wheat Straw (WEH)

To investigate Na_2CO_3 concentration effect on the desilication efficiency, the amount of Na_2CO_3 was varied between 1% and 10%. As the base amount increased, the silica content steadily decreased from $7.7 \pm 0.5\%$ to $1.7 \pm 0.3\%$, leveling off at 5% for $1.3 \pm 0.1\%$. Previous studies on desilication of wheat straw used Na_2CO_3 concentration ranging from 1% to 19% with a distinct preference for 10% [34,84,85]. This trend diverges from Durand et al.'s [20] study on rice husk, where no significant change in silica removal was observed for base concentrations between 1% and 5%. However it does follow the trends reported by Perarovic et al. [34], where low Na_2CO_3 concentration caused leaching of silica. It has been suggested that silica present on the surface of the stem is more accessible and therefore easier to remove. The results are different from those obtained with rice husk due to the morphological differences between husk and straw, directly affecting the availability of silica. In the WEH organosolv pulp, silica interacts with carbonate, leading to the formation of a silicate through the reactions outlined below:



Morphological availability of silica seems to have a stronger effect on silica solubility than pH, as observed by Durand et al. [20]. The sodium carbonate concentration of 7.5%, leading to a residual ash content in cellulosic pulp of $1.3 \pm 0.1\%$, was selected (Figure 5).

3.4.2. Effect of Wheat Straw Cellulosic Pulp:Solvent Ratio on the Silica Removal Efficiency

To investigate the wheat straw cellulosic pulp:solvent ratio effect on the silica removal efficiency, a range from 1:10 to 1:25 (*w/w*) was selected. With the lowest ratio value (1:10), the ash content remained almost unchanged at $7.4 \pm 0.4\%$. But a significant reduction in ash content was obtained above 1:10 to $1.7 \pm 0.3\%$, followed by a slight decrease at 1:25 for $1.0 \pm 0.1\%$. Previous studies on desilication of wheat straw used a ratio ranging from 1:4 to 1:20 [34,84,85]. This trend aligns with Durand et al. [20], where a substantial decrease in ash content was found at a 1:15 ratio, decreasing from $43.48 \pm 0.06\%$ to $2.395 \pm 0.004\%$. Since the wheat straw cellulosic pulp absorbs a significant amount of water, it is important to maintain a solvent ratio of at least 1:10 to ensure that the reaction is adequately supported, even if there are enough reagents present. Previous studies have reported similar trends, but different ratios, explained by this limitation [34,84,85]. After a significant reduction in ash content has been achieved, additional solvent does not result in further decreases in

ash levels. For further studies, a biomass:solvent ratio of 1:20 was maintained, yielding a final ash content of 2% in the cellulosic pulp.

3.4.3. Effect of Extraction Time on the Silica Removal from Wheat Straw Cellulosic Pulp

To assess the influence of extraction duration on ash content, the time was varied from 30 to 240 min. The extraction duration was established once the target temperature was achieved. It was found that time had a negligible impact on the recovery of ash, as a variation of time between 30 and 240 min led to ash content of $1.5 \pm 0.4\%$ and $1.0 \pm 0.1\%$, respectively. Previous studies on desilication of wheat straw were performed at times between 5 and 85 min. A similar trend was observed on rice husk by Durand et al. [20]. However, Perarovic et al. [34] described time as an important parameter and observed an increase in desilication with longer reaction time beyond 30 min, then reaching a plateau. The silica from WEH organosolv pulp is more readily available than from the untreated straw, and silica reaction kinetics, dependent on the specific surface area, make desilication less time-dependent. A shorter reaction time seems to minimally affect the ash removal, while a 240 min reaction time means significant energy consumption. As a result, an extraction duration of 60 min was chosen, leaving 1% ash in the cellulosic pulp.

