

Article

Steam Explosion-Based Method for the Extraction of Cellulose and Lignin from Rice Straw Waste

Víctor M. Serrano-Martínez ^{*}, Henoc Pérez-Aguilar, María Pilar Carbonell-Blasco , Francisca Arán-Ais ^{*} and Elena Orgilés-Calpena

Footwear Technology Centre, Campo Alto Campground, 03600 Alicante, Spain; hperez@inescop.es (H.P.-A.)

^{*} Correspondence: vmserrano@inescop.es (V.M.S.-M.); aran@inescop.es (F.A.-A.);

Tel.: +34-965-395-213 (V.M.S.-M. & F.A.-A.)

Abstract: This paper focuses on the optimisation of an efficient extraction process for cellulose and lignin from rice straw waste from the Albufera of Valencia using the steam explosion method. This method is particularly pertinent given the environmental and economic challenges posed by the current disposal practices of agricultural waste. The technique comprises a high-temperature cooking stage followed by instantaneous decompression, effectively altering the biomass's physical and chemical properties to enhance its surface area and porosity. Our adaptation of the steam explosion technique specifically addresses the challenges of rice straw waste, marking a significant departure from previous applications. This innovation is crucial in addressing the urgent need for more sustainable waste management practices, as it effectively deconstructs the lignocellulosic matrix of rice straw. This facilitates the selective extraction of cellulose at a 70% efficiency, with a 20% yield and the subsequent recovery of lignin. The results of this study are significant for sustainable biomaterial production, offering novel insights into optimising these crucial biomass components. By refining the process and focusing on critical parameters, our work advances the application of steam explosion methods for agricultural waste, enhancing efficiency and sustainability. By utilising rice straw biowaste, this research not only proposes a solution to a pressing environmental issue but also demonstrates the potential to create new market opportunities, increase the economic value for rice producers, and significantly reduce the environmental footprint of existing waste disposal methods. The holistic and ecological approach of this study underscores the vital need for innovative strategies in agricultural waste management, positioning the valorisation of rice straw waste as a key component in the pursuit of environmental sustainability.

Keywords: steam explosion method; cellulose extraction; sustainable biomaterials; lignocellulosic biomass; biocomposites; rice straw waste



Citation: Serrano-Martínez, V.M.; Pérez-Aguilar, H.; Carbonell-Blasco, M.P.; Arán-Ais, F.; Orgilés-Calpena, E. Steam Explosion-Based Method for the Extraction of Cellulose and Lignin from Rice Straw Waste. *Appl. Sci.* **2024**, *14*, 2059. <https://doi.org/10.3390/app14052059>

Academic Editors: Eleni Kastanaki and Kaixin Li

Received: 15 January 2024

Revised: 21 February 2024

Accepted: 23 February 2024

Published: 1 March 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The sustainable management of agricultural waste is an increasingly crucial element in the global effort to balance environmental conservation with economic development. This study examines a novel approach to valorising rice straw waste from the Albufera of Valencia, focusing on the extraction of valuable biopolymers, cellulose, and lignin, using a steam explosion method.

Rice cultivation is a cornerstone of global agriculture, feeding over half of the world's population [1], and with rice straw constituting around 45% of the total volume in rice production, it stands as the most prevalent agricultural byproduct on a global scale [2]. In regions like the Albufera of Valencia, this significant agricultural activity, while crucial for food production, also results in the generation of a considerable amount of straw waste.

Agricultural residues, particularly rice straw, are abundant yet underutilised resources. Historically, this byproduct has been neglected, often disposed of through environmentally detrimental methods like open burning, leading to air pollution and substantial greenhouse

gas emissions [3,4]. In Spain, particularly in areas such as the Valencian Community, rice cultivation generates between 75,000 and 90,000 tons of rice straw annually, leading to the emission of millions of tons of CO₂, CH₄, and NO₂, along with harmful gases like methyl chloride [5,6]. The handling of rice straw waste thus emerges as a critical issue in sustainable agricultural practices [1].

The potential of rice straw extends beyond its role as a waste product. As a lignocellulosic biomass, it is primarily composed of 40–50% cellulose, 25–35% hemicellulose, and 15–20% lignin, presenting an opportunity for extracting valuable materials [7–10]. Cellulose is a key component in biofuel production, biodegradable materials, and high-strength composites, marking a cornerstone in the development of sustainable materials. Lignin offers potential in creating bio-based chemicals and materials, facilitating a transition towards a circular economy, where waste materials are converted into valuable resources [10–14].

Conventional methods for cellulose and lignin extraction are predominantly alkaline pulping, which uses sodium hydroxide for effective lignin removal [15–18]; organosolv pulping, utilising organic solvents for efficient delignification [19–21]; bleaching, involving chemical treatments to purify cellulose [16,22,23]; enzymatic treatment, employing enzymes such as cellulase for hydrolysis [24,25]; TEMPO-mediated oxidation, which oxidises hydroxyl groups to enhance mechanical fibrillation [15,26]; and ionic liquid pretreatment, using ionic liquids for cellulose dissolution [27,28]; among others. Each method's unique conditions contribute to its efficacy. However, these methods often face challenges regarding their energy intensity and environmental impact and typically involve the use of harsh chemicals, elevated temperatures, and a high energy consumption, leading to issues such as environmental pollution and safety concerns [29–31]. These processes also generate large amounts of residues and require expensive and resistant processing equipment as well as considerable waste treatments, thus increasing their environmental footprint. This has driven the scientific community to seek more sustainable and eco-friendly alternatives for cellulose and lignin extraction, such as steam explosion, ultrasound-assisted pretreatment, supercritical fluid pretreatment, and others, in addition to combining both green and conventional methods in order to reduce the impact and optimise the process [31–36]. Furthermore, the extraction of cellulose and lignin from biomass such as rice straw is fraught with challenges, including effectively removing silica and developing a practical method to isolate its key components [37].

The steam explosion technique emerged as a promising alternative, leveraging thermal and mechanical forces to break down the complex structure of rice straw. This method involves treating the biomass with high-pressure steam and then rapidly reducing the pressure, causing an explosive decompression that disrupts the lignocellulosic matrix [34,38]. This hydrothermal method also delignifies the residue and explodes the cellulosic fibrils, maintaining the fibre integrity below 240 °C and yielding separate solutions of lignin, hemicellulose, and cellulose fibres [39]. This approach aligns with green chemistry, enhancing cellulose and lignin accessibility, improving their purity and yield, and reducing the chemical usage and energy requirements [31].

In this sense, the steam explosion technique is not only promising but has already demonstrated its efficacy in various studies. For instance, Gou et al. [9] reported the extraction of cellulose, reaching up to 64.80% of cellulose with a high purity. Similarly, Hong-Zhang et al. [40] achieved an efficient separation of lignin and cellulose, with a lignin extraction yield of 90% and a cellulose yield of 93.2%. These advances represent a milestone in obtaining high-quality materials from biomass.

Furthermore, there has been an increasing trend towards integrating conventional methods and green chemistry approaches in the extraction of cellulose and lignin from natural sources. A notable example of this synergy is demonstrated in the work of Padilla et al. [41], who utilised a combined process of acid pretreatment, followed by steam explosion and enzymatic hydrolysis for the valorisation of olive byproducts into sugars. Their research highlighted the most effective condition for steam explosion to be at 195 °C for a duration of 5 min, thereby emphasising the method's effectiveness in delignifying biomass. This hybrid approach not only maximises the purity and yield but also aligns

with the principles of sustainability and energy efficiency, demonstrating the potential of combining traditional and modern techniques in biomass biorefinery.

This research conducts a thorough assessment and refinement of the multistep cellulose and lignin extraction protocol previously developed at INESCOP [32], particularly focusing on rice straw waste from the Albufera of Valencia. This study not only adapts but significantly enhances the steam explosion technique, specifically targeting the valorisation of rice straw, a biomass source hitherto underexplored in this context. By optimising the extraction process, our work systematically reduces the complexity, time, and resource requirements, setting a new benchmark in agricultural waste utilisation. This methodological advancement emphasises the versatility and potential of the steam explosion method under varied conditions, representing a novel approach to more effective biomass conversion technologies. To validate the efficacy of the optimised protocol, various sophisticated characterisation techniques, including scanning electron microscopy (SEM), thermogravimetric analysis (TGA), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD), have been employed. Ultimately, the research seeks to advance the application of these processes in industry, highlighting the potential for enhanced resource recovery and environmental stewardship.

To further delineate the scope and impact of this study within the field, Table 1 presents a comparative analysis that positions our findings within the context of previous landmark studies. This comparison not only underscores the advancements our research contributes to the domain but also illustrates the continuous journey towards optimising biomass conversion processes. The table below offers a concise yet comprehensive summary of these efforts, directly aligning with our pursuit of more sustainable and efficient extraction methodologies.

Table 1. A concise comparative assessment of biomass valorisation methods.

Biomass Source	Extraction Method	Conditions	Yield	Strategic Advancements	Reference
Rice straw	Sequential execution of alkaline pretreatment, steam explosion with and without supercritical CO ₂ integration, oxidation treatment, and bleaching	Varying conditions on the steam explosion process and oxidation treatment; trying to eliminate alkaline pretreatment	Selective extraction of cellulose at 70% efficiency with a 20% yield; significant recovery of lignin	Advanced a significant leap in biomass valorisation through a novel, optimised steam explosion process, significantly enhancing cellulose extraction efficiency and sustainability, and providing a holistic approach to agricultural waste management	Current research
Phoenix canariensis palm leaves	Sequential processes including alkaline treatment, steam explosion, oxidation, and bleaching	Steam explosion times between 15–33 min; temperature up to 200 °C; alkaline and oxidation conditions for effective delignification	Cellulose yield significantly increased with processing time, with notable efficiency in lignin and hemicellulose removal, obtaining 56% cellulose content	Highlighted the optimised combination of steam explosion with alkaline and oxidation treatments for improved cellulose fibre extraction, suitable for high-value material applications	[32]
Wheat straw	Steam explosion followed by ethanol extraction	Steam explosion at 1.6 MPa for 5.2 min; ethanol for lignin extraction at 160 °C for 2 h with 60% ethanol (<i>v/v</i>)	Hemicellulose fractionation yield of 73%; lignin fractionation yield of 90%; cellulose yield of 93.2%	Demonstrated an effective multilevel composition fractionation process, enabling the high-value utilisation of wheat straw for cellulose extraction and regenerated cellulose film production	[40]

Table 1. Cont.

Biomass Source	Extraction Method	Conditions	Yield	Strategic Advancements	Reference
Olive stones	Acid pretreatment, steam explosion, and enzymatic hydrolysis	Acid pretreatment and steam explosion at various temperatures (120–200 °C) and durations; enzyme specificity adjusted for biomass	Yields of 83% total sugar recovery from raw material, with specific yields for xylose (71% from acid pretreatment)	Integrated approach utilising steam explosion in conjunction with acid pretreatment for enhanced biomass fractionation, applicable to cellulose extraction processes	[41]
Rice straw	Steam explosion followed by ionic liquid treatment, organosolv, and biological treatments	AMIMCl ionic liquid extraction emphasises efficiency	Cellulose purity up to 64.80% and yield of 30.73% for bleached cellulose using AMIMCl	Introduced environmentally friendly methods for cellulose separation from straw, showcasing the potential of rice straw cellulose in enhancing the mechanical properties of composites; demonstrated the effectiveness of steam explosion in preparing biomass for further processing, with a focus on sustainable and recyclable approaches	[9]
Agricultural residues, wood biomass, and aquatic plants	Steam explosion, acid/alkaline treatments, enzymatic hydrolysis, and ionic liquid extraction	Specified to method, e.g., high pressure and temperature for steam explosion, specific pH for chemical treatments, enzyme specificity for enzymatic hydrolysis, and solvent type for ionic liquids	Optimal methods report high cellulose purity (>90%) and significant lignin removal	Highlights the diversity of biomass sources suitable for cellulose extraction, and the evolution of environmentally friendly and efficient extraction methods; emphasises the need for optimisation based on specific biomass characteristics and end-use applications	[31]

2. Materials and Methods

2.1. Materials

Rice straw was sourced from the Albufera of Valencia, a renowned wetland and rice growing area in Spain. This material was generously provided by Unió Llauradors i Ramaders (Valencia, Spain), a prominent organisation representing agricultural professionals in the region, known for its commitment to sustainable farming practices. The Albufera of Valencia is well known for its extensive rice fields, and the rice straw used in this study is a byproduct of these fields, commonly available post-harvest. To illustrate the physical characteristics of the rice straw, Figure 1 shows both its state when harvested and before processing.

For the chemical treatment of the rice straw, several reagents were employed. Sodium hydroxide (NaOH), hydrogen peroxide (H₂O₂), acetic acid (CH₃COOH), potassium hydroxide (KOH), and sodium hypochlorite (NaClO) were all procured from Merck KGaA (Darmstadt, Germany). Additionally, carbon dioxide (CO₂) was used in the form of a compressed gas, sourced from Sociedad Española de Carburos Metálicos, S.A. (Barcelona, Spain), for its application in the steam explosion experiments, although it was not consistently used across all experimental procedures. All chemicals were used as received,

without further purification. The solutions and mixtures were prepared using distilled Milli-Q water to ensure the consistency and reliability of the experimental results.



