DIFFERENT TREATMENTS OF PEARL MILLET BIOMASS FOR CELLULOSE RECOVERY: EFFECTS ON LIGNOCELLULOSIC COMPOSITION

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The objective of this study was to recover cellulose from pearl millet biomass through chemical (alkaline and acidic) pretreatments. The cellulosic fraction was characterized by Fourier transform spectroscopy (FTIR), X-ray diffraction (XRD), and Raman spectroscopy. The cellulose-rich fraction presented 100% and 99.86% of lignin and hemicelluloses removal, respectively, and a high crystallinity index of 82.43% after two 4-hour alkaline extractions with 4% NaOH, followed by 2 h extraction with 5% $C_2H_2O_4$ in an autoclave at a temperature of 125.6 °C and 1.4 bar. According to the FTIR, XRD, and Raman spectroscopy analyses, the removal of hemicelluloses and lignin from pearl millet biomass was confirmed, which indicated the efficiency of the pretreatments evaluated. Therefore, the results presented in this study should contribute to improving the efficiency of the fractionation processes of lignocellulosic biomasses.

Keywords: waste valorization, *Pennisetum glaucum*, oxalic acid, characterization

INTRODUCTION

Lignocellulosic biomass is the most abundant and valuable source of renewable and cheap raw materials.[1–4] Pearl millet (*Pennisetum glaucum*) belongs to the Poaceae family; it is easy to grow and has a short production cycle (120 days), adapting to different climatic conditions. In Brazil, the planted area is approximately 5 million hectares, and the leftovers after harvesting could reach 20 tons per hectare on a dry basis.[5–7] The millet is an important crop in Brazilian agriculture, serving as animal feed and as a ground-cover plant during the off-season. After that, the millet biomass becomes a readily available agricultural waste.[6,8–10]

The main constituents of this agricultural waste are the carbohydrates of cellulose and hemicelluloses, which constitute approximately 75% of the total composition of the lignocellulosic matrix,[1] varying between 30-50% for cellulose and 20-40% for hemicelluloses.[2,11] The plant may have lignin levels between 10-20%, which may vary according to the type of biomass.[11,12]

From a waste valorization perspective, the fractionation of this lignocellulosic residue into cellulose, hemicelluloses, and lignin is the first step for producing numerous products with a higher economic value, such as biofuel,[13–15] cosmetics,[16] biodegradable films,[17] paper and plastic materials,[17,18] large screens,[18] solar panels,[18,19] supercapacitor batteries,[19] medicine,[14,18] pharmaceuticals,[17] aircraft components and automobiles,[18,19] aerogels,[20] hydrogels[20,21] among others, minimizing the associated environmental impacts through the integration of technologies.

However, hemicelluloses are attached to cellulose, and these are surrounded by an amorphous matrix of lignin, which acts as a natural barrier to the attack of microorganisms, providing plant tissues with rigidity, resistance and impermeability.[5–7] In this sense, one of the main steps in the process of biomass fractionation is the pretreatment. Pretreatments disrupt the bonds between cellulose,

hemicelluloses, and lignin to increase the surface area of lignocellulsic biomass, maximise yields, and minimize the formation of inhibitory compounds throughout the process.[11,22,23] However, although the technologies are already well advanced, most of them still encounter technical or economic difficulties. Cellulose, hemicelluloses, and lignin can present impurities after separation and isolation processes.[1,24,25] Therefore, an efficient pretreatment step is necessary to fractionate lignocellulosic wastes, such as millet biomass, maximizing yields and minimizing unwanted compounds.[8,26]

Currently, the pretreatment technologies used can be either physical (milling, steam explosion, hydrothermal),[27–30] chemical (alkaline and acid hydrolysis),[11,26,31,32] biological (enzymatic pretreatment),[29,33,34] or a combination of these methods (acid–steam-explosion),[34–37]. Considering that each pretreatment presents advantages and disadvantages, the choice of the method should take into account the specifics of each approach.[35]

For instance, alkaline pretreatments are efficient for delignification, but they can remove some of the hemicelluloses.[14,23,26] On the other hand, pretreatments with dilute acids and steam explosion are effective to completely solubilize hemicelluloses. Acids are commonly used at low concentrations (<5%) and moderate temperatures (120-160 °C), while steam explosion involves high temperatures and pressures followed by rapid decompression, leading to mechanical rupture of the lignocellulosic material.[1,26,27,36] Another alternative is a sequential alkali-acid pretreatment, where the first step removes lignin using an alkaline agent, and the second step solubilizes hemicelluloses with dilute acid and/or steam explosion.[12,27,29,38–40]

Accordingly, this study aimed to isolate cellulose from millet biomass through chemical (alkaline and acidic), physical (milling, autoclave) and combined pretreatments, maximizing cellulose recovery and hemicelluloses and lignin solubilization. Fourier-transform infrared spectroscopy (FTIR-ATR), X-ray diffraction (DRX) and Raman spectroscopy were utilized to characterize the cellulose after the different pretreatments.