3.4.4. Effect of Temperature on the Silica Removal from Wheat Straw Cellulosic Pulp Samples

To assess the impact of temperature on ash content, the temperature was changed from 100 to 145 °C. At 100 °C, a decrease in ash content to $1.8 \pm 0.7\%$ was determined, followed by a plateau at 110 °C and a decrease to $1.0 \pm 0.1\%$ at 115 °C. Similar trends and temperatures were reported in previous studies on both rice husk and wheat straw [34,84,85]. The silica solubilization process is endothermic, necessitating the provision of sufficient energy to the reaction mixture [87]. This observation accounts for the reduction in ash content at temperatures exceeding 115 °C. As no additional decrease in ash content is observed at higher temperatures, 115 °C was chosen as the optimal temperature.

Based on the results, optimal conditions for silica extraction from the pulp have been determined as follows: 7.5% Na_2CO_3 concentration, a biomass-to-solvent ratio of 1:20, and an extraction time of 60 min at 115 °C. Under these conditions, the resulting cellulosic pulp contains 11% lignin, 2% ash, and 86% cellulose.

3.5. Characterization of the Silica-Free Wheat Straw Cellulosic Pulp

The characteristics of the cellulose extracted following silica removal are detailed in Table 2.

Table 2. Characterization of the WEH organosolv pulp after silica removal and bleaching.

Characterization		Silica-Free Cellulosic Pulp from Extractive-Free Hydrolyzed Wheat Straw	Silica-Free Cellulosic Pulp from Extractive-Free Wheat Straw
Klason lignin (insoluble) Acid-soluble lignin Cellulose Ash		$11.2 \pm 0.4\%$	$3.2 \pm 0.2\%$
		$0.047 \pm 0.001\%$	$0.0589 \pm 0.0003\%$
		86.8%	94.4%
		$2.0 \pm 0.2\%$	$2.32 \pm 0.2\%$
Average composition	Na_2O	22.2%	4.6%
	CaO	27.0%	17.6%
	SiO_2	30.3%	48.8%
	Fe_2O_3	10.0%	24.4%
	Other (Fe_2O_3 , MgO , SO_3 , ...)	10.4%	4.6%

Table 2. Cont.

Characterization	Bleached silica-free cellulosic pulp from extractive-free hydrolyzed wheat straw	Bleached silica-free cellulosic pulp from extractive-free wheat straw
DP	89.09 ± 0.07	648 ± 13
Mw	14446 ± 12 Da	105141 ± 2051 Da
Crystallinity (^{13}C)	$45 \pm 5\%$	$30 \pm 3\%$
Crystallinity (XRD)	87%	81%
Crystallite size	32 ± 2 Å	29 ± 1 Å
I α (FTIR)	-	-
I β (FTIR)	100%	100%

3.5.1. Fourier-Transform Infrared Spectroscopy Performed on the Bleached and Unbleached Cellulosic Samples

Fourier-transform infrared (FTIR) spectroscopy is a commonly employed technique for analyzing cellulose polymorphs and assessing purity. The interpretation and quantification of the cellulose spectral bands were conducted in accordance with established methodologies from prior research [88]. The FTIR spectra reveal bands at 1161 cm^{-1} , 1076 cm^{-1} , 1035 cm^{-1} , and 897 cm^{-1} , which are indicative of C-O and C-O-C vibrations, signifying the presence of hydroxyl and ether functional groups within the cellulose samples. Additionally, the band at 1603 cm^{-1} , corresponding to aromatic backbone vibrations, and the peak at 1264 cm^{-1} , associated with guaiacyl unit deformation and bond elongation, provides evidence of residual lignin (Figure 6). These findings corroborate the results obtained from Klason lignin analysis, as detailed in Table 2 and Figure 5.

