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Influence of Chemical Pre-treatment on *Clitoria fairchildiana* Biomass for Nanocellulose Production

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Abstract

In the past ten years, interest in renewable materials has increased, driving research into plant fibers. This study extracts nanocellulose from Clitoria fairchildiana prunings from the semi-arid region of Alagoas using acid hydrolysis. The biomass was dried, ground, and characterized to determine the cellulose, lignin, and hemicellulose content. The pre-treatment included mercerization with 2% NaOH and bleaching with hydrogen peroxide combined with NaOH. The chemical treatment removed extractives, improving the surface morphology of the plant fiber, making it more suitable for the acid hydrolysis mechanism, removing amorphous and disordered regions, and increasing the frequency of crystalline domain regions, which are important for the extraction of cellulose nanocrystals. FTIR analyses showed an increase in cellulose content, while SEM suggested the removal of impurities such as lignin and hemicellulose, exposing the fibers and confirming modifications in surface morphology. Preliminary tests of cellulose nanocrystal extraction by acid hydrolysis indicated a yield of 54.32% in the mass of nanocellulose produced.

Keywords: Biomass; urban waste prunings; chemical treatment; cellulose nanocrystals.

1. Introduction

Cellulose is a natural macromolecule composed of glucose monomers, with great potential for various applications due to its properties. As a renewable and sustainable resource, it can be obtained from agricultural sources as well as urban and domestic waste (Penloglou, Basna, and Kiparissides, 2023).

The industrial demand for materials utilizing cellulose has been increasing, mainly due to its application in various sectors, such as paper production, packaging, bioplastics, and the manufacturing of products requiring mechanical strength, lightness, and biodegradability. The extraction of nanocellulose has demonstrated that its production cost can be competitive, especially in applications where its unique properties offer significant advantages and economic value. The global nanocellulose market was valued at USD 319.5 million in 2021 and is expected to reach USD 1.063 billion by 2028, with a compound annual growth rate (CAGR) of 22.2% during the estimated period (Joe, 2013, as cited in Penloglou, Basna, and Kiparissides, 2023).

Nanocellulose is obtained by disintegrating cellulose at the nanometric scale and is divided into two main types: nanofibrillated cellulose (NFC) and cellulose nanocrystals (CNC). NFC is generated by mechanical methods such as homogenization and grinding, resulting in long and flexible nanofibers. CNC, on the other hand, is produced by chemical processes such as acid hydrolysis, which creates rigid and highly crystalline nanocrystals. Each type of nanocellulose exhibits distinct properties, such as high strength and stiffness, making them widely used in various industrial and scientific applications. *Clitoria fairchildiana*, essential for the biodiversity of the semi-arid region of Alagoas, plays a crucial role in afforestation and the preservation of native species, contributing to the environmental sustainability of the region (Da Silva, et al., 2024).

The pruning residues of this species can be used as biomass, offering a sustainable alternative for waste management and utilizing an abundant natural resource. The fibers obtained from *Clitoria fairchildiana* prunings are rich in cellulose, hemicellulose, minerals, and lignin, with their content varying depending on the origin of the raw material. The high cellulose content makes this biomass attractive for nanocellulose extraction (Braz et al., 2011).

The objective of this work is to analyze how pre-treatment affects the process of nanocellulose extraction from Clitoria fairchildiana pruning residues using conventional methods such as acid hydrolysis.

2. Methodololy

The Clitoria fairchildiana prunings collected in the semi-arid region of Batalha-AL were stored in plastic bags in a dry and ventilated environment until the chemical pre-treatment. After grinding and drying, the biomass was characterized according to methodologies from the literature: Chen et al. (2016) for extractives, Hojo et al. (2008) and Araújo et al. (2006) for ash content, Gouveia et al. (2009) for lignin, and the Tappi T 203 method (2009) to determine holocellulose, alpha-cellulose, and hemicellulose.