EXPERIMENTAL

Materials

Pearl millet was obtained from the agricultural experimental area of the Federal University of Fronteira Sul – UFFS, Campus Chapecó, Santa Catarina, Brazil (27°11' S and 52°70' W). After harvesting, the millet biomass was dried in an oven (AmericanLab model AL - 102/480) at 60 °C for 48 h, and milled in a Willye-type knife mill (AmericanLab model AL - 032S) to obtain particles of 0.6 mm maximum diameter. All chemicals and solvents used were of analytical grade.

Isolation of cellulose fibers

For performing the pretreatments, an experimental unit, composed of a stirred tank reactor and a mechanical agitator (Fisatom, Model 713D), coupled to a three-propeller naval type impeller, was adapted (Fig. 1). Through this experimental unit, five pretreatment methodologies were performed.

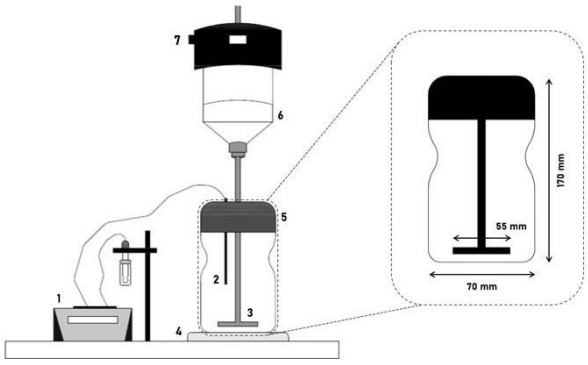

Figure 1: Schematic representation of the experimental setup: (1) bench pH meter, (2) temperature electrode, (3) impeller, (4) heater plate with temperature control, (5) reactor, (6) stirring motor and (7) stirring controller (rpm)

Alkaline pretreatment with sodium hydroxide

The method for cellulose extraction was based on the study reported by Souza *et al*.[23] For this, 20 g of biomass was treated with 200 mL of a 4% (wt/vol) solution of sodium hydroxide (NaOH) at 80 °C and 1200 rpm. Three tests with different reaction times (4, 8, and 12 h) were performed and, at four-hour intervals, the

reaction medium was neutralized with acetic acid (CH_3COOH) 3% (vol/vol) and vacuum filtered using high-quality filter paper. After the extractions, the cellulosic fractions, named 4H, 8H, and 12H, were dried in an oven (Vulcan model EESCRAF-115D) at 60 °C to constant weight for further physicochemical characterization.[41]

Neutralization step

To improve the neutralization step, two new neutralization tests (A and B) were performed with resuspension, based on the 8H pretreatment described above.

In test A, the reaction medium obtained after 4 h of pretreatment was vacuum filtered using high-quality filter paper. The remaining solid fraction underwent the same pretreatment again under the same conditions. At the end of the pretreatment (8 h), the solid fraction was filtered and resuspended in 100 mL of distilled water to then be neutralized with 3% acetic acid (vol/vol).

In test B, the reaction medium was vacuum filtered using high-quality filter paper, resuspended with 100 mL of distilled water, and neutralized with 3% acetic acid (vol/vol) every 4 h of extraction, *i.e.*, the procedure was performed twice between and at the end of the pretreatment (8 h).

After both neutralization tests (A and B), the cellulosic fractions, named Test A and Test B, were dried in an oven (Vulcan model EESCRAF-115D) at 60 °C to constant weight for further physicochemical characterization.[41]

Alkaline pretreatment combined with hydrogen peroxide (CT)

This extraction method was based on the study of Lenhani *et al.*[17] Thus, 20 g of pearl millet biomass was treated with a solution of NaOH 4% (wt/vol) and hydrogen peroxide (H_2O_2) 1% (vol/vol) in a ratio of 1:1. A solid-to-liquid ratio of 1:10 (wt/vol) was utilized in this test.

