CELLULOSE CHEMISTRY AND TECHNOLOGY

EFFECTS OF COMBINED CHEMICAL AND HYDROTHERMAL PRETREATMENT ON PAPYRUS CELLULOSE STRUCTURE

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Papyrus (*Cyperus papyrus* L.) is a lignocellulosic plant suitable for many applications when properly pretreated. This study explores the potential of a two-stage pretreatment (combining hydrothermal and chemical methods) on the characteristics of cellulose fibers derived from papyrus. One-stage and two-stage pretreatments were compared, utilizing sodium hydroxide (NaOH) and ferric chloride ($FeCl_3$) chemical solutions at a 3% (w/v) concentration. Chemical pretreatment was employed for one-stage processing, while hydrothermal pretreatment was introduced prior to chemical pretreatment. As the liquid hot-water process intensified, significant changes in chemical composition and morphology occurred. Hydrothermal pretreatment partially eliminates hemicelluloses and lignin, while increasing the cellulose content and enhancing fiber crystallinity. Following the pulping and bleaching stages, it was determined that the $FeCl_3$-based two-stage pretreatment exhibited the greatest potential for cellulose recovery and hemicelluloses and lignin removal, yielding the highest crystallinity index.

Keywords: papyrus, cellulose fiber, alkaline pretreatment, hydrothermal pretreatment

INTRODUCTION

Wetland systems, which have been recognized for decades, are a natural method for wastewater treatment. A variety of aquatic plants grow in both natural and artificial wetlands. Emergent plants, which need to be removed after reaching maturity and completing the treatment process, are particularly effective in these systems. Instead of discarding them, these plants can be repurposed as innovative natural materials that are gaining popularity in today's world. Tropical wetland ecosystems exhibit biomass production rates that rival those observed in high-input, intensively managed agricultural systems. Notably, wetlands in tropical regions harboring C_4 photosynthetic species demonstrate exceptional levels of primary productivity, surpassing those found in both managed and natural environments.

Papyrus, due to its C_4 photosynthesis and year-round primary productivity, has significant potential output, with aerial net primary productivity ranging from 25.9 to 136.4 tDM/ha-yr in East Africa.[1] Papyrus is classified as lignocellulosic biomass, primarily composed of cellulose fibers, with hemicelluloses acting as an intercellular connector and lignin serving as a binding agent.[2] It has been reported that papyrus pith comprises 29.3% cellulose, 20.8% hemicelluloses, 13.2% Klason lignin, and 2.5% acid-soluble lignin.[2] These primary components form a complex three-dimensional structure, which leads to inefficient applications. In their study,[1] Jones *et al.* highlighted the potential of papyrus as a biofuel for cooking and heating. They found that by crushing or carbonizing papyrus into briquettes, it could serve as an alternative to wood charcoal, albeit with a slightly lower calorific value, approximately one-third less. These findings underscore the promise of papyrus as a viable biofuel option.

Conventional fiber extraction methods remove a significant portion of hemicelluloses and lignin to isolate cellulose. Cellulose, which can be utilized as fibers, is present in various forms, particularly in lignocellulosic materials that require specific processing to become effective for use. The pretreatment process, which employs removing lignin and hemicelluloses, simplifies the production of cellulose fibers by altering the complex structure of lignocellulosic materials. Lignin, a robust component of plant cell walls, impedes the conversion and modification of cellulose fibers. Besides lignin, hemicelluloses are another element that must be eliminated due to its obstruction of lignin diffusion into the pretreatment solution. This approach exposes bio-adsorbents to additional functional groups, enhancing their adsorption capacity, altering their surface charges, and improving certain functional groups for biosorption.[3] Alkaline pretreatment, *e.g.* with sodium hydroxide (NaOH), and inorganic salt pretreatment, *e.g.* with ferric chloride ($FeCl_3$), are commonly used. Alkali pretreatment is characterized by the effective removal of lignin, along with the partial removal of hemicelluloses. In terms of hydrothermal pretreatment, it is an efficient process for hemicelluloses removal.[4] Yaashikaa *et al.* revealed that using NaOH for pretreatment affected the bio-adsorbent shape through saponification, converting esters to alcohols and carboxylates, and removing cell membrane liposomes. Moreover, using salts, including NaCl,[6,7] $ZnCl_2$ and $FeCl_3$[7], as ion-exchangers in the pretreatment caused the binding sites to become saturated with contaminants. Benefits, such as high efficiency, reduced corrosion and recoverability, have been shown for inorganic salt pretreatment.[8] Liu *et al.* reported that $FeCl_3$ could act as a catalyst in the hydrolysis process, especially for the removal of hemicelluloses.[8] However, pretreatment with high concentrations of chemicals or over long periods decreased the content of cellulose.[9] Therefore, owing to the importance of an efficient and rational use of lignocellulose biomass, a two-step pretreatment was considered.[10]