Figure 1. Rice waste: (a) fresh harvested rice straw and (b) wet chopped rice straw.

2.2. Extraction of Cellulose Fibres from Rice Straw

In this study, a multistep method, based on the sequential execution of several chemical and physical processes, was employed for the isolation of cellulose fibres from rice straw. The methodological framework, adapted from Pérez-Limiñana et al. [32], was comprehensively optimised to enhance the extraction efficacy while striving for a reduction in the number of stages, duration, and reagent use.

Figure 2 shows the schematic representation of the process, illustrating the sequential steps followed, tailored specifically for rice straw. The process begins with the collection of rice straw, a plentiful agricultural residue from rice (Step 0), which undergoes preparatory treatments to facilitate the subsequent extraction phases (Step 1–4). In order to maximise the surface area in contact with the treatment solutions, rice straw is initially cut longitudinally to 10 cm in size, as shown (in solution) in Step 1. Each stage is described in detail below, as well as its function.

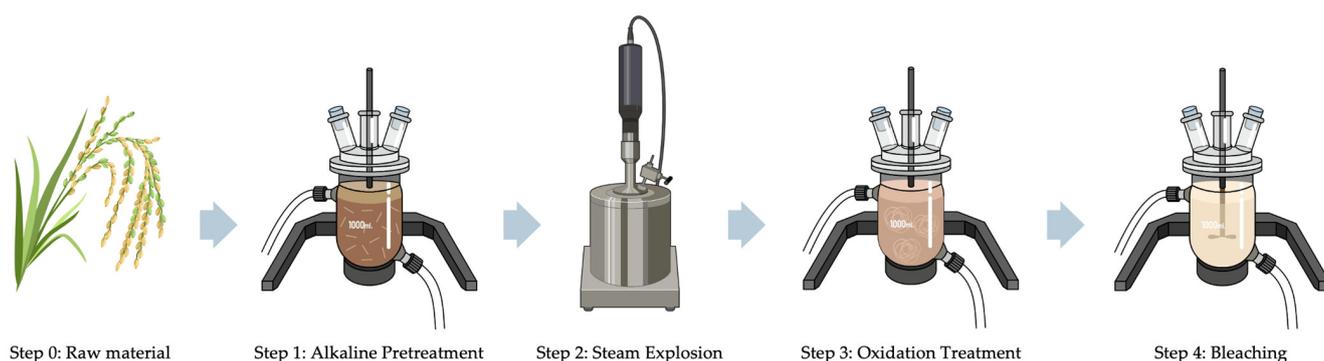


Figure 2. Extraction process followed to obtain cellulose fibres and lignin from rice straw.

Step 1: Alkaline Pretreatment: This process involves immersing the rice straw in an aqueous sodium hydroxide (NaOH) solution, which acts on the fibre structure of the straw. The primary purpose of this treatment is to modify the hydrogen bonds within the cellulose, increasing the surface roughness and facilitating the removal of the lignin, waxes, and oils [42–44]. This is chemically represented by the reaction:



In our experimental approach, 10 g of dried chopped rice straw were introduced in a jacketed glass reactor of 1 L with 600 mL distilled water and heated to 70 °C, with a pH maintained at 12.5 using NaOH for a duration of 3 h and stirring at 500 rpm. This step has been traditionally considered essential for enhancing the mechanical properties of the extracted fibres. The solid residue is filtered and washed several times in order to standardise the condition of the biomass before the steam explosion stage, ensuring consistency in our experimental results.

Step 2: Steam Explosion and Supercritical CO₂ Integration: In this phase of the study, the rice straw, regardless of having undergone the pretreatment or not, is subjected to a steam explosion process within a high-temperature/high-pressure reactor (HPHT) (novoclave, Büchi AG—Pilot Plant & Reactor Systems; Uster, Switzerland). In this process, a water-to-raw material ratio of 10:1 is employed, with 10 g of rice straw added in every instance. The reactor, equipped with a thermal jacket of 600 mL, is sealed and heated to maintain an internal temperature of 200 °C, ensuring that no pressure is released. To assess the impact of time on the effectiveness of lignin depolymerisation and hemicellulose degradation, the reactor's temperature is sustained at 200 °C for varying durations, ranging from 30 min up to 4 h. Following this treatment, the reactor is methodically depressurised and left to cool to an ambient temperature over the course of the night.

This step involves both mechanical action through shear forces caused by rapid pressure release and chemical action resulting from autohydrolysis, primarily the hydrolytic attack by acetic acid formed from acetyl groups in the hemicelluloses at high temperatures [9].

The innovative inclusion in this step is the application of supercritical carbon dioxide (ScCO₂). ScCO₂ is commonly utilised for cellulose modification and as a pretreatment for enzymatic hydrolysis [45–47]. The diffusion of ScCO₂ can be penetrative, impacting the structural and physicochemical properties of cellulose derived from rice straw. Therefore, in this study, ScCO₂ has been chosen as an alternative medium for cellulose acetylation to explore its potential to improve the process.

Post steam-explosion treatment, approximately 70% of the plant material is obtained as a solid residue, primarily consisting of cellulose and lignin, along with some residual hemicelluloses. The solid residue is filtered and washed several times in order to standardise the condition of the biomass before oxidation, ensuring consistency in our experimental results. The supernatant from this step, a lignin-rich solution, is reserved for further extraction, fractionation, and purification, thereby recovering the entire biomass [43,48,49]. The effectiveness of the cellulose fibre extraction depends on the swelling of the cellulose, as well as the softening and/or removal of the lignin induced by the steam treatment.

Step 3: Oxidation Treatment: Following the hydrothermal process, the remaining solid undergoes a subsequent treatment phase involving a 60 mL 30% hydrogen peroxide solution, with a water-to-raw material ratio of 40:1 (mL:g). This phase is conducted in a jacketed glass reactor of 1 L at a steady temperature of 50 °C, stirring at 400 rpm and with a pH of 11.5 over a period of 3 to 5 h. The application of hydrogen peroxide is a critical step, as peroxides are known to decompose readily into hydroxyl radicals (HO·) through the reaction:



These hydroxyl radicals then interact with the hydrogen in the cellulose matrix and fibres, leading to the reaction:



As a result of this treatment with hydrogen peroxide, there is a notable decrease in the hydrophilicity of the fibres, as well as an increase in their tensile properties [42,50,51]. Together with the previously described steps, a focal point of this study is the examination of the processing time variation during this peroxide treatment, from 3 to 5 h. This investigation aims to identify the optimal duration that maximises both the efficiency

and efficacy, thereby contributing to the overall optimisation of the cellulose and lignin extraction process from rice straw.

To facilitate the purification of the fibres, post-peroxide treatment, a thorough washing process is employed. The fibres are initially washed with distilled water, followed by a water:ethanol mixture in a 1:1 ratio, and finally, with absolute ethanol. This stepwise purification approach ensures the removal of residual chemicals and impurities, thus enhancing the quality and applicability of the cellulose fibres extracted from the rice straw [52].

Step 4: Bleaching Process: This step involves the processing of the extracted cellulose by immersing it in a 3.7 wt% NaClO aqueous solution to eliminate lignin and amorphous cellulose, using a 15:1 (mL:g) solution-to-fibres ratio [53,54]. The mixture is agitated continuously at 500 rpm for an hour at 70 °C. After stirring, the product is filtered and washed with water for neutralisation, then lyophilised to a constant weight. Given the overall aim of optimising the extraction process, this stage remains unchanged, as it is crucial for producing a final bleached product that meets industrial application standards.

In line with the circular economy principles, the process aims to utilise all constituents of rice straw. The refined method detailed herein builds on the procedures established by Pérez-Limiñana et al. (2022) [32] while introducing new process optimisations suitable for the rice straw feedstock. The process efficiency has been evaluated based on the yield and purity of the extracted cellulose and the utility of the byproducts in lignin valorisation.

2.3. Experimental Techniques

The characterisation of the cellulose fibres and lignin from the waste solutions generated during the different steps has been carried out using the following:

2.3.1. Fourier Transform Infrared Spectroscopy (FTIR)

A Varian 660-IR infrared spectrophotometer (Varian Australia PTY LTD; Mulgrave, Australia) was used to determine the composition of the cellulosic fibres, the process followed, and the lignin obtained. The transmission infrared spectroscopy technique was utilised, involving the preparation of samples using potassium bromide (KBr) pellets. This approach entailed conducting 12 scans at a resolution of 4 cm⁻¹, allowing for a comprehensive analysis of the sample's infrared absorption [55].

2.3.2. Thermogravimetric Analysis (TGA)

The thermal stability of cellulose and its solutions was evaluated using a TGA 2 STARe System thermal balance, equipped with STARe v16.4 software (provided by Mettler-Toledo, Columbus, OH, USA). Each sample, weighing between 5 and 7 mg, was placed in an alumina crucible. The analysis involved heating the sample incrementally from 30 to 600 °C at a rate of 10 °C/min under a nitrogen atmosphere, with a flow rate set at 30 mL/min [56]. To ensure accuracy, each sample underwent a triplicate analysis, and the displayed results represent the average of these three trials. The margin of error in these tests was maintained at less than ±0.05%.

The TGA data underwent a baseline correction to address instrumental drifts. Different weight loss steps were identified and quantitatively analysed to determine the percentage of each constituent (hemicellulose, cellulose, and lignin). This was based on the total weight loss at specific temperature intervals corresponding to the known decomposition or volatilisation temperatures of the constituents [57]. The residual mass at the end of the TGA run provided estimates of non-volatile components. This methodology enabled the precise quantification of sample constituents, essential for understanding their composition and thermal behaviour.

2.3.3. X-ray Diffraction (XRD)

A non-destructive approach was adopted for the quantitative assessment of crystalline fibres. This involved using wide-angle X-ray diffraction (XRD) technology, specifically

a Bruker D8-Advance Göebel mirror system (Billerica, MA, USA) designed for non-flat samples. The system, which includes a high-temperature chamber capable of reaching 900 °C, was coupled with a Siemens Bruker Kristalloflex K 760-80F X-ray generator. This generator operates at a power of 3000 W, with an adjustable voltage ranging from 20–60 kV and a current between 5–80 mA, and is equipped with a copper anode XR tube [58]. The crystallinity of the samples was determined using the crystallinity index (*CI*), calculated as per the methodology described by Khan et al. [59], which is defined as:

$$CI = \frac{A_c}{A_c + A_a}, \quad (4)$$

where A_c is the area under crystalline peaks, and A_a is the area of amorphous hollows. This approach involved extracting individual crystalline peaks through a curve-fitting process from diffraction intensity profiles using a tool for fitting peaks included in OriginLab software, with Gaussian functions assumed for each peak. Iterations were repeated until a maximum F number, indicative of a high correlation coefficient (R^2 value of 0.997), was achieved. Furthermore, the crystallite size was determined using the Scherrer Equation [60], given by:

$$D_p = \frac{0.94\lambda}{\beta \cdot \cos\theta} \quad (5)$$

where D_p is the average crystallite size, β is the line broadening in radians, θ is the Bragg angle, and λ is the X-ray wavelength. This formula is widely recognised in X-ray diffraction analysis for estimating the sizes of crystallites in a sample.

Finally, diffractograms were indexed using peak positions corresponding to cellulose I β (ICDD patent PDF-2 database, File n° 00-050-2241) [61,62].

2.3.4. Scanning Electron Microscopy (SEM)

The examination of the morphology was conducted using a Phenom ProX scanning electron microscope (Phenom World, Eindhoven, The Netherlands). An electron beam with a potential ranging from 5 to 15 keV was employed, while operating under high vacuum conditions to enhance the image resolution [63].

2.3.5. Statistical Analysis

The comparative analysis of the different process conditions was supported by carrying out using an analysis of variance. The analysis of variance (ANOVA) is a statistical technique employed to determine whether significant differences exist between groups or treatments for a variable of interest. It uses a within-group variability to assess whether the observed differences are larger than would be expected by chance [64].

To conduct the aforementioned statistical analysis, the software STATGRAPHICS Centurion 18 was used (confidence level of 95%, $p < 0.05$). In this software, a multifactorial and simple ANOVA are used, where the influence of the chosen factors (in this case, different times for the oxidation and steam explosion stages and the use or not of a pretreatment stage and ScCO_2) on the dependent variable (in this case, the % of cellulose) is studied. Then, the software automatically calculates the ANOVA statistics to assess whether there are significant differences between the means of the groups [65].

The F-ratio of the ANOVA table, as well as the p -value, which indicates the probability that the results observed in the experiment are due to chance rather than to the effects of the factors, determine whether there are significant differences between the means. Comparisons of the results are made at a predefined overall significance level: $\alpha = 0.05$. This means that the probability value (p) must be less than 0.05 for the factors and combinations of factors studied to be considered statistically significant in relation to the dependent variable [66].