Accordingly, two 4-hour extraction cycles were performed at 80 °C and 1200 rpm under mechanical stirring. At the end of each extraction cycle, the solution was vacuum filtered using high-quality filter paper, resuspended with 100 mL of distilled water, neutralized with 3% acetic acid (vol/vol), and filtered again. Finally, the isolated cellulose, named CT, was dried in an oven (Vulcan model EESCRAF-115D) at 60 °C to constant weight for further physicochemical characterization.[41]

Alkali-acid pretreatment

The acid pretreatment was based on the study described by Hong *et al.*[42] The pretreatment with oxalic acid ($C_2H_2O_4$) was performed in two different concentrations: 3.8% and 5% (wt/vol) at a solid-liquid ratio of 1:10 (wt/vol).

Thus, for the alkali-acid pretreatment, two alkaline extractions were performed with sodium hydroxide (4% wt/vol) – as described in Test B (in Neutralization step) –, followed by an acid extraction for both acid concentrations (3.8% and 5% (wt/vol)) under agitation at 80 °C and 1200 rpm for 2 h. Subsequently, the cellulose fractions, named OA 3.8% and OA 5%, were resuspended with 100 mL of distilled water, neutralized with NaOH 12% (wt/vol), vacuum filtered using high-quality filter paper, and dried in an oven (Vulcan model EESCRAF-115D) at 60 °C to constant weight for further physicochemical characterization.[41]

Alkali-acid pretreatment in autoclave

In order to achieve better results, the pretreatment of the millet biomass was evaluated in an autoclave. The test was performed in the presence of three different chemical agents: sodium hydroxide (NaOH 4% wt/vol), oxalic acid ($C_2H_2O_4$ 5% wt/vol) and sulfuric acid (H_2SO_4 1% vol/vol). For the treatment, two alkaline extractions were performed with sodium hydroxide (4% wt/vol) – as described in Test B (in Neutralization step) –, followed by an extraction for each chemical agent ((NaOH 4% wt/vol), ($C_2H_2O_4$ 5% wt/vol) and (H_2SO_4 1% vol/vol)) in a vertical autoclave (AV Phoenix Luferco – 75 L) for 2 hours at 1.4 bar and 125.6 °C. The cellulose obtained – named ATA 4%, OAA 5%, SAA 1% corresponding to the treatments with NaOH, $C_2H_2O_4$, and H_2SO_4, respectively – was vacuum filtered using high-quality filter paper, resuspended with 100 mL of distilled water, neutralized with 3% acetic acid (vol/vol), filtered again and dried in an oven (Vulcan model EESCRAF-115D) at 60 °C to constant weight for physicochemical characterization.[41]

Characterization of cellulose fibers

Physicochemical characterization

Moisture, ash, extractives, total lignin and carbohydrate contents were determined by the analytical methods described by the National Renewable Energy Laboratory.[41]

To determine lignin, cellulose and hemicelluloses contents, 3.0 mL of sulfuric acid (72% vol/vol) were added for every 0.3 g of sample. The samples were placed in a thermostatic bath at 30 °C for 1 h to carry out the acid hydrolysis, being homogenized every 5-10 min. After the time of the concentrated acid hydrolysis, the samples were removed from the bath and the concentration of the medium was diluted to 4% using 84 mL of distilled water. For the complete hydrolysis of the oligomers, the flasks were sealed and autoclaved for 1 h at 121 °C and

1.1 bar. Finally, the solid and liquid phases were separated by vacuum filtration.[43] The liquid fractions used were filtered using nylon filters with 0.45 µm to quantify soluble lignin, carbohydrates, acetyl groups, furfural, and 5-hydroxymethylfurfural.

For the quantification of carbohydrates and decomposition products, a high-performance liquid chromatograph (HPLC) model LC-MS 2020 of the Shimadzu brand was used, equipped with a refractive index detector (RID-1, Shimadzu) and a column for organic acids (Aminex HPX-87H, Bio-Rad). The mobile phase used 5 mM sulfuric acid at 50 °C with a flow rate of 0.6 mL/min. The concentrations of the decomposition products were determined using an SPD-M20A detector operated with an NST-18 column, eluted with 85:15 acetonitrile/water and acetic acid 1% at 40 °C and flow rate of 0.8 mL/min.[43,44]

The proportions of cellulose and hemicelluloses were estimated according to Equations 1 and 2, respectively.[43]

$$\% \text{ Cellulose} = (((0.95 \times Ce) + (0.90 \times G) + (1.29 \times HMF) + (3.35 \times FA)) \times V \times (1- (E/100) \times 100)) / B \quad (1)$$

$$\% \text{ Hemicelluloses} = (((0.88 \times X) + (1.37 \times F) + (0.72 \times AA)) \times V \times (1- (E/100) \times 100)) / B \quad (2)$$

where Ce = cellobiose; G = glucose; HMF = 5-hydroxymethylfurfural; FA = formic acid; V = final filtration volume (L); E = extractives; B = biomass without extractives; X = xylose; F = furfural; AA = acetic acid.