A new, ecologically friendly, mild, and efficient separation method is needed for high-value lignocellulosic biomass utilization. Hydrothermal pretreatment offers several advantages over mechanical methods, including lower energy consumption, elimination of recycling requirements, and reduction of the environmental impact associated with chemical pretreatments. This versatile approach can accommodate a diverse array of biomass types, though its effectiveness may differ among them. Moreover, when processing larger chip materials, hydrothermal pretreatment can further reduce the energy demands of mechanical refining.[11] It was reported that hemicelluloses could be removed using hydrothermal pretreatment procedures, and lignin and amorphous cellulose only mildly deteriorated.[12] The hydrothermal method could also reduce ash and extractives, such as silicon, and increased fiber tensile strength.[13] Furthermore, it has been reported that the hydrothermal pretreatment could improve the chemical composition and structure of coffee husk for biomethanization at 120-210 °C for 1-60 min.[14] Other researchers also studied the effect of thermochemical pretreatment on the composition of hazelnut shells with the steam pretreatment method at different temperatures (120, 150, and 200 °C) and found high cellulose recovery with this method.[15] Adekunle *et al.* conducted a study on the effects of compressed hot water pretreatment on corn stalks at various temperatures (100, 120, 140, 160, 180, and 200 °C), and discovered that the ability to remove hemicelluloses improved as the temperature increased.[16] However, to the best of the authors' knowledge, the advancement of a two-stage pretreatment that incorporates an eco-friendly hydrothermal approach, coupled with a known chemically efficient pretreatment solution, such as sodium hydroxide or ferric chloride, is both innovative and effective.

Papyrus is an exceptionally productive plant, capable of multiple harvest cycles and boasting high cellulose content. It offers numerous advantages, particularly in terms of wastewater treatment, and is also suitable for planting for landscape decoration and various other applications. There are many applications of biomass after pretreatment. Previous research has demonstrated the possibility of making lignocellulosic nanofibers from wheat straw using various fibrillation techniques.[17] Rangseesuriyachai *et al.*[18] showed the potential of pretreated Napier grass co-digested with elephant dung to produce high yield of methane production. Nanda *et al.*[19] extracted nanocellulose from wheat straw soda pulp. Wheat straw was processed in 7% NaOH (on dry matter) at a liquid/solid ratio of 10/1 at 100 °C for 150 minutes to produce an unbleached semi-chemical pulp with a yield of 42.31%. Based on the author's review of literature, the pretreatment of papyrus sedge for cellulose production has been minimally explored, with limited studies comparing the product characteristics to those of commercially available cellulose. This research examines the impact of hydrothermal pretreatment (in the initial stage) and chemical pretreatment using NaOH and $FeCl_3$ (in the subsequent stage) on the properties of papyrus cellulose fibers. An optimal pretreatment has the potential to improve the transformation of lignocellulosic material into cellulose fibers, and may lead to a more sustainable process and accelerated production.

EXPERIMENTAL

Raw material and chemicals

Papyrus (*Cyperus papyrus* L.) was sourced from a natural swamp in Suphan Buri province, Thailand. Only the culm and umbel of three-month-old plants were utilized. The biomass was oven-dried at 80 °C for 24 hours, ground, passed through no. 60-100 mesh sieve, and subsequently, stored in a dry location. Sodium hydroxide (NaOH) and ferric chloride ($FeCl_3$) were procured from Loba Chemie, Thailand, while hydrogen peroxide (H_2O_2) at a 30% (w/w) concentration was obtained from Chem-Supply, Thailand.

Pretreatment

One-stage pretreatment

In this study, different chemicals were used to compare the efficiency of one-stage pretreatment using NaOH (N1) and $FeCl_3$ (F1). Dried papyrus was mixed with each chemical (NaOH/$FeCl_3$) solution (3% w/v) at the ratio of solid to liquid at 1:10, and then heated in an autoclave at 121 °C (1 bar) for 1 h. After filtering the slurry, the solid fraction was rinsed with DI water until it reached a neutral pH and dried at 60 °C 24 h.