2.1. Characterization of untreated and pre-treated biomass.

The characterization of untreated and pre-treated biomass involved several steps. For extractive analysis, the ground biomass was placed in a Soxhlet apparatus with hexane/ethanol for 7 hours, and the extractives were dried and weighed. The moisture content was determined by heating the sample at 105°C until constant weight was achieved. The ash content was obtained by heating the biomass at 550°C. Lignin was isolated after treatment with sulfuric acid and autoclaving. Holocellulose was extracted using sodium chlorite and acetic acid, and alpha-cellulose was isolated by washing the holocellulose with sodium hydroxide. The hemicellulose content was determined by the difference between holocellulose and alpha-cellulose.

2.2 Chemical Pre-treatment of Biomass

The chemical treatment involved mercerization of 50 g of biomass in 1 L of 2% (w/v) NaOH at 80°C for 2 hours. Three methods of Bleaching was tested using three methods: 1) H_2O_2 30% (v/v) at 80°C for 1.5 hours, followed by filtration, neutralization, and drying at 55°C for 24 hours; 2) repetition of bleaching with 30% hydrogen peroxide under the same conditions as 1; 3) combination of 5% (w/v) NaOH and 30% (v/v) H_2O_2 at 80°C for 1 hour. The samples were then characterized and compared.

The untreated and pre-treated biomass were characterized by Scanning Electron Microscopy (SEM) and FTIR spectroscopy in the range of 600 to 4000 cm^{-1}.

2.3 Nanocellulose Extraction by Acid Hydrolysis

Cellulose nanocrystals were extracted from the pruning biomass by acid hydrolysis using 55% sulfuric acid. A mixture of 1 g of bleached biomass with 5% NaOH and 30% H_2O_2 and 50 mL of acid was stirred at 150 rpm and 45°C for 60 minutes. After the reaction, the solution was centrifuged three times and then dialyzed until reaching a pH of 6-7.

3. Resulted

The results concerning the elemental composition of the biomass are presented in Table 1.

Table1. Chemical Characterization of untreated

Components	Untreated Percentage (mean ± deviation)
Extractives	5,79± 0,10
Moisture	7,91±0,11
Ash	14,36±0,84
Lignin	12,22±0,59
Cellulose	37,20 ± 0,48
Hemicellulose	21,83 ± 1,01

Source: Authors, 2024

The raw pruning material contains percentages of cellulose, hemicellulose, and lignin, which are crucial for optimizing the process. A high lignin content acts as a natural binder, complicating the cellulose hydrolysis reactions, protecting the original fibers, and providing rigidity and strength to the plant material. Reducing the amount of lignin facilitates the production of cellulose nanocrystals (Martins, 2016; Almeida, 2023). In nanocellulose production, it is desirable to reduce the lignin content to obtain a purer material with specific advantageous properties. After the removal of extractives, lignin was also eliminated impurities, and the native cellulose was converted to type II cellulose, which has higher thermal stability. The yield of the biomass after mercerization was 64%, resulting in a fraction richer in cellulose.

The results presented in Table 2 demonstrated the efficiency of the chemical pre-treatment, evidenced by the reduction in lignin, hemicellulose, and other constituents considered undesirable for the nanocellulose extraction process (Chaves, 2023). The components of the fiber—cellulose, hemicellulose, and lignin—among other extractives and non-extractives, can affect the quality of the extracted nanocellulose.

Table2. Characterization of Biomass After Chemical Pre-treatment (Percentage Content)

Components	Lignin *	alphacellulose *	Hemicellulose *
Mercerized	27,72±0,23	64,548±1,93	3,00±1,04
Bleached 1x c/ H_2O_2	26,21±0,66	67,181±0,09	5,88±0,56
Bleached 2x H_2O_2	21,19±0,51	67,140±0,71	6,64±1,42
Bleached H_2O_2 + NaOH	23,16±1,93	66,839±0,84	7,26±0,05

* Mean value ± Deviation

Source: Authors, 2024.

Figure 1. untreated Biomass (A), Mercerized (B), Bleached with H_2O_2 (C), and Bleached with NaOH and H_2O_2 (D).