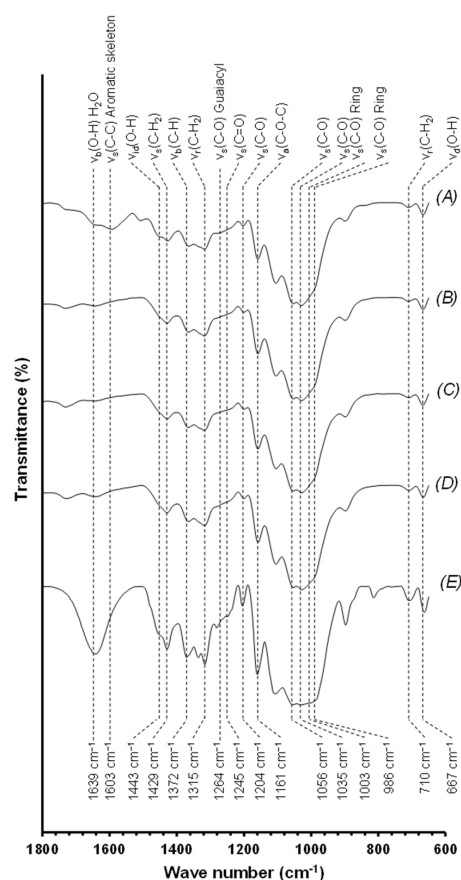


Figure 6. FTIR spectra of extractive-free, hydrolyzed organosolv wheat straw pulp and cellulosic pulp as a function of bleaching duration. The signal at 1603 cm^{-1} represents the stretching of C-C bonds within

the aromatic structure, indicating the presence of lignin. (A) Unbleached silica-free wheat straw pulp, (B) silica-free wheat straw pulp bleached for 1 h, (C) silica-free wheat straw pulp bleached for 2 h, (D) silica-free wheat straw pulp bleached for 3 h, (E) Avicel®. ν_b bending vibration, ν_s stretching vibration, ν_{id} in-plane deformation vibration, ν_r rocking vibration, ν_a asymmetrical vibration.

FTIR analysis of the bleached cellulose provides valuable insights into the distribution of allomorph phases I α and I β present within the cellulosic sample's crystalline structure. As in Durand et al. [20], the predominant presence of the I β (99%) allomorph in the crystalline phase aligns with existing studies on cellulose derived from wood and higher plants. The dominance of the I β form within the crystalline structure of cellulose can likely be ascribed to its tendency to shift towards the most stable allomorphic phase when exposed to elevated temperatures or acidic conditions [15,89].

3.5.2. ^{13}C Nuclear Magnetic Resonance Analyses of the Bleached Wheat STRAW Cellulose Samples

In addition to providing precise details on the ratio of different forms, ^{13}C NMR analysis also allows for the quantification of the amorphous, para-crystalline, crystalline phase, allomorphs (I α , I β), surfaces accessible and inaccessible fiber present in the bleached cellulose samples (Figure S4). The signal cluster ranging from 102 to 98 ppm corresponds to the C-1 carbon and facilitates a semiquantitative assessment of different forms in bleached cellulosic pulp. By deconvoluting both signal clusters between 91 ppm and 80 ppm, it is possible to evaluate the crystallinity of bleached cellulose, with one cluster representing the crystalline phase and the other representing the amorphous phase (Figure S4). A similar problem to that reported by Durand et al. [20] is again observed here on wheat straw. When attempting to deconvolute the signals using the MestRenova line fitting function, it was found that deconvolution into multiple components led to a significant increase in residual error, as well as a loss of reliability and repeatability. The MAS/ ^{13}C NMR spectra of cellulose from cell walls of green algae or acetobacter cellulose are characterized by their pronounced clarity due to the substantial size of the crystallites, while cellulose from higher plants, with smaller crystallites, shows broader and less informative bands. Thus, our focus was limited to the quantification of crystallinity. The carbon signals at 74 ppm are mainly attributed to the carbons present in the C-2, C-3, and C-5 positions of the anhydroglucose units, while the peak at 64 ppm corresponds to the carbon at the C-6 position. To determine the crystallinity of the bleached cellulosic pulp samples, the area of the peak within the range of 92–86 ppm, representing the crystalline region, was divided by the sum of the areas within the ranges of 86–80 ppm (amorphous region) and the crystalline peak. This analysis was conducted through Lorentzian and Gaussian peak fitting using the MestRenova software version 14.3.1. A crystallinity of $45 \pm 5\%$ was determined for silica-free WEH organosolv pulp, almost 15% higher compared to the measurements acquired using a comparable approach for silica-free cellulosic pulp from WE ($30 \pm 3\%$). The crystallinity of the cellulosic samples obtained here were determined to be higher compared to those determined for FiloCell (32%) and cotton cellulose (33%), but lower than that determined for microcrystalline cellulose Avicel (56%). This difference in crystallinity between the hydrolyzed and non-hydrolyzed samples confirms the earlier assumption that hydrolysis is mainly affecting the amorphous regions in cellulosic samples. Under acidic and elevated-temperature conditions, cellulose undergoes hydrolysis, with the initial cleavage of glycosidic bonds occurring primarily in the more accessible amorphous areas, leaving behind a higher proportion of crystalline phase. This results in a decrease in the ratio of amorphous phase in the cellulosic samples, mirrored by an increase in the ratio of crystalline domain.