Two-stage pretreatment

A two-stage pretreatment process was also investigated, combining hydrothermal and chemical methods. In the first stage, hydrothermal pretreatment (H) (121 °C, 1 bar for 1 hour) was applied by adding 40 g of dried papyrus to 400 mL of distilled water, maintaining a solid-to-liquid ratio of 1:10. The solid matter was then separated and dried at 60 °C for 24 hours before proceeding to the second stage. In the second stage, a comparison was made between NaOH (HN2) and $FeCl_3$ (HF2) chemical pretreatments to determine their effectiveness.

Pulping and bleaching

Pulping and bleaching are the steps made in order to: 1) separate and treat the raw material to produce pulp, and 2) lighten the color of the pulp, whitening it. These processes were done after both one-stage NaOH (PN1) and $FeCl_3$ (PF1) pretreatment and two-stage NaOH (PHN2) and $FeCl_3$ (PHF2) pretreatment. First, pretreated papyrus was bleached with a 1:1 solution of 2% (w/v) NaOH and 2.6% (v/v) H_2O_2 (a solid-liquid ratio of 1:20) at 70 °C. Next, the mixture was stirred by mechanical stirring at 100 rpm for 2 h. Finally, the samples were filtered and washed using distilled water until the pH was neutral.

Chemical composition

The chemical composition of fiber was determined using the standards of the Technical Association of the Pulp and Paper (TAPPI). The TAPPI standard procedures T222 om-88 and T203 om-88 were used for the lignin test method and the cellulose and hemicelluloses test methods, respectively.[20]

Bleaching causes further delignification of the fiber, resulting in white cellulose.[21] The percentages of solid recovery, cellulose recovery, hemicelluloses recovery, and lignin removal were calculated based on the dry weight of papyrus. The solid recovery obtained from the pretreatment at each stage of NaOH and $FeCl_3$ was expressed in grams of the dry weight of total solid for one-stage pretreatment and two-stage pretreatment. Moreover, since pulping and bleaching caused further delignification of the fiber, resulting in white cellulose, the solid recovery was also calculated after this step.[21] The calculation for % solid recovery (S_{re}), component recovery in pretreated biomass as cellulose (C_{re}), and hemicelluloses or lignin removal (H_r or L_r) are expressed in Equations (1)-(3):[9]

$$\%S_{re} = \frac{100*(1 - grams\ total\ solids\ after\ pretreatment)}{grams\ total\ solids\ before\ pretreatment} \tag{1}$$

$$\%C_{re} = \frac{\%cellulose\ residue}{\%\ untreated\ cellulose} \times \%S_{re} \tag{2}$$

$$\%\mathrm{H}_r\ or L_r = 100 - \left(\frac{\%lignin\ residue\ or\ hemicellulose}{\%\ untreated\ lignin\ or\ hemicellulose} \times \%S_{re} \right) \tag{3}$$

Characterization of initial biomass and extraction cellulose fibers

The initial biomass and cellulose fibers that were obtained in this manner were analyzed using X-ray diffraction (XRD), attenuated total reflectance–Fourier transform infrared spectroscopy (ATR-FTIR), and scanning electron microscopy (SEM). XRD analysis (Empyrean, Mavern Panalytical, Malvern, UK) was performed with a CuKα radiation source. Scans were performed at a 2θ diffraction angle of 10-40° and a scan speed of 0.02°/sec. From the intensity of the peaks, the crystallinity index (CrI) was estimated using the peak height method, which is widely used to study the crystallinity of native cellulose, using Equation (4):[22]

$$CrI(\%) = \frac{I_{(002)} - I_{(101)}}{I_{(002)}} \times 100 \tag{4}$$

where $I_{(002)}$ is the maximum intensity of the peak corresponding to the plane in the sample at a diffraction angle of 22° 2θ and $I_{(101)}$ is the intensity of the peak at a diffraction angle of 15° 2θ.

ATR-FTIR analysis (Bruker Tensor 27 System, Germany) of native papyrus biomass, after pretreatment, and of extracted cellulose fibers was performed. The samples were dried, milled, and sieved (60 mesh), and then kept in air-tight sealed containers until analysis. FTIR spectra of each sample were acquired in the range between 4000 cm^{-1} and 500 cm^{-1} at 4 cm^{-1} spectral resolution.

Morphological changes in papyrus subjected to different treatments were observed using a JEOL JSM-5410LV scanning electron microscope (Tokyo, Japan), in comparison with native biomass, under the following operating conditions: low vacuum of the order of 90 Pa, and an accelerating voltage of 1.5 kV. Samples of cellulose fibers were prepared by milling and sieving (60 mesh), and were kept in sealed containers at air-dry moisture content.