3. Results and Discussion

In this section, a comparative analysis of the different variations of the process implemented is carried out in order to exclude variables and reduce the number of results to be analysed in more detail. Once the most outstanding results for obtaining cellulose are determined, they are rigorously assessed for the optimisation of the extraction process.

Additionally, the results obtained for the extraction of lignin from the selected processes are shown.

3.1. Comparative Analysis

In the search for an optimised process for the extraction of cellulose from rice straw, a series of experiments were conducted to evaluate the efficacy of various treatment stages, namely alkaline pretreatment, steam explosion, oxidation, and bleaching. The primary aim was to streamline the process by assessing the impact of each stage on the cellulose yield and overall efficiency, as indicated by the thermogravimetric analysis (TGA). The yield of cellulose was calculated based on the mass yield, which involves dividing the weight of the final cellulose product by the weight of the initial raw material used. Thus, this analysis provided insights into the compositions of hemicellulose, cellulose, and lignin, and the yield of each sample [67].

The standard initial process consisted of an alkaline pretreatment followed by a 33 min steam explosion, a 5 h oxidation period, and finally, a bleaching stage [32]. From this baseline, modifications were explored, such as omitting the pretreatment, varying the steam explosion duration (33 min, 1 h, 2 h, and 4 h), implementing or excluding ScCO₂, and adjusting the oxidation times (3, 4, and 5 h). The bleaching stage remained constant throughout the experiments. This experimental work was guided by a thermogravimetric analysis (TGA), which measured the mass loss at varying temperatures, providing a percentage composition of hemicellulose, cellulose and lignin, and the yield, while informing on decisions regarding process adjustments.

The initial comparative analysis focused on samples with and without the alkaline pretreatment stage, as shown in Figure 3. The comparison between samples revealed a negligible effect on the cellulose content, which was supported by the ANOVA analysis (p -value > 0.05). Therefore, omitting the pretreatment did not adversely affect the cellulose content, suggesting that the process could be streamlined by removing this stage, thus achieving reagent and time savings. Moreover, the yield did not show any notable decrease, indicating that the removal of the pretreatment stage could lead to savings in both reagents and time without compromising the cellulose yield. Therefore, the decision to omit the alkaline pretreatment stage was substantiated by these findings.

Subsequent experiments explored the reduction in the oxidation time. Figure 4 shows the comparison between 3, 4, and 5 h oxidation periods, which, supported by the ANOVA analysis (p -value < 0.05), revealed a decrease in the cellulose percentage with the reduction of time to 3 h. This slight decrease in the cellulose content is considered acceptable when balanced against the benefits of a reduced operational time and lower costs. Furthermore, the yield remained relatively stable, supporting the decision to reduce the oxidation period to 3 h.

In the final set of experiments, the steam explosion stage was examined under different reaction times and the presence or absence of ScCO₂. The results shown in Figure 5 suggest that the introduction of ScCO₂ did not significantly enhance the cellulose yield. This was supported by the means of the ANOVA analysis, which were p -value < 0.05. Additionally, a 33 min steam explosion appeared to be as effective as longer durations, as there were no substantial gains in the cellulose concentration or yield with increased reaction times. Consequently, it was deemed reasonable to proceed without ScCO₂ and limit the steam explosion to 33 min. The avoidance of ScCO₂ not only simplifies the process but also enhances its environmental sustainability by reducing the carbon footprint associated with CO₂ utilisation and handling.

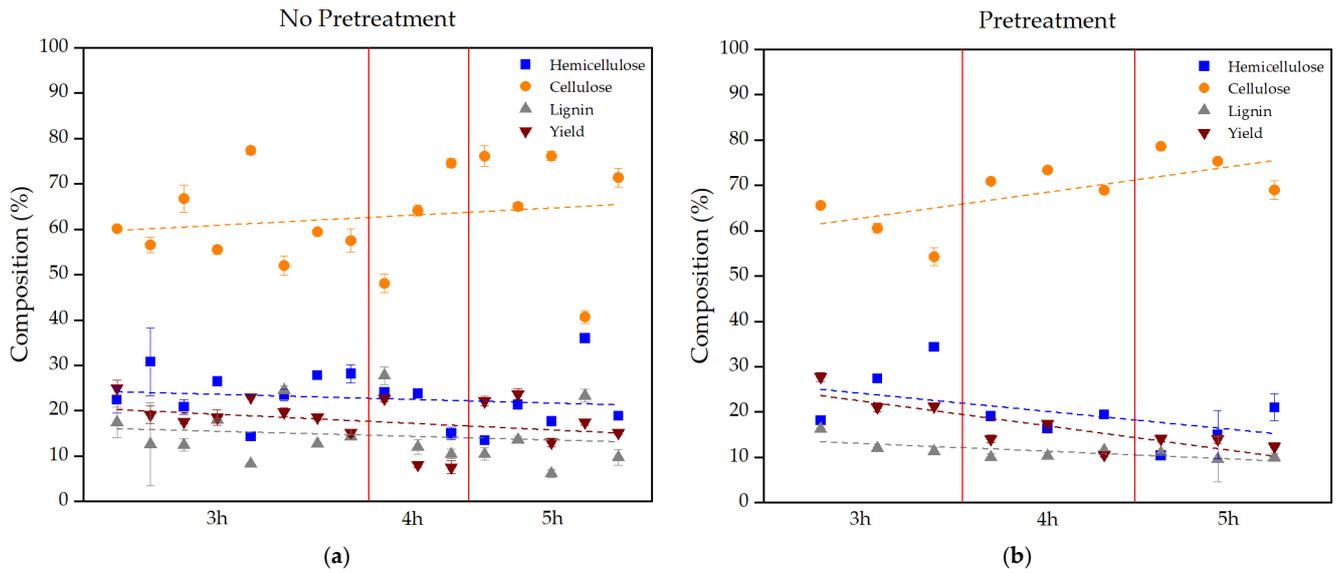


Figure 3. Comparison between the obtained results for different processes performed (a) without and (b) with the alkaline pretreatment stage, indicating the duration of the oxidation stage for each batch of experiments.

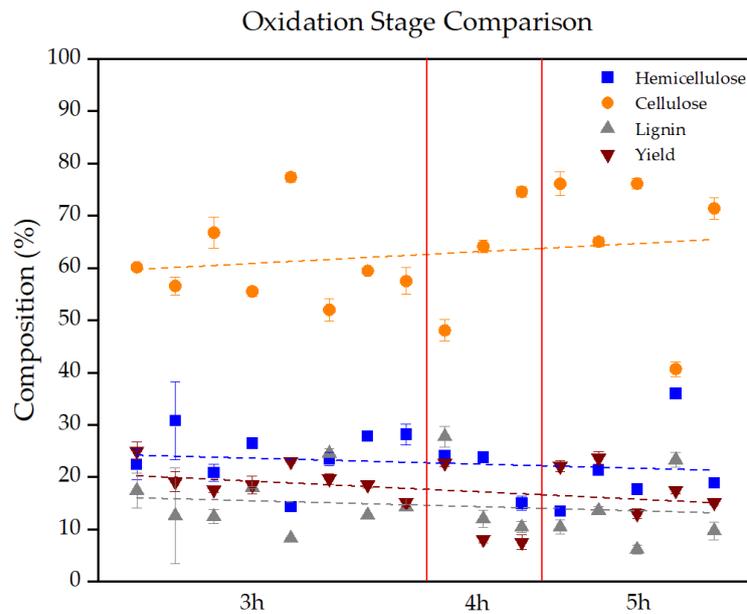


Figure 4. Comparison of the results obtained for different times of the oxidation stage, previously eliminating the alkaline pretreatment stage.

Based on the comprehensive analysis of the cellulose percentage and yield, the process optimisations can be justified. The exclusion of the alkaline pretreatment and the reduction of the oxidation stage to 3 h streamlined the process without detriment to the final product. The decision to forgo ScCO₂ and adhere to a 33 min steam explosion aligns with the objective of maximising the cellulose extraction while maintaining a balance with other process variables.

These conclusions, while preliminary, will be scrutinised in the subsequent section using three processes, Fourier transform infrared spectroscopy (FTIR), TGA, and scanning electron microscopy (SEM), of the eight under consideration. This rigorous analysis will ensure that the optimised process is not only cost effective but also maintains the desired cellulose yield and quality.

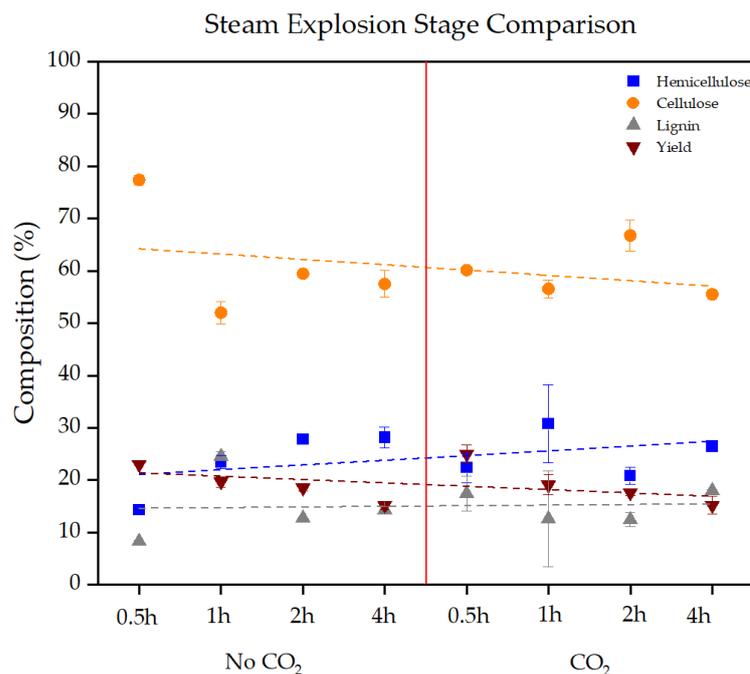


Figure 5. Comparison of the results obtained for the steam explosion stage at different times and with or not without ScCO₂.

3.2. Characterisation of Cellulose Fibres

An FTIR spectroscopic analysis was conducted to examine the chemical properties of the cellulose extracted from rice straw after a four-stage process, including steam explosion with and without ScCO₂ addition. The FTIR spectra of both the extracted cellulose fibres and the rice straw waste are displayed below. The most characteristic peaks associated with typical functional groups found in biomass confirm the presence of cellulose in the samples, as outlined in Table 2.

Table 2. Infrared band assignments for cellulose, hemicellulose, and lignin.

Wavenumber (cm ⁻¹)	Assignment	References
3400	-OH group stretching	[7]
2900	C-H stretching vibration	[8]
1640	Water absorption	[27,32]
1429	Symmetric deformation of -CH ₂	[27]
1375	Symmetric deformation of -CH ₃	[68]
897	Bending vibrations of β-glycosidic C-H bonds	[27,32]
1054–1155	Stretching vibrations of C-O and C-O-C	[29,69]

The FTIR spectrum of the untreated rice straw serves as a baseline, presenting an -OH stretching band at 3400 cm⁻¹, attributed to the hydroxyl groups in cellulose, hemicellulose, and lignin [7]. Water absorption is evidenced by a peak at 1640 cm⁻¹ [32]. Aliphatic C-H stretching vibrations are observed around 2900 cm⁻¹ [8]. The peaks at 1429 cm⁻¹ and 1375 cm⁻¹ correspond to the symmetric deformation of -CH₂ and -CH₃, respectively [27,68], while bending vibrations of β-glycosidic C-H bonds are identified at 897 cm⁻¹ [8,27]. This spectrum serves as a comparison to identify the effects of the treatments applied to rice straw. Figure 6 shows the FTIR spectra for the rice straw raw material and the cellulose fibres extracted after different steam explosion treatments using ScCO₂.

The samples treated with CO₂ exhibit significant changes in their vibration bands. At 33 min of treatment, there is a notable decrease in the ratio between the -OH stretching band at 3400 cm⁻¹ and the aliphatic C-H stretching vibration at 2900 cm⁻¹, compared to the rice straw raw material spectrum, suggesting potential hydrogen bond breaking and initial

depolymerisation [7,8]. As the treatment time increases, the ratio between the C–H stretching vibration at 2900 cm^{-1} and the C–O stretching bands at 1054 cm^{-1} and C–O–C at 1155 cm^{-1} progressively increases, indicating cellulose and hemicellulose degradation [29,32].