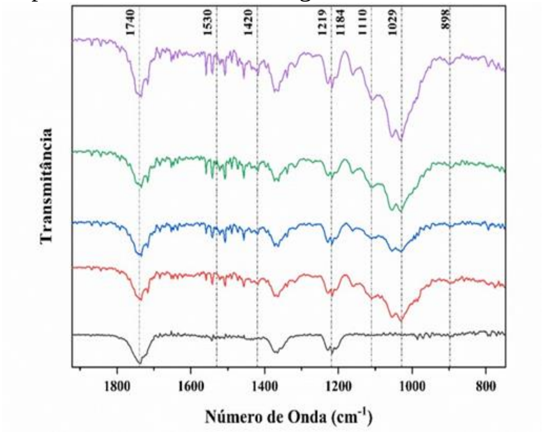
Source: Authors, 2024

Figure 1 presented satisfactory physical results, showing that the pre-treatment enabled a change in the biomass color, indicating alterations in its structure and the removal of compounds responsible for pigmentation. The color changed from medium brown to white.

Based on the results obtained through FTIR (Graphs 1), it is possible to observe the intensity of the signals, indicating a modification in the material's structure after mercerization and bleaching of the biomass.

Bleaching with NaOH combined with hydrogen peroxide was the most effective pre-treatment, as evidenced by the increase in cellulose peaks compared to the untreated sample and other pre-treatments. The prominence of the most evident peaks, corresponding to the main absorption bands of functional groups present in the samples, allows for the comparison of chemical changes that occurred after each treatment.

Graphs 1. Main absorption peaks, facilitating the comparison of chemical changes after each treatment.



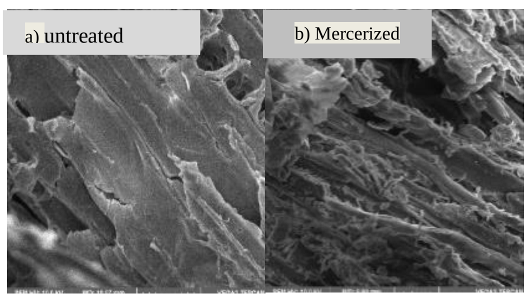
■ H_2O_2+NaOH ■ 2x H_2O_2 ■ 1x H_2O_2 ■ Mercerized ■*untreated*

Source: Authors, 2024

The peaks at 1219 and 898 cm^{-1} are associated with the C-O and C-H stretching of cellulose. It is important to note that between the peaks of 898 and 1184 cm^{-1}, there was no cellulose present before the pre-treatment. The stretching at the peak of 1530 cm^{-1} tends to increase with biomass treatment; this peak is characteristic of the presence of lignin, which is due to the vibration of aromatic C=C rings (Flauzino Neto, 2012). The peak at 1740 cm^{-1} refers to the stretching of acetyl groups and ester bonds in hemicellulose and lignin (Doh et al., 2020).

The results from Figures 2A and 2B show that the process led to a gradual change in morphology and exposure of the fibers.

Figure 2. Scanning Electron Microscopy (SEM) a) untreated b) Mercerized



In Figure 2A, the more regular surface and cellulose microfibrils connected indicated the removal of lignin and hemicellulose. Figure 2B showed finer and interconnected microfibrils, suggesting that the combined chemical treatment removed almost all the components of the cellulosic matrix, facilitating the separation of the fibers.

Preliminary tests of cellulose nanocrystal extraction by acid hydrolysis from the pruning of *Clitoria fairchildiana* revealed a nanocellulose mass yield of 54.32%. The results obtained are shown in Table 3.

Table 3- Yield of cellulose nanocrystals from acid hydrolysis

Biomass	Temperature °C	yield (%)
C. fairchildiana	45	54,32±4,30

Source: Authors, 2024

4. Conclusion

We conclude that the pre-treatment of *Clitoria fairchildiana* biomass proved effective, as evidenced by the significant reduction in lignin and hemicellulose after the chemical treatment. FTIR analyses revealed changes in the absorption bands of functional groups, allowing a detailed comparison of the chemical changes that occurred. SEM, in turn, highlighted the structural modifications on the fiber surface. The 54.32% yield reinforces the effectiveness of the pre-treatment and underscores the importance of considering different operational conditions to optimize the process.

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