RESULTS AND DISCUSSION

Effects of treatments on chemical composition

Plants primarily consist of cellulose, hemicelluloses, and lignin. Cellulose functions as a structural component in plant tissue and constitutes a significant portion of plant cell walls. Hemicellulose is another polysaccharide found in plant cell walls, but it has shorter sugar chains than cellulose. Lignin is a complex aromatic polymer that provides structural support within plant cell walls. Composition analysis of the raw papyrus, performed according to TAPPI standard test methods, showed that it had a cellulose content of 2.49 ± 0.059 g/g biomass, hemicelluloses content of 1.01 ± 0.031 g/g biomass, and lignin content of 1.50 ± 0.117 g/g biomass. The papyrus pretreated by the hydrothermal process in the two-stage pretreatment exhibited a slightly higher cellulose level (2.66 ± 0.031 g/g biomass) and lower hemicelluloses (0.924 ± 0.025 g/g biomass) and lignin levels (1.40 ± 0.027 g/g biomass). The pretreatment process involving NaOH and $FeCl_3$ significantly altered the papyrus composition. The treated papyrus had a much higher cellulose content (3.36 ± 0.019 g/g biomass) and lower hemicelluloses (0.15 ± 0.009 g/g biomass) and lignin levels (0.50 ± 0.036 g/g biomass). Likewise, the papyrus subjected to the pulping and bleaching process demonstrated a higher cellulose content (3.24 ± 0.019 g/g biomass) and lower hemicelluloses (0.24 ± 0.030 g/g biomass) and lignin levels (0.58 ± 0.042 g/g biomass). The study concluded that papyrus treated with a hydrothermal process exhibited a marginally higher cellulose content and slightly lower hemicelluloses and lignin contents compared to raw papyrus. Additionally, the NaOH treatment yielded a greater amount of cellulose (2.72 g/g biomass) than the $FeCl_3$ treatment (2.51 g/g biomass). Conversely, the $FeCl_3$ treatment resulted in a lower hemicelluloses content (0.15 g/g biomass), compared to the NaOH treatment (0.80 g/g biomass). A comparison of the chemical compositions after one-stage (chemical pretreatment) and two-stage pretreatment (hydrothermal and chemical pretreatment) using different chemical solutions is illustrated in Figure 1. Figure 1 illustrates that, when considering only the pretreatment step, NaOH was more effective in reducing lignin and hemicelluloses than $FeCl_3$. Moreover, the two-stage process showed potential for producing slightly higher cellulose content. This two-stage pretreatment, which included an additional hydrothermal step, resulted in a significant reduction of hemicelluloses, compared to the one-stage, chemical-only pretreatment.

Pulping and bleaching are crucial steps in producing high-quality cellulose fibers, which are utilized in a wide array of products, such as paper, cardboard, and tissue paper. These processes enhance the strength, brightness, and overall quality of the fibers, making them suitable for various end products. Notably, the hemicelluloses content results after pulping and bleaching processes revealed differences between single-stage and two-stage pretreatments. The effects on cellulose and lignin were also consistent. Thus, the hemicelluloses content increased when papyrus was pretreated using the hydrothermal method, while it decreased when the chemical pretreatment was used alone. After pulping and bleaching performed after the pretreatments with both NaOH and $FeCl_3$, the hemicelluloses content differed between the one-stage and two-stage pretreatment processes; specifically, the hemicelluloses content increased when using NaOH. Hydrogen peroxide (H_2O_2), a common bleaching agent, has been shown to potentially increase the hemicelluloses content in plant tissue. Pudjiastuti *et al.*[23]discovered that treating coffee waste pulp with alkaline H_2O_2 led to an increase in hemicelluloses levels within the cell walls. This increase is likely due to the release of hemicelluloses from unassociated lignin and the overall macromolecular structure. The process responsible for the increase in the hemicelluloses content is known as peroxidase-mediated oxidative polymerization, which involves enzymes called peroxidases found in plant cells. These enzymes can utilize H_2O_2 as a substrate.

During the bleaching process, lignin and other organic components in the pulp are broken down, releasing hemicelluloses. Alkaline bleaching conditions may solubilize hemicelluloses, resulting in an increase in their content within the pulp. However, it is essential to consider the optimal conditions for achieving the desired cellulose yield, which depends on the total solid recovery. The data of solid recovery (S_{re}), cellulose recovery (C_{re}), hemicelluloses removal (H_r), and lignin removal (L_r) of papyrus treated by different routes, using one-stage and two-stage pretreatments, are shown in Table 1.