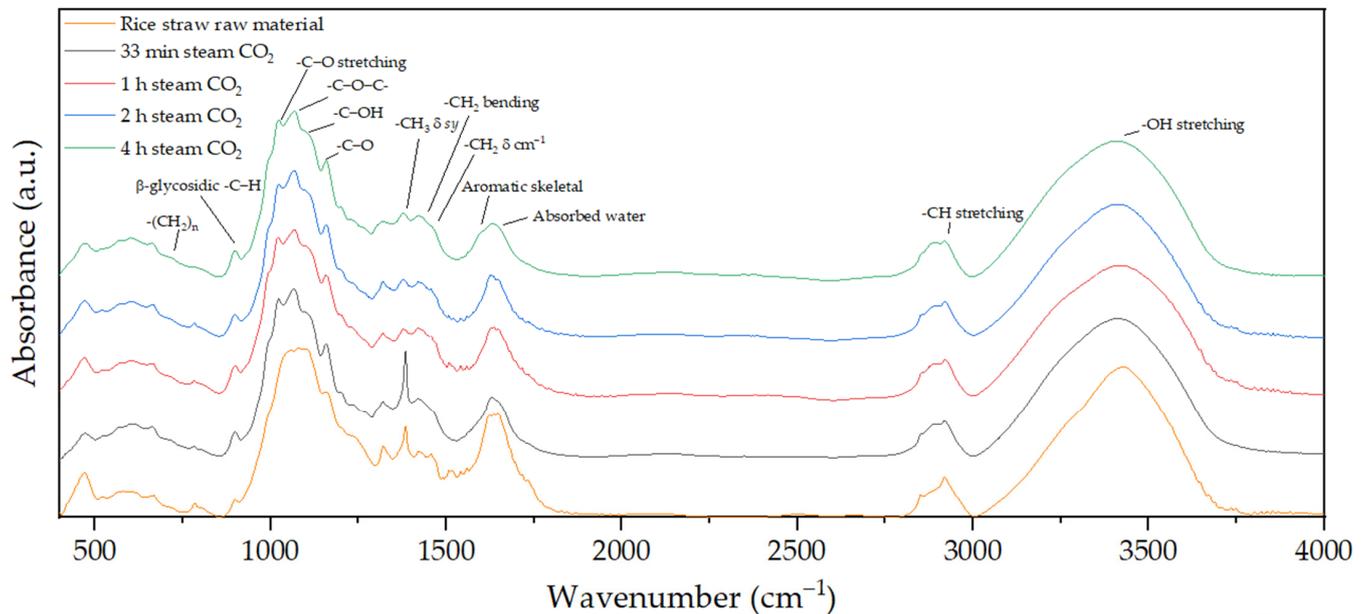


Figure 6. FTIR spectra of rice straw waste and the cellulose fibres extracted after different durations of steam explosion treatment using ScCO_2 .

Conversely, Figure 7 shows the samples treated without CO_2 , compared to the raw material, which show a smaller decrease in the ratio between the -OH and C–H stretching bands, suggesting a more effective preservation of the cellulose structure. At 33 min, the ratio between the -CH stretching vibration at 2900 cm^{-1} and the C–O stretching band at 1054 cm^{-1} experiences a slight decrease, indicating minor cellulose structure alterations, but at 4 h, a recovery in its intensity is observed, which might reflect a stabilisation of the cellulose structure after undergoing oxidation and bleaching treatments.

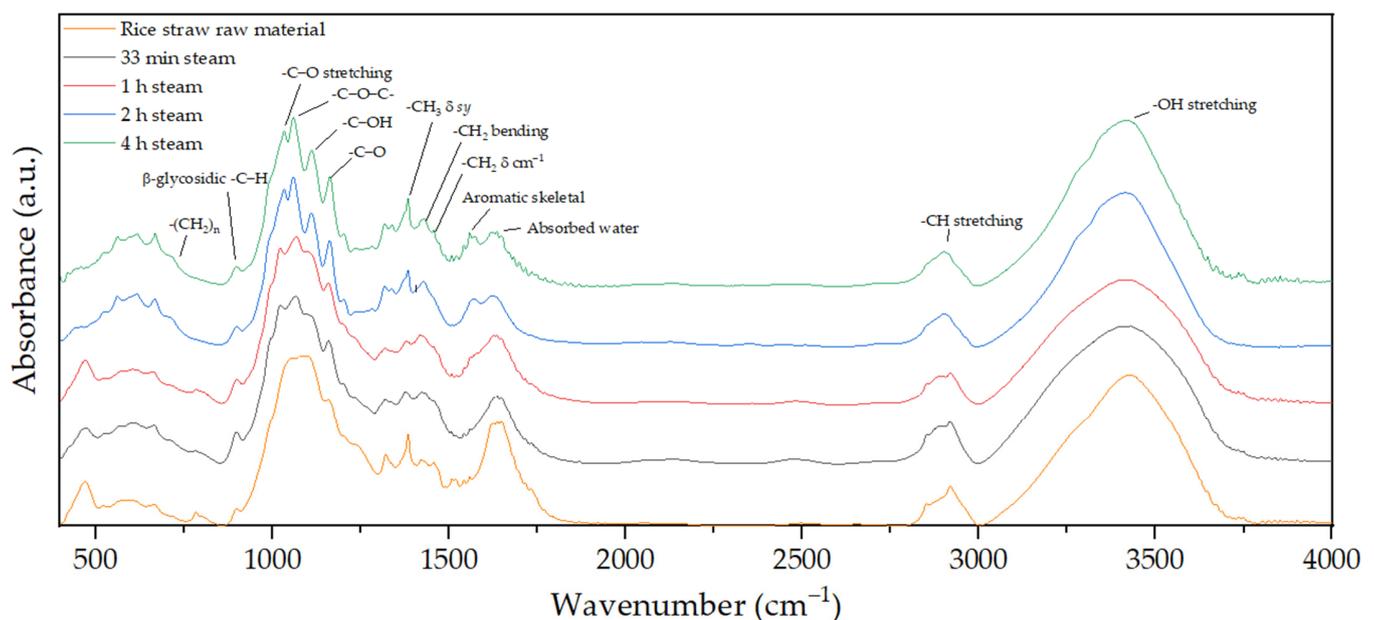


Figure 7. FTIR spectra of rice straw waste and the cellulose fibres extracted after different durations of steam explosion treatment.

Likewise, the thermogravimetric analysis (TGA) demonstrated a remarkable efficacy in identifying and quantifying the components of plant-based materials, specifically β -cellulose, lignin, and hemicellulose. This technique surpasses traditional wet chemical methods in accuracy [67] and is particularly adept for the high-throughput examination of polymers such as cellulose and lignin.

The data in Figure 8 elucidate the thermal degradation patterns of both biomass waste and the fibres derived from its processing in the research, as analysed using a TGA. The differential thermal decomposition behaviours observed in cellulose, hemicellulose, and lignin can be attributed to their unique molecular structures and chemical properties. Hemicellulose, comprised of a variety of sugars such as xylose, mannose, glucose, and galactose, presents a disordered, non-crystalline, and branched architecture [70]. This structure is responsible for its earlier decomposition into volatile substances (including CO, CO₂, and various hydrocarbons) at lower temperatures, typically in the 200–300 °C range [71]. Contrasting with hemicellulose, cellulose—a glucose-based polymer linked by β -1,4 glycosidic bonds—exhibits an enhanced strength and thermal resistance, decomposing mainly between 300–370 °C [71]. Lignin, out of the three, shows the most substantial thermal stability, a characteristic stemming from its complex network of aromatic rings and diverse branching patterns, resulting in a wider decomposition temperature spectrum (200–500 °C) [32]. The weight loss observed below 110 °C is primarily due to the release of moisture associated with the hydroxyl and carboxyl groups in the fibres [72].

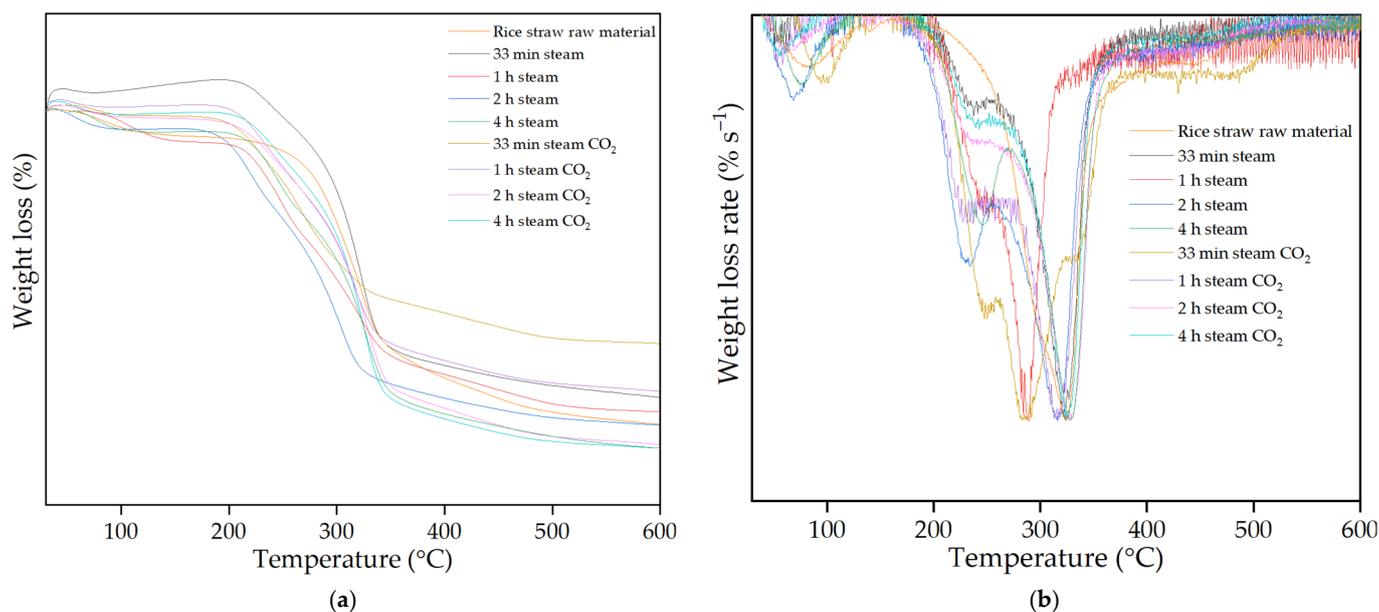


Figure 8. (a) TGA and (b) DTA curves corresponding to rice waste and the resulting fibres.

In the context of optimising the biomass pretreatment, Figure 8b displays the differential thermal analysis (DTA) curves derived from the TGA data, which have facilitated the determination of specific degradation ranges for the principal lignocellulosic components: hemicellulose, cellulose, and lignin. Based on these data, the TGA weight loss curves shown in Figure 8a were standardised, enabling the extraction of precise compositional percentages across varying durations of steam explosion treatment, both with and without the application of ScCO₂ [57]. The yields for each condition have also been provided. Table 3 illustrates the compositional breakdown and yields of the biomass subjected to different treatment durations.

When examining the influence of the reaction time on the results shown in Table 3, notable trends become apparent. In both cases, with and without ScCO₂, as the reaction time increases, the yield consistently decreases. However, the behaviour of the cellulose

content differs significantly. In the ScCO₂-involved reactions, there is a clear trend of maintaining the cellulose percentages regardless of the reaction time. Conversely, in the absence of ScCO₂, the cellulose percentage decreases with longer reaction times.

Table 3. Compositions and yields of the rice straw waste subjected to different treatment conditions.

Steam Explosion Process	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Yield (%)
ScCO ₂ 33 min	22.40 ± 2.84	60.12 ± 0.50	17.48 ± 3.34	25.00 ± 1.70
ScCO ₂ 1 h	30.79 ± 7.47	56.55 ± 1.67	12.66 ± 9.14	19.10 ± 1.90
ScCO ₂ 2 h	20.82 ± 1.63	66.74 ± 3.01	12.44 ± 1.38	17.60 ± 0.45
ScCO ₂ 4 h	26.49 ± 0.70	55.49 ± 0.89	18.02 ± 0.19	15.20 ± 1.70
33 min	14.31 ± 0.84	77.36 ± 0.94	8.33 ± 0.10	23.00 ± 0.15
1 h	23.42 ± 1.27	52.00 ± 2.10	24.58 ± 0.83	19.69 ± 1.00
2 h	27.79 ± 0.21	59.44 ± 0.24	12.77 ± 0.03	18.55 ± 0.55
4 h	28.15 ± 2.00	57.49 ± 2.54	14.36 ± 0.54	15.19 ± 0.75

When comparing the conditions, the data indicate that a 33 min steam explosion without ScCO₂ yields the highest percentage of cellulose (77.36% ± 0.94%) and the lowest percentage of lignin (8.33% ± 0.10%), with a notable yield of 23.00% ± 0.15%. This suggests an efficient delignification and the preservation of cellulose content, which is crucial for subsequent conversion processes. In contrast, longer durations and the introduction of ScCO₂ do not significantly enhance the cellulose content or yield. Specifically, ScCO₂ treatments appear to retain higher percentages of hemicellulose and lignin, with a subsequent decrease in the cellulose percentage. Likewise, as mentioned before, the yield consistently declines with extended treatment times, particularly noticeable at the 4 h mark with both ScCO₂ and non-ScCO₂ processes.

Therefore, the 33 min steam explosion treatment stands out as the most favourable, achieving a balance between cellulose preservation, lignin removal, and overall yield, thereby positioning as a key parameter for efficient biomass conversion.