3.5.3. X-ray Diffraction Analyses of the Bleached Wheat STRAW Cellulose Samples

By analyzing the XRD diffractogram (Figure 7) of the bleached cellulosic samples, important information regarding crystallinity, the ratio of amorphous cellulose to crystalline

and crystallite size can be obtained [38,90]. The Segal method (Equation (3)) was used to determine crystallinity of the bleached cellulose samples through XRD analysis. The Scherrer equation (Equation (2)) was used to determine the crystallite size, while the peak patterns were examined to evaluate the crystalline phase. In the X-ray diffraction pattern of bleached wheat straw cellulose, diffraction peaks were observed at 15.1° , 16.6° , and 22.3° , corresponding to the $(1\bar{1}0)$, (110) , and (200) planes, respectively, indicating the presence of native cellulose (I). In a diffractogram, cellulose II is characterized by distinct peaks at 12.0° , 20.0° , and 22.0° , whereas cellulose III displays peaks at 11.7° , 17.3° , and 21.0° , along with less prominent peaks around 14.5° and 16.5° [38,90] (Figure 7).

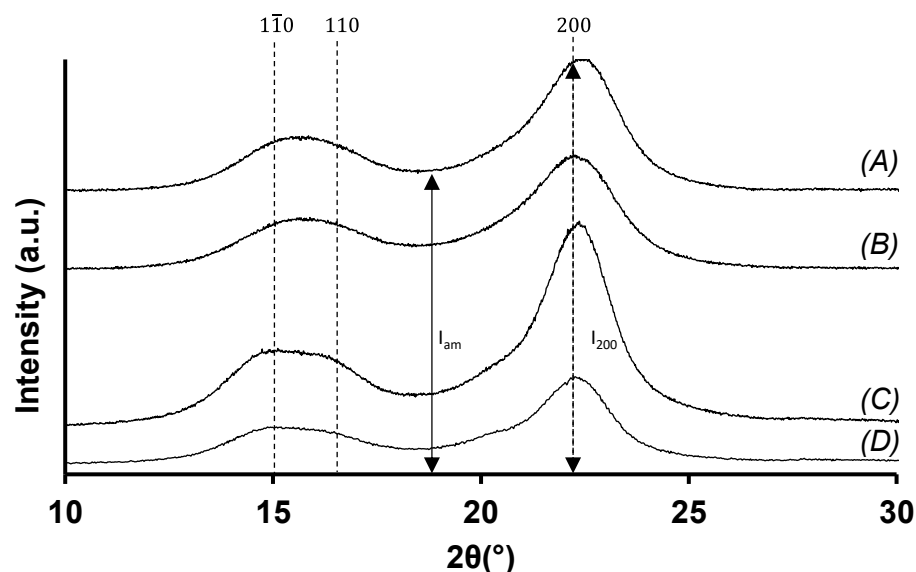


Figure 7. X-ray diffractogram of the bleached silica-free WEH cellulosic pulp (A), bleached silica-free WE cellulosic pulp (B), Avicel® (C), FiloCell® (D) and Miller indices associated with the diffraction peak.