Fourier transform infrared spectroscopy (FTIR)

Infrared spectra of dry millet biomass and isolated cellulose fraction were obtained with a spectrophotometer equipped with a total attenuated reflectance accessory (FTIR-ATR Irtrace-100, Shimadzu). Spectra were obtained in the region of 4000-800 cm^{-1}, after 45 readings at a resolution of 4 cm^{-1}.[17,23,45–47]

X-ray diffraction (XRD) and crystallinity index (CI)

The crystallinity index (CI) of millet biomass and isolated cellulose fraction were obtained with an XRD-7000 X-ray diffractometer (Shimadzu), operated at 40 kV and 20 mA with nickel-filtered copper radiation (Cukα, λ = 1.5418 Å). The samples were scanned in the 2θ range of 10-30°, at a rate of $2°\cdot min^{-1}$. The crystallinity index (CI) was calculated using Equation (3) according to the Segal method:[12,48,49]

$$CI\ (\%) = (I_C - I_A / I_c) \times 100 \quad (3)$$

where I_C is the maximum intensity of the crystalline peak of 2θ between 22° and 23° and I_A the minimum intensity peak of 2θ around 18°.

Raman spectroscopy

The pearl millet and the isolated cellulose fractions were analyzed by a Raman spectrophotometer (SENTERRA, Bruker), operated with a 540 nm and 5.0 mW laser source. The spectra were obtained at three different points of the sample with 50x magnification, in a range of 4000-500 cm^{-1}, with 32 readings at a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSION

Characterization of the pearl millet

The percentage content (dry basis) of ash, extractives, total lignin, cellulose, and hemicelluloses in pearl millet biomass was 3.03 ± 0.26, 15.39 ± 0.09, 18.55 ± 0.65, 43.89 ± 2.62, and 21.08 ± 1.31, respectively. For the same biomass, another study reported 36.43% for cellulose and 25.28% for hemicelluloses.[50] Total lignin content was similar to that obtained in another study: 21.81%.[5] The extractive content obtained for the pearl millet also agrees with the value found in another work: 15.63%.[6] The ash content is in the range reported elsewhere: 1.8% to 9.46%.[5–7] The differences reported in the aforementioned studies can be explained by the variation in the composition of lignocellulosic materials according to species, cultivars, soil type, climatic condition, and time of cultivation.[6]

Characterization of cellulose isolated after pretreatment steps

Table 1 presents the lignocellulosic composition after and before alkaline pretreatment with NaOH 4% (wt/vol) at 80 °C and 1200 rpm for 4, 8, and 12 h under agitation, in addition to the volume of acetic acid 3% (vol/vol) used to neutralize the samples.

After performing the three extractions of 4, 8, and 12 h with NaOH, the cellulose increased from 43.89% to 72.42%, 72.27%, and 72.60%, respectively. The pretreatments led to an apparent increase in the cellulose content owing to the partial or total removal of the other compounds.[17,51] This is due to the solubilization of lignin by breaking its ether bonds with cellulose and hemicelluloses, making these components available.[6,52] As expected, lignin was completely removed with NaOH 4% (wt/vol) after

two and three extractions due to the complete solubilization of lignin and the lignin-carbohydrate complex after the chemical pretreatment. Chemical pretreatments are the most efficient to remove lignin.[52] In addition, NaOH is shown in different studies as one of the most widely used alkali agents in the process of delignification, being more efficient than others, such as calcium oxide, calcium hydroxide, and ammonium hydroxide.[4,34,36] Although the pretreatment with NaOH is efficient for this purpose, in the 4H pretreatment lignin was not completely removed because chemical pretreatments need longer reaction times.[34] Therefore, the minimum pretreatment time that must be performed to obtain a complete delignification of the millet biomass is 8 h. As expected, the chemical pretreatment with NaOH was ineffective for total solubilization of hemicelluloses, remaining 14.86%, 14.14%, and 14.90% after 4H, 8H, and 12H treatments, respectively. The chemical pretreatment may even solubilize a portion of hemicelluloses, but the most effective pretreatments to provide a high rate of hemicelluloses degradation are those with diluted acids.[29,33]