These results are in line with the previously shown FTIR spectra, evidencing the structural preservation of cellulose in the biomass at the 33 min mark without ScCO₂ (Figure 7). This is evidenced by the stable ratio between the -OH stretching band at 3400 cm⁻¹ and the C-H stretching band around 2900 cm⁻¹ in the FTIR spectrum, which are associated with the hydroxyl groups of cellulose and its overall molecular structure. This aligns with the high cellulose content observed in the TGA. Conversely, the increasing intensity in the ratio between the -CH stretching vibration at 2900 cm⁻¹ and the C-O stretching band at 1054 cm⁻¹—representing cellulose and hemicellulose bonds—in the CO₂-treated samples over time aligns with the reduced cellulose yield from the TGA, suggesting cellulose destabilisation.

Furthermore, X-ray diffraction (XRD) patterns, shown in Figures 9 and 10, revealed the presence of crystalline cellulose Iβ in the extracted samples, with peaks at 16.5°, 22.6°, and 32.5° 2θ aligning with the (110), (200), and (004) planes, supporting the identification of this polymorph, which is consistent with the diffraction pattern of the reference cellulose (ICDD patent PDF-2 database, File no. 00-050-2241). Cellulose Iβ is the most dominant allomorph in higher plants and is frequently targeted for the production of high-quality nanocellulosic materials due to its crystalline nature [73]. The additional diffraction peaks observed throughout the 90° 2θ range indicate the presence of other crystalline constituents such as silica, a known component of rice straw. The presence of broad peaks throughout the diffractograms is indicative of a substantial amorphous fraction, which must be considered when interpreting the crystallinity indices (CI) and crystallite sizes (CS) provided in Table 4.

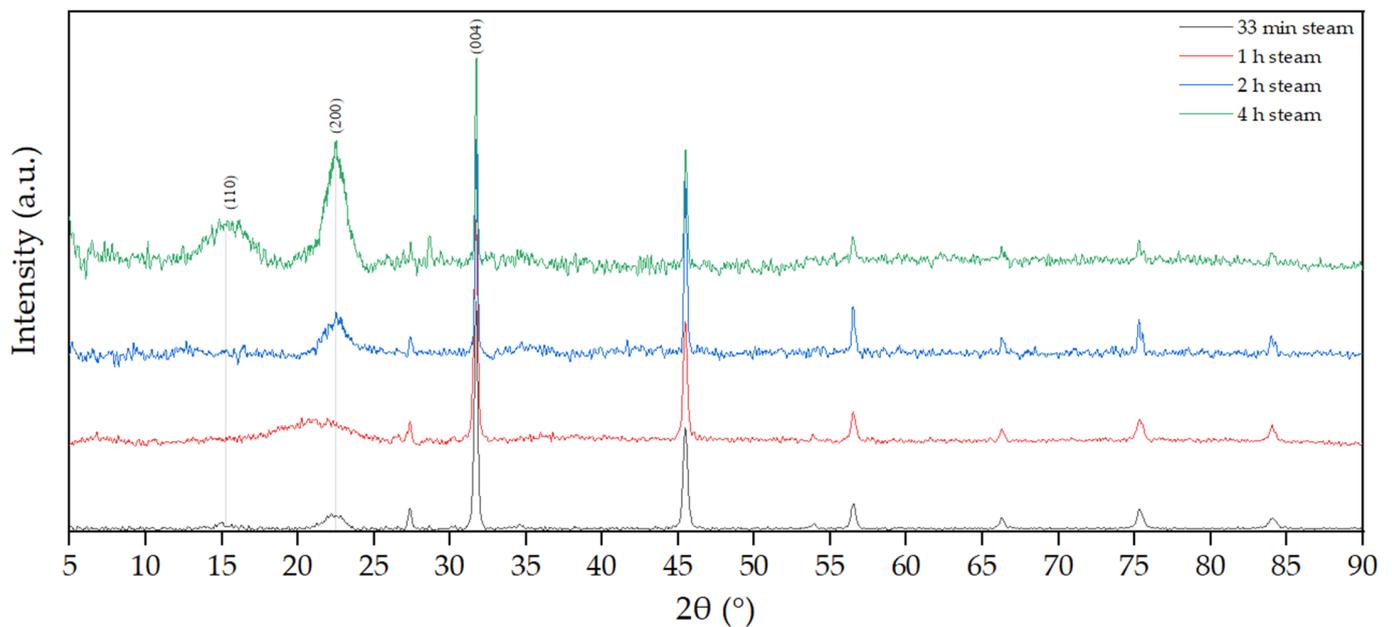


Figure 9. XRD profiles of extracted fibres after different durations of the steam explosion stage.

When comparing the steam explosion processes, the data evidence the influence of ScCO_2 on the crystalline and amorphous contents of cellulose. A longer exposure to steam in the presence of ScCO_2 is associated with a reduced crystallinity and crystallite size, as reflected by the calculated values. The steam-treated sample for 33 min without ScCO_2 exhibits a lower crystallinity index compared to the one with ScCO_2 , which could be affected by the amorphous broad component on the crystalline peaks. In that sense, the presence of broader peaks along the ScCO_2 diffractograms suggests that the absence of ScCO_2 potentially favours the preservation of the crystalline structure of cellulose.

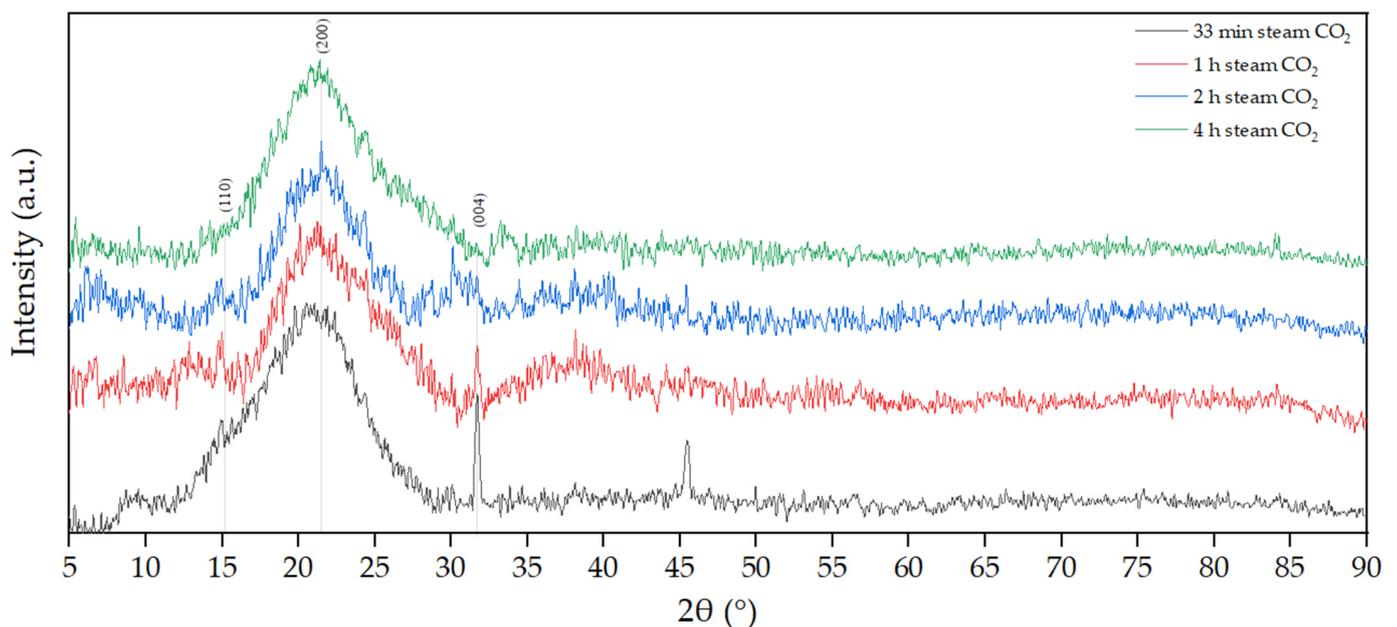


Figure 10. XRD profiles of extracted fibres after different durations of the steam explosion stage using ScCO_2 .

These findings highlight the importance of peak broadening and the amorphous background when evaluating the crystallinity of the samples. The higher crystallinity index observed in the steam-treated sample for 33 min without CO₂, despite not being the highest, may be more reflective of the true crystalline content, as it is less influenced by the broadening effects seen in other samples. This finding is consistent with the untreated natural fibres and demonstrates that the cellulose extracted from rice straw exhibits a crystallinity index that matches that which was reported for untreated natural fibres [32,69], suggesting that rice straw cellulose could be a suitable candidate for high-quality nanocellulosic materials.

Table 4. Crystallinity index (CI) and crystallite size (CS) obtained for the different steam explosion processes performed.

Steam Explosion Process	CI (%)	CS (nm)
ScCO ₂ 33 min	66.47	12.18
ScCO ₂ 1 h	49.90	12.90
ScCO ₂ 2 h	64.32	4.37
ScCO ₂ 4 h	37.03	1.65
33 min	46.20	11.30
1 h	53.57	14.14
2 h	36.31	13.34
4 h	52.72	15.51

Figure 11 contains SEM images illustrating the morphology of the extracted cellulose fibres. The rice straw image depicts its structure before undergoing treatment, revealing the presence of a cell wall composed of hemicellulose and lignin, which serves to protect the cellulose.

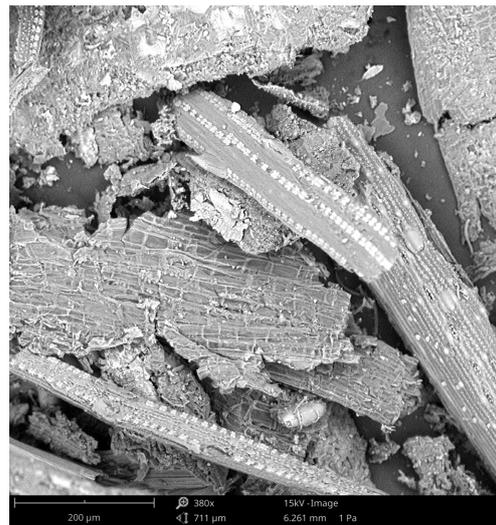
Throughout the analysis, significant differences have been noted between the treatments with and without ScCO₂. The samples treated with ScCO₂ exhibited an increased compactness in the cellulose fibres, which is indicative of a higher degree of amorphousness, in line with the previous discussion on the XRD. These fibres appeared denser and less defined, suggesting a profound alteration in the cellulose structure due to the presence of ScCO₂. This effect is likely attributed to an enhanced breakdown of cellulose's crystalline structure, leading to the predominance of amorphous cellulose.

Contrastingly, the samples treated without ScCO₂, especially the 33 min treatment, presented better preserved fibrous structure of cellulose [32,74,75]. These fibres are more defined and less compact, indicating the retention of crystalline structures and consequently, less amorphousness. As confirmed in the FTIR and XRD tests, this treatment was more effective in removing lignin and hemicellulose without significantly altering the crystalline structure of cellulose.

The SEM images also reveal varying degrees of defibrillation in the cellulose fibres. The treatments, particularly those involving ScCO₂, facilitated a more complete defibrillation, breaking down the fibres into finer structures. In contrast, the treatments without ScCO₂ led to the fibres retaining more of their original, less fragmented form.

Furthermore, this analysis indicated the presence of possible remnants of cell wall materials or silica oxide crystals, characteristic of rice straw. These remnants were more evident in the samples treated less aggressively, preserving more of the original biomass structure.

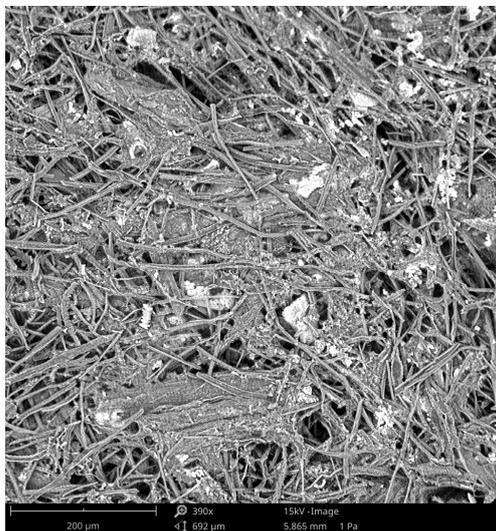
These findings provide valuable insights into the effects of steam explosion treatments with and without ScCO₂ on the structure of the cellulose obtained from rice straw. Therefore, they underline the crucial role of the treatment conditions in determining the final characteristics of the cellulose fibres.