As previously observed for rice husk cellulosic pulp [20], the extraction of the wheat straw cellulosic samples with Na_2CO_3 allowed for the extraction of silica while preserving polymorph cellulose I. However, it should be noted that it is difficult to totally prevent conversion of native cellulose into cellulose II under basic treatment conditions. While the diffraction peak of cellulose II allomorph remained undetectable on the diffractogram, it is likely that a small percentage of cellulose I had been converted into cellulose II. Similar crystallite size was determined for both studied cellulose samples using the Sherrer equation: 3.2 ± 0.1 nm for WEH cellulose sample and 2.9 ± 0.1 nm for WE sample. This confirms again that hydrolysis had limited effect on cellulose crystallinity. Earlier research has determined that the crystallite size of wheat straw cellulose varies from 3.5 nm to 5.0 nm depending on pre-treatment [91–93]. This indicates that the basic treatments did not adversely affect the integrity of the crystalline regions.

Employing the Segal method, the crystallinity index was determined to be 87% for the bleached and silica-free WEH cellulosic pulp and 81% for the WE wheat straw cellulosic pulp counterpart. While these results follow the trend previously seen for hydrolyzed vs. untreated wheat straw, they diverge from the trends observed for Avicel microcrystalline cellulose (80%). As reported in Durand et al. [20], the accuracy and reliability of crystallinity calculation based solely on the relationship between peak heights and degree of crystallinity have been questioned in previous studies [94,95]. This method does not take into account the impact of specific peaks and their respective widths, casting doubt on its validity. This discrepancy can also be attributed to the fundamental differences in the principles and sensitivities of the two techniques. XRD mainly measures the crystalline regions of cellulosic samples, providing information on the long-range order and the degree of crystallinity. It is highly sensitive to the periodicity and orientation of the cellulose crystals, which may

lead to an overestimation of crystallinity in samples with a high degree of alignment or a significant presence of crystalline regions. On the other hand, MAS/ ^{13}C NMR provides a more comprehensive view by probing both the crystalline and amorphous regions of cellulose. The technique measures the local molecular environment and can detect short-range order, making it more sensitive to the presence of amorphous content that XRD might overlook. Therefore, MAS/ ^{13}C NMR can sometimes show a lower degree of crystallinity compared to XRD, especially in samples with significant amorphous regions [96]. In conclusion, while the Segal method shows higher crystallinity for hydrolyzed wheat straw, contrasting trends are observed for microcrystalline cellulose samples, raising concerns about the method's accuracy suggest that these results should be interpreted with caution.

3.5.4. Viscometry Measurement Performed on the Bleached Cellulosic Samples

In this study, viscometry was used to investigate the polymer characteristics of bleached cellulosic samples. Intrinsic viscosity measurements indicated that the degree of polymerization and as a result the molecular weight (Mw) of silica-free WEH cellulosic sample (89.09 ± 0.07 Da) are lower compared to the silica-free WE cellulosic sample (648 ± 13 Da), FiloCell (593 ± 9 Da) Avicel cellulose (157.5 ± 0.7 Da), and cotton samples (2062 ± 356 Da) studied here as references. These findings can be explained by the effect of hydrolysis on cellulose.