After selecting the 8H pretreatment for the following experiments, two neutralization tests (A and B) were performed to reduce the consumption of reagent, the need for energy, and the generation of residues without affecting the cellulose yield: neutralization with resuspension and washing after 8 h of pretreatment (Test A), and neutralization with resuspension and washing every 4 h of pretreatment (Test B). The effect of each neutralization test on the lignocellulosic composition of the millet biomass is presented in Table 2, as well as the volume of 3% acetic acid (vol/vol) used.

Table 1
Lignocellulosic composition of pearl millet biomass before and after the three alkaline treatments at 80 °C and 1200 rpm, using NaOH 4% (wt/vol), followed by neutralization with 3% acetic acid (vol/vol)

Treatment	Chemical composition (%, dry basis)			Volume (mL)
	Cellulose	Hemicelluloses	Lignin	Acetic acid
Untreated	43.89 ± 2.62	21.08 ± 1.31	18.60 ± 0.65	-
4H	72.42 ± 1.22	14.86 ± 0.17	6.58 ± 1.25	300.00
8H	72.27 ± 1.68	14.14 ± 0.42	-	354.00
12H	72.60 ± 1.22	14.90 ± 0.16	-	350.00

4H: one extraction, 8H: two extractions, 12H: three extractions

Table 2
Effect of neutralization and washing steps on lignocellulosic composition of millet biomass and the volume of acetic acid (3% vol/vol) utilized

Treatment	Chemical composition (%, dry basis)			Volume of acetic acid (mL)	Cellulose removal (g/20 g)
	Cellulose	Hemicelluloses	Lignin		
Test A	62.75 ± 2.42	13.37 ± 2.01	6.07 ±1.64	12.00	7.95
Test B	71.82 ± 0.80	14.62 ± 0.22	-	15.00	5.66

From Table 2, both tests A and B promoted a large reduction in the volume of acetic acid 3% (vol/vol) to neutralize the samples. The volume of acetic acid used reduced from 354.00 mL to 12.00 mL (Test A) and 15.00 mL (Test B), decreasing the consumption of reagent used in the neutralization process.

Regarding lignin, Test A presented 6.07% of lignin, while for Test B 100% of lignin was removed. This emphasizes the importance of performing the neutralization and washing steps of the solid fraction every 4 h of treatment. In both neutralizing tests, the 8H pretreatment was used and this did not present lignin in its composition after the end of the treatment. Therefore, the phase of neutralization and washing is fundamental to eliminate the lignin from the process, ensuring that it does not adhere to the solid fraction of cellulose.[25] In addition, Test B still presented a lower cellulose loss (5.66 g cellulose/20 g biomass), compared to Test A (7.95 g cellulose/20 g biomass), proving to be favorable, since the objective of the study is to maximize the yield of this carbohydrate.

After obtaining an effective method of delignification (Test B), combined treatments with diluted acids and in an autoclave were investigated to solubilize the remaining hemicelluloses in the lignocellulosic matrix. Table 3 shows the results in terms of their composition, loss, and removal of the lignin-polysaccharide complex for the new treatments performed.

According to the results shown in Table 3, CT was inefficient to remove the remaining hemicelluloses. The test presented a percentage of 13.58% for hemicelluloses, and when compared with Test B (Table 2), this reduction was equivalent to only 1.04%. This low removal may be due to the fact that H_2O_2 is a bleaching agent. The main purpose of bleaching is to bleach and clean the pulp by removing light-absorbing substances, such as lignin, and this agent can even remove small percentages of hemicelluloses, but its main objective is to let the polysaccharides remain preserved in the lignocellulosic matrix.[53,54]

The hemicelluloses removal after OA 3.8% and OA 5% pretreatments was only 12.68% and 11.82%, respectively. According to the literature, pretreatment with diluted acids is an efficient way to perform hemicelluloses degradation. However, higher temperatures, in the range of 120-160 °C, must be employed to obtain a high removal.[26,55] In the study conducted by Hoang *et al*.,[42] the authors found that its optimal condition to solubilize 95.74% of hemicelluloses was using 178.4 °C for 28.4 min, employing a concentration of 3.68% oxalic acid. The ATA 4% pretreatment removed only 3.56% of the hemicelluloses compared with Test B. This result reinforces that the utilization of diluted acids is a more efficient way to perform hemicelluloses degradation compared with alkaline treatments.[34,51]