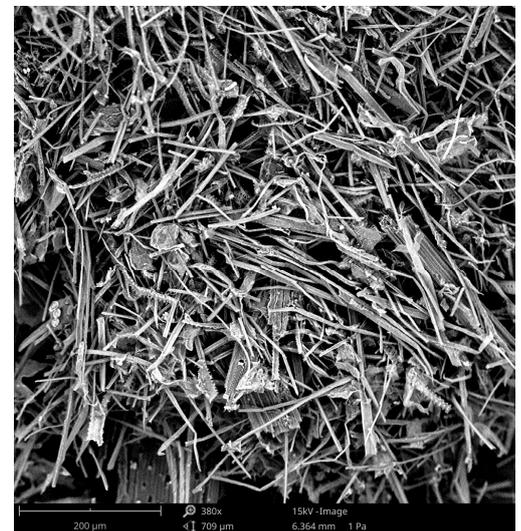
(a)



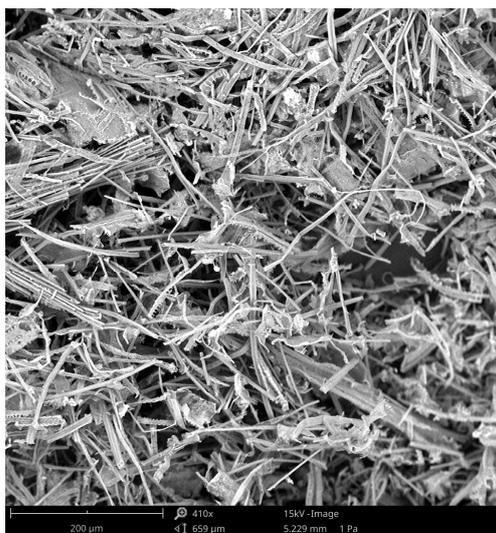
(b)



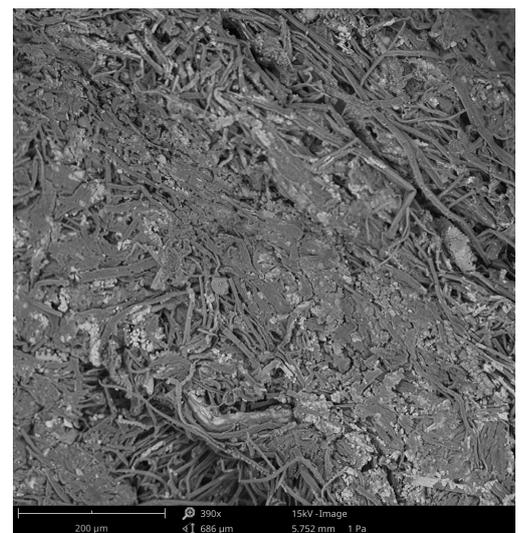
(c)



(d)



(e)



(f)

Figure 11. Cont.

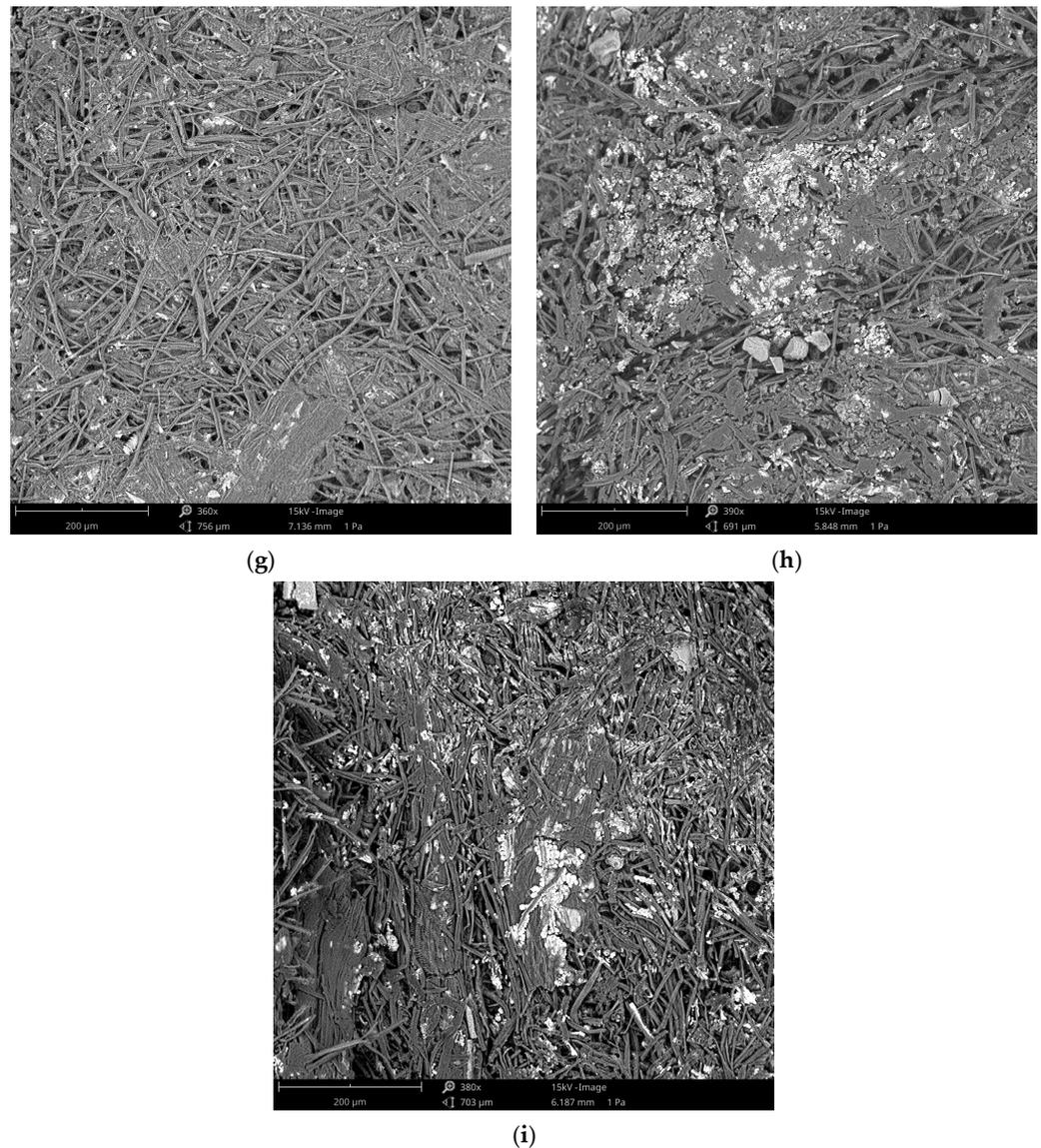


Figure 11. SEM images of the fibres extracted at different steam explosion conditions. (a) Untreated rice straw, (b) 33 min, (c) 1 h, (d) 2 h, (e) 4 h, (f) 33 min ScCO₂, (g) 1 h ScCO₂, (h) 2 h ScCO₂, and (i) 4 h ScCO₂.

3.3. Characterisation of Lignin

Transitioning from the characterisation of cellulose to the exploration of lignin extraction, this study narrows its scope to samples that showed exceptional outcomes in the cellulose extraction. Specifically, samples subjected to 33 min and 1 h of steam explosion with and without ScCO₂ were selected for lignin isolation from the lignin- and hemicellulose-rich supernatant post-steam explosion. The rationale for this selection was underpinned by the FTIR, TGA, XRD, and SEM analyses, which justified the preferential choice of the 33 min steam explosion without ScCO₂, reducing the time of this stage. Utilising the supernatant from the steam explosion stage, which contains high concentrations of lignin and hemicellulose, the main goal was to isolate lignin for future purification steps.

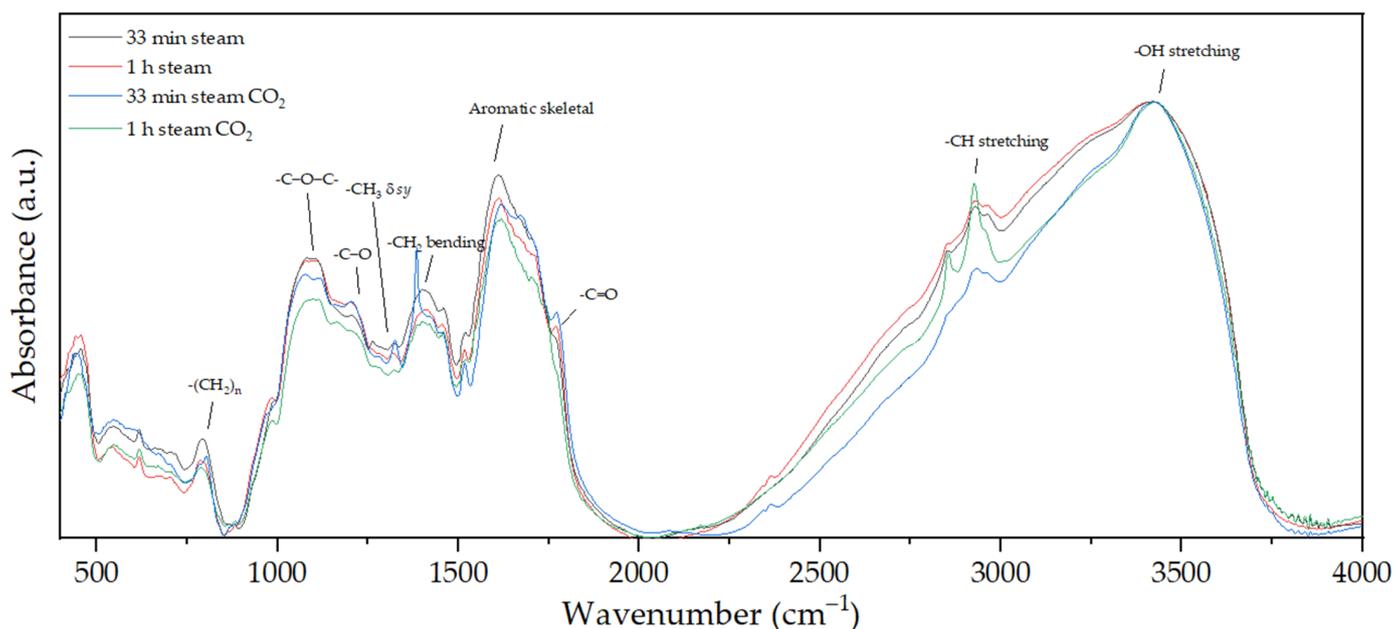
Table 5 indicates the percentage composition of hemicellulose, cellulose, and lignin in each sample, derived from the established ranges in the TGA derivatives and standardised TGA data.

Table 5. Compositions and yields of the steam explosion supernatant at different treatment conditions.

Steam Explosion Process	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Yield
33 min	23.78 ± 1.78	24.57 ± 1.19	51.65 ± 2.88	19.96 ± 0.47
1 h	25.99 ± 1.46	27.27 ± 1.33	46.74 ± 2.79	13.64 ± 1.58
33 min ScCO ₂	26.96 ± 0.49	31.17 ± 0.92	41.87 ± 0.43	14.12 ± 1.53
1 h ScCO ₂	32.04 ± 0.96	23.52 ± 0.85	44.44 ± 1.81	17.21 ± 0.80

A comparative analysis of the TGA data revealed notable differences between the samples treated with and without ScCO₂. The samples subjected to steam explosion for 33 min without ScCO₂ demonstrated a more favourable lignin yield compared to their counterparts. Moreover, the absence of ScCO₂ in the steam explosion process appeared to enhance the selectivity of the lignin extraction, as evidenced by the lignin content. Notably, the lignin content in the samples without ScCO₂ was consistently higher across the stages, particularly in the optimised 33 min condition.

Furthermore, the FTIR analysis performed (Figure 12) provides a molecular-level insight into the lignin structure of rice straw treated with steam explosion under various conditions. The FTIR spectra show the analysis of the lignin extracted from a biomass substrate through a steam explosion process at 33 min and 1 h, with and without the presence of ScCO₂. The four spectra show different peaks that can be correlated with lignin content. These include the aromatic skeletal vibrations around 1500 cm⁻¹, the -C-O stretching indicative of guaiacyl and syringyl lignin units, and the -CH stretching which suggests aliphatic hydrocarbons [49]. The prominence of -OH stretching near 3400 cm⁻¹ suggests a high concentration of phenolic and aliphatic OH groups, typically abundant in lignin [76].

**Figure 12.** FTIR spectra performed for the supernatants obtained after carrying out the different steam explosion processes.

When comparing the 33 min without ScCO₂ spectrum to the other treatments, the former displays sharper and more defined peaks, suggesting less degradation and a higher preservation of the aromatic ring structure of lignin. The addition of ScCO₂ in the 1 h treatment seems to affect the lignin structure, as evidenced by the slight shifts and changes in the peak intensities, particularly in the functional groups associated with the aromatic

ring and the -C–O stretching, which might indicate alterations in the ether bonds within the lignin.

Considering the TGA data provided, they indicate a higher yield of lignin in the 33 min sample, which is corroborated by the FTIR analysis. The sharper peaks and their positions closely resemble those of pure lignin, suggesting that the shorter steam treatment preserves lignin better than the longer one and that the absence of ScCO₂ prevents further modifications of the lignin structure during the process.

This preliminary study lays the groundwork for future endeavours in lignin purification and underlines the efficacy of the optimised steam explosion conditions in lignin extraction from rice straw [8,48,49].