Silica-free WEH organosolv pulp, while not completely pure, contains a significant amount of cellulose and has a low ash content. The application of acid treatments to wheat straw led to a decrease in both the molecular weight (Mw) and number-average molecular weight (Mn). However, the basic treatments applied to the cellulose did not have any effect on its crystallinity or its detectable conversion into cellulose II. The crystalline phase of bleached silica-free WEH cellulosic pulp contains cellulose I, lacking inter-sheet hydrogen bonding. The unique properties of WEH organosolv cellulosic pulp render it well-suited for the production of diverse cellulose nanoparticles through various treatments designed to effectively isolate the fibers [97]. Additionally, it increases the digestibility of silica-free WEH cellulose making it suitable for enzymatic treatments [26,58,71]. High crystallinity has been listed as the most important property of microcrystalline cellulose and nanocrystalline cellulose samples as it implies mechanical and thermal stability. High-crystallinity cellulose has been previously used for pharmaceutical and biomedical applications (tissue engineering, wound healing, and drug delivery), polymer composites (reinforcing agents in composite and nanocomposite), food packaging (biodegradable packaging), and environment applications (water purification and air filtration) [98,99].

3.5.5. Fourier-Transform Infrared Spectroscopy Performed on the Silica Samples Isolated Following the Sodium Silicate Extraction of the Wheat Straw Cellulosic Pulp

To confirm the composition of the solid material produced through the process of acidification with 2 M HCl and heating at 90 °C for 2 h of the sodium silicate solution derived from wheat straw cellulosic pulp silica extraction, Fourier-transform infrared (FTIR) spectroscopy was used for its characterization. The identification and quantification of the observed spectral peaks of the silica samples were in agreement with a prior investigation on silica [42,44]. The presence of characteristic bands corresponding to the asymmetric stretching vibration of Si-O-Si bonds (1060 cm^{-1}) and the symmetric bending vibration of Si-O bonds (812 cm^{-1}) in the obtained spectra (Figure S5) provide conclusive evidence for the presence of silica in the studied material, thereby confirming its nature as silica.

The conversion of sodium silicate to silica can be easily achieved by treating the alkaline extract by acid, and this transformation can be improved by manipulating the reaction time, type of acid, and its concentration, allowing for the production of silica particles with desired sizes and properties. This approach provides a route to easier modification compared to the conventional method of calcination commonly used in the literature. Furthermore, it offers the advantage of generating an adjustable silica precursor suitable for diverse applications [42,44].

4. Conclusions

This investigation presented a promising prospect of using extracted and hydrolyzed wheat straw (WEH) in a sequential extraction approach with the objective of generating xylose, high-purity organosolv lignin, cellulosic pulp, and silica. This method maximizes the value derived from each component, which is a step forward compared to published studies that focused on one or two components. The results obtained in this study showed that by implementing an optimized catalytic organosolv process on WEH, it is possible to efficiently extract high-purity organosolv lignin with a recovery rate of 41%. These findings indicate the feasibility of adapting existing processes designed originally for wood to other lignocellulosic biomasses, such as wheat straw that has been previously extracted and hydrolyzed (WEH).

The organosolv lignin samples isolated from WEH exhibited structural and compositional characteristics comparable to those of lignin obtained from organosolv-treated wheat straw (WE). Additionally, the silica content in the ash-rich pulp produced via the organosolv process was effectively removed through a reaction with sodium carbonate. The optimized conditions for each stage of the process were identified to ensure high recovery rates and purity of the isolated material, which is of importance for potential industrial applications and commercial scalability. The characteristics of both wheat organosolv lignin and cellulosic pulp analyzed indicated promising potential for their transformation into high-value chemicals and advanced materials.