The OAA 5% and SAA 1% pretreatments removed about 99.86% and 100% of the remaining hemicelluloses from the lignocellulosic matrix, respectively. In addition, the cellulose content in the residual fraction was 79.23% with the use of oxalic acid and 79.63% using sulfuric acid. Therefore, among all treatments performed to remove hemicelluloses, the best results obtained were those carried out in the autoclave at 125.6 °C and 1.4 bar for 2 h. However, even if a higher percentage of cellulose and a greater removal of hemicelluloses can be achieved using sulfuric acid in this process, the use of oxalic acid is more attractive as it led to lower losses of cellulose throughout the isolation process (6.85), compared to sulfuric acid (7.47).

The high efficiency of cellulose recovery (79.23%) using the alkali-acid pretreatment and the pretreatment performed in the autoclave is a promising result. There are few studies discussing the use of oxalic acid as a strategy in the removal of hemicelluloses.[50] Imman *et al*.[56] and Qing *et al*.[38] reported in their studies the advantages of using oxalic acid as a promoter in the pretreatment of lignocellulosic biomasses. In addition to oxalic acid being a weak acid and less toxic to the environment, it is able to achieve the same high levels of yield obtained with the use of sulfuric acid, without presenting a high level of inhibitors generated by the process. However, most studies still report as more traditional treatments those based on alkali agents or stronger acids, such as H_2SO_4.[13,31,50]

Table 3
Effect of different pretreatments on lignocellulosic composition of pearl millet biomass

Treatment	Chemical composition (%, dry basis)			Component removal (g/20 g)		
	Cellulose	Hemicelluloses	Lignin	Cellulose	Hemicelluloses	Lignin
CT	72.39 ± 3.90	13.58 ± 0.82	-	6.41	14.69	20.00
OA 3.8%	80.57 ± 0.34	12.68 ± 0.12	-	5.14	15.13	19.12
OA 5%	83.39 ± 0.15	11.82 ± 0.35	-	6.23	15.94	18.99
OAA 5%	79.23 ± 1.77	0.08 ± 0.11	-	6.85	19.97	20.00
SAA 1%	79.63 ± 0.72	-	-	7.47	20.00	20.00
ATA 4%	85.47 ± 5.05	11.06 ± 1.46	-	5.05	15.97	20.00

CT: two 4-hour alkaline extractions with NaOH 4% and H_2O_2 1%; OA: two 4-hour alkaline extractions with 4% NaOH and a 2-hour acid extraction with $C_2H_2O_4$ at two concentrations (3.8% and 5%); OAA: two 4-hour alkaline extractions with 4% NaOH and a 2-hour extraction with $C_2H_2O_4$ 5% in an autoclave; SAA: two 4-hour alkaline extractions with 4% NaOH and a 2-hour extraction with H_2SO_4 1% in an autoclave; ATA: two 4-hour alkaline extractions with 4% NaOH and a 2-hour extraction with NaOH 4% in an autoclave

Fourier transform infrared spectroscopy (FTIR)

The infrared spectra of untreated millet biomass and isolated cellulose are reported in Figure 2.

All samples presented absorption band intervals for two main regions: the first between 3750 and 3000 cm^{-1}, and the second between 1750 and 800 cm^{-1}. The bands between 3500 and 3000 cm^{-1} are assigned to the stretching of the hydroxyl groups –OH.[6,57–59] For all treatments, this stretching width -OH reduced, which may suggest a rupture of intermolecular hydrogen bonds in lignin.[26,60] For OAA 5% and 8H pretreatments, the absorption band at 2900 cm^{-1} decreased. It corresponds to the vibration

and stretching of C-H and CH_2 in cellulose molecule. Hence, that reduction may indicate a severe pretreatment able to degrade cellulose.[57,61,62]

The absorption bands at 1515, 1230, and from 860 to 817 cm^{-1} correspond to the lignin phenolic compounds, the vibration of the lignin aromatic ring, and out-of-plane C-H curvature, respectively.[62–65] Regarding hemicelluloses, the bands that represent them are those at 1730 and 1245 cm^{-1}, assigned to, respectively, the acetyl and uronic group of hemicelluloses and the vibration of the hemicelluloses stretch.[57,61,62,64,65] For all pretreatments, it was possible to observe the disappearance of one or more absorption bands related to lignin and hemicelluloses, thus proving the cellulose isolation indicated by the physicochemical characterization.