4. Conclusions

This study explores the extraction of cellulose and lignin from rice straw through a four-stage process, focusing on varying and optimising each stage to determine the most efficient conditions. Upon completion of the experimentation, it was concluded that the ideal approach excludes the alkaline pretreatment stage and involves three hours of oxidation and one hour of bleaching. The study also evaluates the effect of ScCO₂ in the steam explosion stage over different durations (from 33 min to 4 h) based on different characterisation techniques.

The consolidated findings from various analyses indicate significant chemical, thermal, and structural changes in the cellulose extracted from rice straw. The FTIR and SEM analyses revealed that non-ScCO₂ treatments effectively retained the cellulose structure while upholding a superior fibrous structure in the samples. This observation aligns with the TGA results, which identified a 33 min steam explosion treatment without ScCO₂ as optimal for maintaining the cellulose integrity and for effective lignin removal. XRD patterns confirmed the presence of crystalline cellulose I β , with non-ScCO₂ treated samples exhibiting a more effective preservation of the crystalline structure of cellulose. These results demonstrate the significant influence of the treatment conditions on the properties of rice straw-derived cellulose.

Furthermore, regarding the extraction of lignin from the supernatant of the steam explosion stage, the four processes considered most optimal for cellulose extraction were selected and analysed using FTIR and TGA. The TGA results showed that the 33 min steam explosion condition without ScCO₂ yields a higher lignin content compared to other conditions, and the absence of ScCO₂ improved the selectivity of lignin extraction. FTIR analysis also revealed that the 33 min condition better preserves the aromatic ring structure of lignin compared to longer treatments or those with ScCO₂.

To conclude, the study highlights the effectiveness of specific treatment conditions in preserving the cellulose integrity, which is crucial for its application in the production of high-quality nanocellulosic materials, while laying the groundwork for future efforts in lignin purification, emphasising the effectiveness of the optimised steam explosion conditions in lignin extraction from rice straw.

Author Contributions: Conceptualisation and investigation, V.M.S.-M. and H.P.-A.; methodology, V.M.S.-M.; validation and formal analysis, V.M.S.-M. and H.P.-A.; writing—original draft preparation, V.M.S.-M.; writing—review and editing, V.M.S.-M., H.P.-A. and M.P.C.-B.; supervision, F.A.-A. and E.O.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science and Innovation (MCIN) and the State Research Agency (AEI) through the grant number TED2021-129932B-C22, BioTech-RICE.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ramos, M.; Laveriano, E.; San Sebastián, L.; Perez, M.; Jiménez, A.; Lamuela-Raventos, R.M.; Garrigós, M.C.; Vallverdú-Queralt, A. Rice Straw as a Valuable Source of Cellulose and Polyphenols: Applications in the Food Industry. *Trends Food Sci. Technol.* **2023**, *131*, 14–27. [[CrossRef](#)]
2. Thongsomboon, W.; Baimark, Y.; Srihanam, P. Valorization of Cellulose-Based Materials from Agricultural Waste: Comparison between Sugarcane Bagasse and Rice Straw. *Polymers* **2023**, *15*, 3190. [[CrossRef](#)] [[PubMed](#)]
3. Singh, G.; Gupta, M.K.; Chaurasiya, S.; Sharma, V.S.; Pimenov, D.Y. Rice Straw Burning: A Review on Its Global Prevalence and the Sustainable Alternatives for Its Effective Mitigation. *Environ. Sci. Pollut. Res.* **2021**, *28*, 32125–32155. [[CrossRef](#)] [[PubMed](#)]
4. Yodkhum, S.; Sampattagul, S.; Gheewala, S.H. Energy and Environmental Impact Analysis of Rice Cultivation and Straw Management in Northern Thailand. *Environ. Sci. Pollut. Res.* **2018**, *25*, 17654–17664. [[CrossRef](#)]
5. Yang, S.; He, H.; Lu, S.; Chen, D.; Zhu, J. Quantification of Crop Residue Burning in the Field and Its Influence on Ambient Air Quality in Suqian, China. *Atmos. Environ.* **2008**, *42*, 1961–1969. [[CrossRef](#)]
6. Ribo, M.; Albiach, R.; Pomares, F.; Canet, R. Alternativas de Gestión de La Paja de Arroz En La Albufera de Valencia. ReDivia. 2017. Available online: <http://hdl.handle.net/20.500.11939/5895> (accessed on 22 February 2024).
7. Kumari, D.; Singh, R. Rice Straw Structure Changes Following Green Pretreatment with Petha Wastewater for Economically Viable Bioethanol Production. *Sci. Rep.* **2022**, *12*, 10443. [[CrossRef](#)] [[PubMed](#)]
8. Dinh Vu, N.; Thi Tran, H.; Bui, N.D.; Duc Vu, C.; Viet Nguyen, H. Lignin and Cellulose Extraction from Vietnam's Rice Straw Using Ultrasound-Assisted Alkaline Treatment Method. *Int. J. Polym. Sci.* **2017**, *2017*, 1063695. [[CrossRef](#)]
9. Gou, G.; Wei, W.; Jiang, M.; Zhang, S.; Lu, T.; Xie, X.; Meng, F.; Zhou, Z. Environmentally Friendly Method for the Separation of Cellulose from Steam-Exploded Rice Straw and Its High-Value Applications. In *Pulp and Paper Processing*; InTech: Rijeka, Croatia, 2018. [[CrossRef](#)]
10. Islam, M.; Saini, P.; Das, R.; Shekhar, S.; Sinha, A.S.K.; Prasad, K. Rice Straw as a Source of Nanocellulose for Sustainable Food Packaging Materials: A Review. *Bioresources* **2022**, *18*, 2351–2385. [[CrossRef](#)]
11. Yang, J.; Ching, Y.; Chuah, C. Applications of Lignocellulosic Fibers and Lignin in Bioplastics: A Review. *Polymers* **2019**, *11*, 751. [[CrossRef](#)]
12. Agustin, M.B.; Ahmmad, B.; Alonzo, S.M.M.; Patriana, F.M. Bioplastic Based on Starch and Cellulose Nanocrystals from Rice Straw. *J. Reinf. Plast. Compos.* **2014**, *33*, 2205–2213. [[CrossRef](#)]
13. Dhali, K.; Ghasemlou, M.; Daver, F.; Cass, P.; Adhikari, B. A Review of Nanocellulose as a New Material towards Environmental Sustainability. *Sci. Total Environ.* **2021**, *775*, 145871. [[CrossRef](#)]
14. Urbina, L.; Corcuera, M.Á.; Gabilondo, N.; Eceiza, A.; Retegi, A. A Review of Bacterial Cellulose: Sustainable Production from Agricultural Waste and Applications in Various Fields. *Cellulose* **2021**, *28*, 8229–8253. [[CrossRef](#)]
15. Gu, J.; Hsieh, Y.-L. Alkaline Cellulose Nanofibrils from Streamlined Alkali Treated Rice Straw. *ACS Sustain. Chem. Eng.* **2017**, *5*, 1730–1737. [[CrossRef](#)]
16. Goswami, S. Cellulose Nanofibers from Rice Straw: Process Development for Improved Delignification and Better Crystallinity Index. *Trends Carbohydr. Res.* **2018**, *9*, 1–12.
17. Tang, P.L.; Hassan, O.; Yue, C.S.; Abdul, P.M. Lignin Extraction from Oil Palm Empty Fruit Bunch Fiber (OPEFBF) via Different Alkaline Treatments. *Biomass Convers. Biorefin* **2020**, *10*, 125–138. [[CrossRef](#)]
18. Bali, G.; Meng, X.; Deneff, J.I.; Sun, Q.; Ragauskas, A.J. The Effect of Alkaline Pretreatment Methods on Cellulose Structure and Accessibility. *ChemSusChem* **2015**, *8*, 275–279. [[CrossRef](#)] [[PubMed](#)]
19. Rodríguez, A.; Moral, A.; Serrano, L.; Labidi, J.; Jiménez, L. Rice Straw Pulp Obtained by Using Various Methods. *Bioresour. Technol.* **2008**, *99*, 2881–2886. [[CrossRef](#)]
20. Inkrod, C.; Raita, M.; Champreda, V.; Laosiripojana, N. Characteristics of Lignin Extracted from Different Lignocellulosic Materials via Organosolv Fractionation. *Bioenergy Res.* **2018**, *11*, 277–290. [[CrossRef](#)]
21. Zhang, H.; Wu, S. Generation of Lignin and Enzymatically Digestible Cellulose from Ethanol-Based Organosolv Pretreatment of Sugarcane Bagasse. *Cellulose* **2015**, *22*, 2409–2418. [[CrossRef](#)]
22. Rizwan, M.; Gilani, S.R.; Durrani, A.I.; Naseem, S. Cellulose Extraction of *Alstonia Scholaris*: A Comparative Study on Efficiency of Different Bleaching Reagents for Its Isolation and Characterization. *Int. J. Biol. Macromol.* **2021**, *191*, 964–972. [[CrossRef](#)]
23. Kaur, D.; Bhardwaj, N.K.; Lohchab, R.K. Reduction in Chlorophenolic Compounds during Bleaching of Rice Straw Pulp by Replacing Elemental Chlorine with Chlorine Dioxide. *Int. J. Environ. Sci. Technol.* **2018**, *15*, 1113–1122. [[CrossRef](#)]
24. Karim, Z.; Afrin, S.; Husain, Q.; Danish, R. Necessity of Enzymatic Hydrolysis for Production and Functionalization of Nanocelluloses. *Crit. Rev. Biotechnol.* **2017**, *37*, 355–370. [[CrossRef](#)] [[PubMed](#)]
25. Hassan, M.; Berglund, L.; Hassan, E.; Abou-Zeid, R.; Oksman, K. Effect of Xylanase Pretreatment of Rice Straw Unbleached Soda and Neutral Sulfite Pulps on Isolation of Nanofibers and Their Properties. *Cellulose* **2018**, *25*, 2939–2953. [[CrossRef](#)]
26. Isogai, A.; Hänninen, T.; Fujisawa, S.; Saito, T. Review: Catalytic Oxidation of Cellulose with Nitroxyl Radicals under Aqueous Conditions. *Prog. Polym. Sci.* **2018**, *86*, 122–148. [[CrossRef](#)]
27. Lan, W.; Liu, C.-F.; Sun, R.-C. Fractionation of Bagasse into Cellulose, Hemicelluloses, and Lignin with Ionic Liquid Treatment Followed by Alkaline Extraction. *J. Agric. Food Chem.* **2011**, *59*, 8691–8701. [[CrossRef](#)]