We have successfully accomplished the extraction of all components from hydrolyzed and extracted wheat straw (WEH), which were determined to have structural characteristics comparable to the biopolymers isolated from non-hydrolyzed wheat straw. For organosolv lignin samples, they were determined to have similar properties to those of lignin isolated from non-hydrolyzed wheat straw, maintaining the functionality, value, and indicating potential for further conversion through a biorefinery concept. It was determined by Durand et al. [20] that organosolv lignin isolated from hydrolyzed rice husks had similar properties to the lignin isolated from untreated rice husks, while cellulose underwent extensive degradation, indicated by a change in crystallinity and a decrease in average molecular weight. Again, the composition of the biopolymers from both hydrolyzed and untreated wheat straw were compared, and similar trends in the crystallinity of cellulosic pulp was determined. Wheat straw cellulose crystallinity increased after the hydrolysis pre-treatment. But the hydrolysis process extensively degraded cellulose, especially in amorphous domains, leading to shorter average cellulose chains with increased crystallinity. The comprehensive nature of the process, coupled with the detailed evaluation of the effects on biopolymer properties, makes it a strong candidate for industrial scale-up, potentially transforming the wheat straw use.

However, some properties of the wheat straw biopolymers suggest their suitability for specific uses. The lignin extracted from WEH showed promise for the production of lignin nanoparticles via electrospray, as shown in Durand et al.'s [20] study on rice husk lignin [100]. The silica-free and bleached cellulose recovered from WEH is an excellent candidate for transformation into nanoparticles due to the composition mainly consisting of cellulose I. The mechanical and thermal stability of microcrystalline and nanocrystalline cellulose were mainly determined by their high crystallinity, recognized as their most important property. High-crystallinity cellulose has been applied in diverse areas, including pharmaceuticals and biomedicine (tissue engineering, wound healing, and drug delivery), polymer composites (serving as reinforcing agents in both composites and nanocomposites), biodegradable food packaging, and environmental solutions (such as water purification and air filtration) [98,99]. Furthermore, it can be enzymatically digested to produce biofuel [8]. The relatively mild and environmentally friendly process used to recover silica allows for its further investigation. It can be directly used for different applications or modified using diverse methods, including acid precipitation [42,44]. The recovery of silica as part of the process is innovative, as silica is often overlooked in biomass processing or highlighted only as detrimental to wheat straw common valorization by combustion. Using our biorefinery

process on global wheat straw production would lead to a potential production of between 253 and 273 million tons of silica-free extractive-free organosolv wheat straw cellulose [9] suitable for applications in nanoparticle-containing materials. By offering an alternative to burning wheat straw as fuel, which contributes to air, water, and soil pollution, this process provides a sustainable solution to manage this abundant agricultural waste, reducing its environmental impact and contributing to circular economy principles. The biorefinery of wheat straw produces two biopolymers from both hydrolyzed and non-hydrolyzed biomass, as well as silica, which is inevitably associated with the cellulosic pulp of wheat straw. Despite some silica remaining in the lignin, most of the silica was found to be associated with the cellulosic pulp. Consequently, the cellulose was washed with sodium carbonate and the remaining lignin was removed by bleaching to obtain purified cellulose, suitable for further transformation into nanoparticles. However, our process has some drawbacks. Although it allows for the sequential extraction of all components of wheat straw, the lignin recovery rate is lower than that reported in the literature. Although possible and already achieved in previous studies, the recovery of reagents and catalyst was not evaluated in this study and would require further investigation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/macromol4030039/s1>, Figure S1: Fourier transform infrared (FTIR) spectra of organosolv lignins isolated from pre-extracted and hydrolyzed wheat straw (WEH) and extractive-free wheat straw (WE); Figure S2: 2D NMR spectra of organosolv lignin isolated from WEH; Figure S3: 2D NMR spectra of organosolv lignin from WE; Table S1: Assignment of $^1\text{H}/^{13}\text{C}$ correlation signals in the 2D HSQC spectra of organosolv lignin from WEH; Table S2: GPC analysis data of wheat straw lignin samples; Table S3: Characterization of cotton, Avicel®, and FiloCell® cellulose samples; Figure S4: MAS/ ^{13}C NMR spectra of bleached organosolv cellulosic pulp from pre-hydrolyzed wheat straw; Figure S5: Fourier transform infrared (FTIR) spectrum of silica extracted from wheat straw pulp.

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