In addition, the bands at 1454, 1420, 1370, 1315, 1150, 1110, 988 and 895 cm^{-1},[6,46,57,58,62,63,66] corresponding to the cellulose molecule also demonstrates the efficiency of OAA 5%, SAA 1%, ATA 4% and 8H pretreatments for cellulose isolation, given the appearance of the peaks compared with untreated dry biomass.

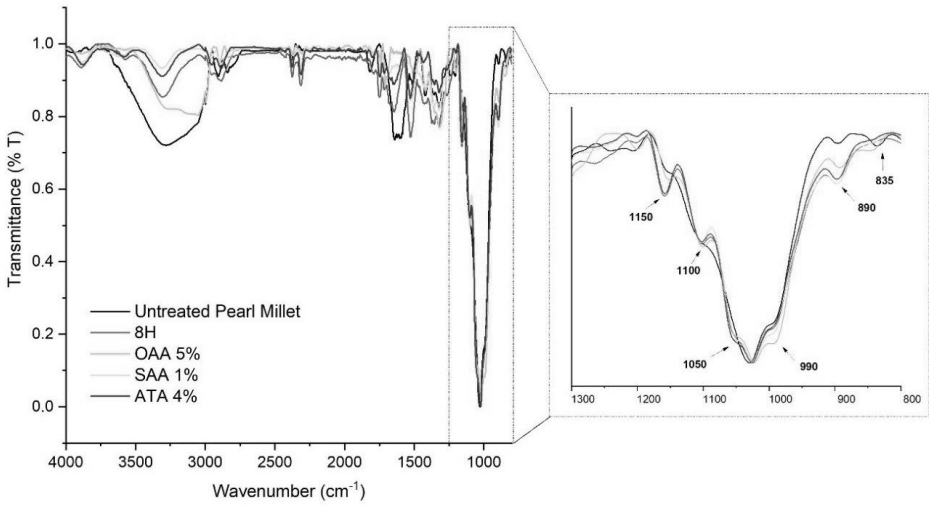

Figure 2: FTIR-ATR spectra of untreated pearl millet biomass and cellulose isolated through treatments of 8H (two 4-hour alkaline extractions with 4% NaOH), OAA 5%, SAA 1% and ATA 4% (two 4-hour alkaline extractions with 4% NaOH and a 2-hour extraction with $C_2H_2O_4$ 5%, H_2SO_4 1% and NaOH 4% in an autoclave, respectively)

X-ray diffraction (XRD)

The X-ray diffractograms of the untreated pearl millet biomass and of the OAA 5% and ATA 4% pretreated samples, which achieved the best performance (corresponding to the absence of the peak assigned to lignin and hemicelluloses and to a greater increase in the peak assigned to the cellulose band), are shown in Figure 3.

The diffractograms have two main peaks at 2θ of 15.5°and 22.4°, corresponding to the structure of crystalline cellulose type I.[35,67] The appearance of these two peaks show that the alkaline and acid pretreatments performed on pearl millet biomass did not reduce the native structure of crystalline cellulose in the fibers.[24]

According to the diffractograms, the untreated pearl millet biomass had a crystallinity index (CI) of 66.79%, which rose to 82.43% and 77.40% with OAA 5% and ATA 4%, respectively. This CI increase is mainly attributed to the removal of the amorphous portion of lignin and hemicelluloses.[68,69] The removal of these amorphous components causes greater accessibility of groups -OH, increasing crystalline arrangements in the structure through intramolecular and intermolecular hydrogen bonds.[6,17,70]

Raman spectroscopy

The raw pearl millet biomass and the OAA 5% and ATA 4% pretreated samples were submitted to Raman spectroscopy analyses, as depicted in Figure 4.