28. Yang, D.; Zhong, L.-X.; Yuan, T.-Q.; Peng, X.-W.; Sun, R.-C. Studies on the Structural Characterization of Lignin, Hemicelluloses and Cellulose Fractionated by Ionic Liquid Followed by Alkaline Extraction from Bamboo. *Ind. Crops Prod.* **2013**, *43*, 141–149. [[CrossRef](#)]
29. Shi, S.-C.; Liu, G.-T. Cellulose Nanocrystal Extraction from Rice Straw Using a Chlorine-Free Bleaching Process. *Cellulose* **2021**, *28*, 6147–6158. [[CrossRef](#)]
30. Magalhães, S.; Fernandes, C.; Pedrosa, J.F.S.; Alves, L.; Medronho, B.; Ferreira, P.J.T.; Rasteiro, M.d.G. Eco-Friendly Methods for Extraction and Modification of Cellulose: An Overview. *Polymers* **2023**, *15*, 3138. [[CrossRef](#)]
31. Abolore, R.S.; Jaiswal, S.; Jaiswal, A.K. Green and Sustainable Pretreatment Methods for Cellulose Extraction from Lignocellulosic Biomass and Its Applications: A Review. *Carbohydr. Polym. Technol. Appl.* **2024**, *7*, 100396. [[CrossRef](#)]
32. Pérez-Limiñana, M.A.; Pérez-Aguilar, H.; Ruzafa-Silvestre, C.; Orgilés-Calpena, E.; Arán-Ais, F. Effect of Processing Time of Steam-Explosion for the Extraction of Cellulose Fibers from Phoenix Canariensis Palm Leaves as Potential Renewable Feedstock for Materials. *Polymers* **2022**, *14*, 5206. [[CrossRef](#)]
33. Kempainen, K.; Inkinen, J.; Uusitalo, J.; Nakari-Setälä, T.; Siika-aho, M. Hot Water Extraction and Steam Explosion as Pretreatments for Ethanol Production from Spruce Bark. *Bioresour. Technol.* **2012**, *117*, 131–139. [[CrossRef](#)] [[PubMed](#)]
34. Ouyang, X. Effect of Simultaneous Steam Explosion and Alkaline Depolymerization on Corn Cob Lignin and Cellulose Structure. *Chem. Biochem. Eng. Q.* **2018**, *32*, 177–189. [[CrossRef](#)]
35. Dai, N.H.; Huynh, K.T.T.; Nguyen, T.A.D.; Do, V.V.T.; Van Tran, M. Hydrothermal and Steam Explosion Pretreatment of Bambusa Stenostachya Bamboo. *Waste Biomass Valorization* **2021**, *12*, 4103–4112. [[CrossRef](#)]
36. Pirozzi, A.; Ferrari, G.; Donsi, F. Cellulose Isolation from Tomato Pomace Pretreated by High-Pressure Homogenization. *Foods* **2022**, *11*, 266. [[CrossRef](#)]
37. Aggarwal, N.; Pal, P.; Sharma, N.; Saravanamurugan, S. Consecutive Organosolv and Alkaline Pretreatment: An Efficient Approach toward the Production of Cellulose from Rice Straw. *ACS Omega* **2021**, *6*, 27247–27258. [[CrossRef](#)]
38. Ziegler-Devin, I.; Chrusciel, L.; Brosse, N. Steam Explosion Pretreatment of Lignocellulosic Biomass: A Mini-Review of Theoretical and Experimental Approaches. *Front. Chem.* **2021**, *9*, 705358. [[CrossRef](#)] [[PubMed](#)]
39. Sun, D.; Lv, Z.-W.; Rao, J.; Tian, R.; Sun, S.-N.; Peng, F. Effects of Hydrothermal Pretreatment on the Dissolution and Structural Evolution of Hemicelluloses and Lignin: A Review. *Carbohydr. Polym.* **2022**, *281*, 119050. [[CrossRef](#)] [[PubMed](#)]
40. Chen, H.-Z.; Liu, Z.-H. Multilevel Composition Fractionation Process for High-Value Utilization of Wheat Straw Cellulose. *Biotechnol. Biofuels* **2014**, *7*, 137. [[CrossRef](#)] [[PubMed](#)]
41. Padilla-Rascón, C.; Ruiz, E.; Romero, I.; Castro, E.; Oliva, J.M.; Ballesteros, I.; Manzanares, P. Valorisation of Olive Stone By-Product for Sugar Production Using a Sequential Acid/Steam Explosion Pretreatment. *Ind. Crops Prod.* **2020**, *148*, 112279. [[CrossRef](#)]
42. Li, X.; Tabil, L.G.; Panigrahi, S. Chemical Treatments of Natural Fiber for Use in Natural Fiber-Reinforced Composites: A Review. *J. Polym. Environ.* **2007**, *15*, 25–33. [[CrossRef](#)]
43. Tribot, A.; Amer, G.; Abdou Alio, M.; de Baynast, H.; Delattre, C.; Pons, A.; Mathias, J.-D.; Callois, J.-M.; Vial, C.; Michaud, P.; et al. Wood-Lignin: Supply, Extraction Processes and Use as Bio-Based Material. *Eur. Polym. J.* **2019**, *112*, 228–240. [[CrossRef](#)]
44. Tanis, M.H.; Wallberg, O.; Galbe, M.; Al-Rudainy, B. Lignin Extraction by Using Two-Step Fractionation: A Review. *Molecules* **2023**, *29*, 98. [[CrossRef](#)] [[PubMed](#)]
45. Putrino, F.M.; Tedesco, M.; Bodini, R.B.; de Oliveira, A.L. Study of Supercritical Carbon Dioxide Pretreatment Processes on Green Coconut Fiber to Enhance Enzymatic Hydrolysis of Cellulose. *Bioresour. Technol.* **2020**, *309*, 123387. [[CrossRef](#)] [[PubMed](#)]
46. Nanta, P.; Kasemwong, K.; Skolpap, W.; Shimoyama, Y. Influence of Supercritical Carbon Dioxide Treatment on the Physicochemical Properties of Cellulose Extracted from Cassava Pulp Waste. *J. Supercrit. Fluids* **2019**, *154*, 104605. [[CrossRef](#)]
47. Wimmer, Z.; Zarevúcka, M. A Review on the Effects of Supercritical Carbon Dioxide on Enzyme Activity. *Int. J. Mol. Sci.* **2010**, *11*, 233–253. [[CrossRef](#)] [[PubMed](#)]
48. Boeriu, C.G.; Fițișău, F.I.; Gosselink, R.J.A.; Frissen, A.E.; Stoutjesdijk, J.; Peter, F. Fractionation of Five Technical Lignins by Selective Extraction in Green Solvents and Characterisation of Isolated Fractions. *Ind. Crops Prod.* **2014**, *62*, 481–490. [[CrossRef](#)]
49. D’Orsi, R.; Di Fidio, N.; Antonetti, C.; Raspolli Galletti, A.M.; Operamolla, A. Isolation of Pure Lignin and Highly Digestible Cellulose from Defatted and Steam-Exploded *Cynara cardunculus*. *ACS Sustain. Chem. Eng.* **2023**, *11*, 1875–1887. [[CrossRef](#)] [[PubMed](#)]
50. Zeronian, S.H.; Inglesby, M.K. Bleaching of Cellulose by Hydrogen Peroxide. *Cellulose* **1995**, *2*, 265–272. [[CrossRef](#)]
51. Mun, J.S.; Mun, S.P. Alkaline Hydrogen Peroxide Delignification of Three Lignocellulosic Biomass under Atmospheric Pressure. *Bioresources* **2023**, *19*, 998–1009. [[CrossRef](#)]
52. Bitwell, C.; Indra, S.S.; Luke, C.; Kakoma, M.K. A Review of Modern and Conventional Extraction Techniques and Their Applications for Extracting Phytochemicals from Plants. *Sci. Afr.* **2023**, *19*, e01585. [[CrossRef](#)]
53. Sutrisno, E.; Tanpichai, S.; Phiriyawirut, M.; Chuangchote, S. Investigation of Cellulose Nanofibers (CNFs) from Macaranga Hypoleuca Pulp in Chemical Treatment Phase. *Mater. Today Proc.* **2020**, *23*, 674–680. [[CrossRef](#)]
54. Aurelia, C.; Murdiati, A.; Ningrum, A. Effect of Sodium Hydroxide and Sodium Hypochlorite on the Physicochemical Characteristics of Jack Bean Skin (*Canavalia ensiformis*). *Pak. J. Nutr.* **2019**, *18*, 193–200. [[CrossRef](#)]
55. Świątek, K.; Gaag, S.; Klier, A.; Kruse, A.; Sauer, J.; Steinbach, D. Acid Hydrolysis of Lignocellulosic Biomass: Sugars and Furfurals Formation. *Catalysts* **2020**, *10*, 437. [[CrossRef](#)]

56. Ferrández-García, A.; Ferrández-Villena, M.; Ferrández-García, C.E.; García-Ortuño, T.; Ferrández-García, M.T. Potential Use of Phoenix Canariensis Biomass in Binderless Particleboards at Low Temperature and Pressure. *Bioresources* **2017**, *12*, 6698–6712. [CrossRef]
57. Song, C.; Hu, H.; Zhu, S.; Wang, G.; Chen, G. Nonisothermal Catalytic Liquefaction of Corn Stalk in Subcritical and Supercritical Water. *Energy Fuels* **2004**, *18*, 90–96. [CrossRef]
58. Carbonell-Blasco, P.; Martín-Martínez, J.M.; Antoniac, I.V. Synthesis and Characterization of Polyurethane Sealants Containing Rosin Intended for Sealing Defect in Annulus for Disc Regeneration. *Int. J. Adhes. Adhes.* **2013**, *42*, 11–20. [CrossRef]
59. Khan, A.; Toufiq, A.M.; Tariq, F.; Khan, Y.; Hussain, R.; Akhtar, N.; Rahman, S.U. Influence of Fe Doping on the Structural, Optical and Thermal Properties of α -MnO₂ Nanowires. *Mater. Res. Express* **2019**, *6*, 65043. [CrossRef]
60. Vinila, V.S.; Isac, J. Synthesis and Structural Studies of Superconducting Perovskite GdBa₂Ca₃Cu₄O_{10.5+ δ} Nanosystems. In *Design, Fabrication, and Characterization of Multifunctional Nanomaterials*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 319–341. [CrossRef]
61. French, A.D. Idealized Powder Diffraction Patterns for Cellulose Polymorphs. *Cellulose* **2014**, *21*, 885–896. [CrossRef]
62. Abou-Sekkina, M.M.; Sakran, M.A.; Saafan, A.A. Development of Correlations among the Spectral, Structural, and Electrical Properties and Chemical Treatment of Egyptian Cotton Fabric Strips. *Ind. Eng. Chem. Prod. Res. Dev.* **1986**, *25*, 676–680. [CrossRef]
63. Silvestre, C.R.; Blasco, M.P.C.; López, S.R.; Aguilar, H.P.; Limiñana, M.Á.P.; Gil, E.B.; Calpena, E.O.; Ais, F.A. Hydrophobic Leather Coating for Footwear Applications by a Low-Pressure Plasma Polymerisation Process. *Polymers* **2021**, *13*, 3549. [CrossRef] [PubMed]
64. Batanero, C.; Batanero, M.C.D. *Análisis de Datos Con Statgraphics*; Departamento de Didáctica de la Matemática, Universidad de Granada: Granada, Spain, 2008; ISBN 9788469147962.
65. Statpoint Technologies, Inc. STATGRAPHICS®Centurion XVII: Main Manual. 2014. Available online: <https://www.statgraphics.net/wp-content/uploads/2015/03/Centurion-XVII-Manual-Principal.pdf> (accessed on 22 February 2024).
66. Leenen, I. La Prueba de La Hipótesis Nula y Sus Alternativas: Revisión de Algunas Críticas y Su Relevancia Para Las Ciencias Médicas. *Investig. Educ. Médica* **2012**, *1*, 225–234. [CrossRef]
67. Lupoi, J.S.; Singh, S.; Parthasarathi, R.; Simmons, B.A.; Henry, R.J. Recent Innovations in Analytical Methods for the Qualitative and Quantitative Assessment of Lignin. *Renew. Sustain. Energy Rev.* **2015**, *49*, 871–906. [CrossRef]
68. Shaikh, H.M.; Anis, A.; Poulouse, A.M.; Al-Zahrani, S.M.; Madhar, N.A.; Alhamidi, A.; Alam, M.A. Isolation and Characterization of Alpha and Nanocrystalline Cellulose from Date Palm (*Phoenix dactylifera* L.) Trunk Mesh. *Polymers* **2021**, *13*, 1893. [CrossRef]
69. Sheeba, K.R.J.; Priya, R.K.; Arunachalam, K.P.; Shobana, S.; Avudaiappan, S.; Flores, E.S. Examining the Physico-Chemical, Structural and Thermo-Mechanical Properties of Naturally Occurring Acacia Pennata Fibres Treated with KMnO₄. *Sci. Rep.* **2023**, *13*, 20643. [CrossRef]
70. Abik, F.; Palasingh, C.; Bhattarai, M.; Leivers, S.; Ström, A.; Westereng, B.; Mikkonen, K.S.; Nypelö, T. Potential of Wood Hemicelluloses and Their Derivates as Food Ingredients. *J. Agric. Food Chem.* **2023**, *71*, 2667–2683. [CrossRef]
71. Diez, D.; Urueña, A.; Piñero, R.; Barrio, A.; Tamminen, T. Determination of Hemicellulose, Cellulose, and Lignin Content in Different Types of Biomasses by Thermogravimetric Analysis and Pseudocomponent Kinetic Model (TGA-PKM Method). *Processes* **2020**, *8*, 1048. [CrossRef]
72. Lu, X.; Zhu, X.; Guo, H.; Que, H.; Wang, D.; Liang, D.; He, T.; Hu, C.; Xu, C.; Gu, X. Investigation on the Thermal Degradation Behavior of Enzymatic Hydrolysis Lignin with or without Steam Explosion Treatment Characterized by TG-FTIR and Py-GC/MS. *Biomass Convers. Biorefin* **2022**, *12*, 5825–5834. [CrossRef]
73. Del Mundo, J.T.; Rongpipi, S.; Yang, H.; Ye, D.; Kiemle, S.N.; Moffitt, S.L.; Troxel, C.L.; Toney, M.F.; Zhu, C.; Kubicki, J.D.; et al. Grazing-Incidence Diffraction Reveals Cellulose and Pectin Organization in Hydrated Plant Primary Cell Wall. *Sci. Rep.* **2023**, *13*, 5421. [CrossRef] [PubMed]
74. Hou, X.; Sun, F.; Zhang, L.; Luo, J.; Lu, D.; Yang, Y. Chemical-Free Extraction of Cotton Stalk Bark Fibers by Steam Flash Explosion. *Bioresources* **2014**, *9*, 6950–6967. [CrossRef]
75. Ma, P.; Lan, J.; Feng, Y.; Liu, R.; Qu, J.; He, H. Effects of Continuous Steam Explosion on the Microstructure and Properties of Eucalyptus Fibers. *Bioresources* **2015**, *11*, 6950–6967. [CrossRef]
76. Diop, A.; Jradi, K.; Daneault, C.; Montplaisir, D. Kraft Lignin Depolymerization in an Ionic Liquid without a Catalyst. *Bioresources* **2015**, *10*, 4933–4946. [CrossRef]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.