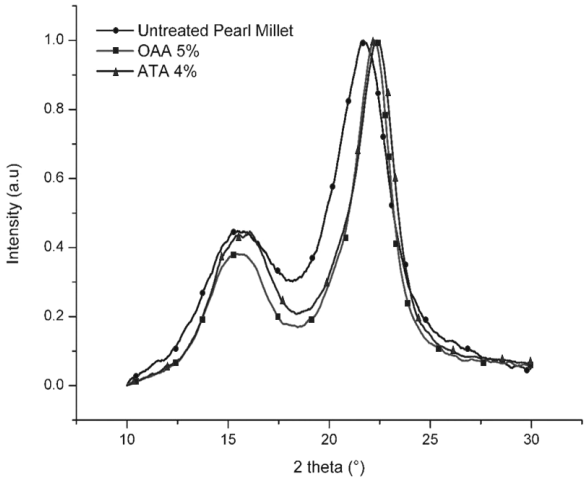

Figure 3: X-ray diffractograms of untreated pearl millet biomass and cellulose isolated through treatments of OAA 5% and ATA 4% (two 4-hour alkaline extractions with 4% NaOH and a 2-hour extraction with $C_2H_2O_4$ 5% and NaOH 4% in an autoclave, respectively)

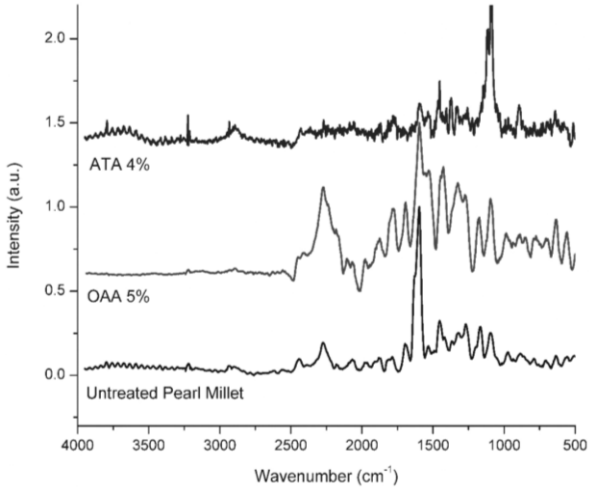

Figure 4: Raman spectroscopy of untreated pearl millet biomass and cellulose isolated through treatments of OAA 5% and ATA 4% (two 4-hour alkaline extractions with 4% NaOH and a 2-hour extraction with $C_2H_2O_4$ 5% and NaOH 4% in an autoclave, respectively)

The Raman spectra obtained for the OAA 5% and ATA 4% pretreated samples indicated an increase in the cellulose content, compared with the untreated pearl millet biomass. The characteristic peaks of 1096 cm^{-1} and 2900 cm^{-1} – related to the vibrations of cellulose C-O, C-C, and C-H bonds – increased their intensity for both treatments.[71–73] The 577 cm^{-1} and 1460 cm^{-1} peaks, which relate to cellulose type II also increased their intensity for both treatments, proving the efficiency of the cellulose isolation process.[74–76]

In addition, for the ATA 4% and OAA 5% treatments, there was a decline in the intensity of the peaks in the region from 1800 to 1500 cm^{-1}, a characteristic region of C=C and C=O bonds of the aromatic rings present in the lignin molecule.[77] Thus, it indicates lignin removal after the alkali and acid treatments performed, as also evidenced by the lignocellulosic composition characterization and FTIR spectra.

CONCLUSION

Cellulose was isolated from pearl millet by two alkaline extractions of 4 h with NaOH 4%, followed by an extraction with oxalic acid in an autoclave at 125.6 °C and 1.4 bar for 2 hours. With this treatment, it was possible to obtain a higher yield of pure cellulose with a high crystallinity index (82.43%). After the treatment, the cellulose content increased from 43.89% to 79.23%, while removing 100% of lignin and 99.86% of hemicelluloses. The analyses of FTIR, XRD, and Raman spectroscopy confirmed the removal of lignin and hemicelluloses, as well as the cellulose increment after the treatment. This removal is due to the combination of alkaline and acid treatments, sequentially, performed at high temperature (>100 °C) and moderate working pressure.

This treatment was chosen precisely with the intention of preserving the crystalline structure of the cellulose, and it can be observed that the use of an organic acid, such as oxalic acid, was beneficial for avoiding the intense degradation of the cellulose structure, given the high rate of crystallinity obtained after treatment.

Therefore, the cellulose obtained is pure and can contribute to the valorization of pearl millet, a biomass that has never been studied for this purpose, being easy to cultivate, combined with high productivity and yield, which is not competitive with human and animal food compared to other crops, such as sugar cane. In addition, the cellulose isolated from pearl millet can be transformed into nanocellulose, which has a wide range of applications in the industrial sector, such as plastic for furniture, large screens, solar panels, supercapacitor batteries, cosmetics, medicine, pharmaceuticals, aircraft components and automobiles, aerogels, hydrogels, among others.

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