Table 1 presents the recovery and removal of various components (cellulose, hemicelluloses, and lignin) during the processing of papyrus using different methods. The total solid recovery after one-stage pretreatment (chemical pretreatment) and two-stage pretreatment (hydrothermal and chemical pretreatment) were similar for both NaOH and $FeCl_3$ solutions. However, NaOH pretreatment demonstrated higher solid recovery than $FeCl_3$ pretreatment. The results indicated a substantial loss of raw material during pretreatment, leading to lower yields. Additionally, cellulose fiber production after one-stage pretreatment with NaOH, followed by pulping and bleaching processes, yielded less product than other pretreatments. Conversely, the two-stage pretreatment increased cellulose recovery by incorporating a hydrothermal process before the chemical pretreatment. Despite exhibiting the highest lignin removal, the cellulose recovery percentage was the least, making it less suitable for producing cellulose fiber. According to the two-stage pretreatment results, hemicelluloses and lignin contents were initially reduced by the hydrothermal pretreatment, resulting in a 1.50% increase in cellulose and a 1.30% and 1.32% decrease in hemicelluloses and lignin contents, respectively. These findings indicate that the hydrothermal pretreatment can remove lignin and hemicelluloses, without using chemicals, thus reducing fiber preparation costs.

In general, pretreatment methods aim to modify the structure of plant biomass to enhance the efficiency of downstream processes, such as fermentation or enzymatic hydrolysis. Vassilev *et al.*[24] noted that lignin solubility may potentially be enhanced at temperatures above 90 °C, causing it to dissolve into soluble fractions and simultaneously weakening its recalcitrant structure. Moreover, Toscan *et al.*[10] showed an improved cellulose content of elephant grass by pretreating it with the hydrothermal process (160 and 180 °C). The specific objectives of the pretreatment depend on the intended end use of the product and the starting material's properties.

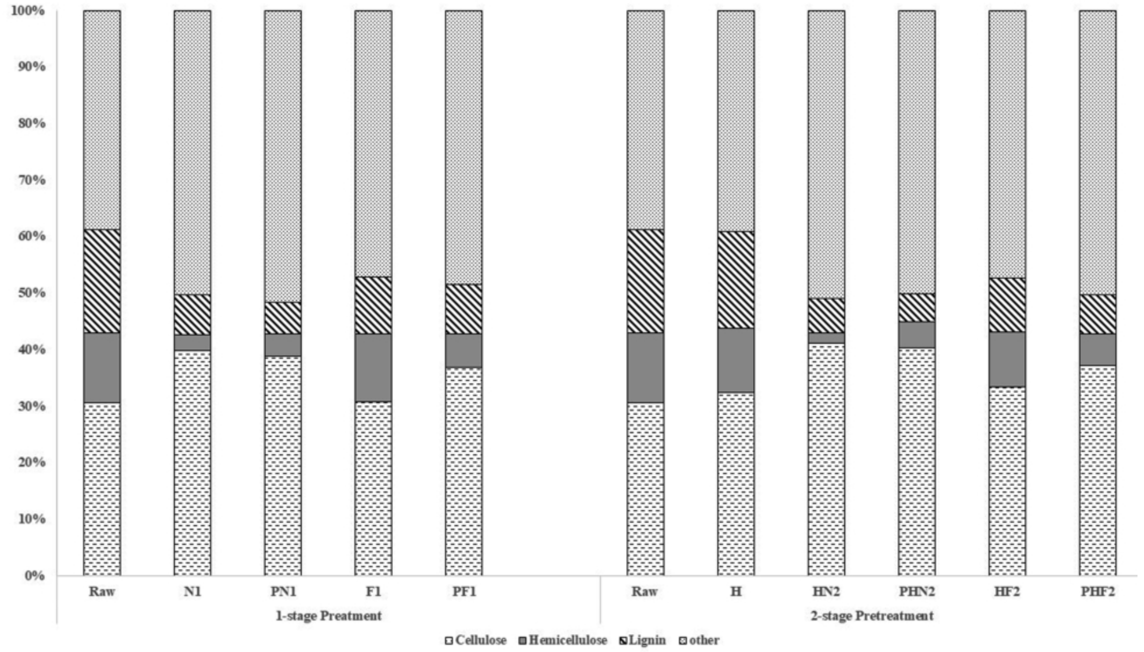

Figure 1: Chemical composition of untreated and pretreated papyrus by different pretreatment methods (one-stage and two-stage pretreatments)

Table 1
Percentages of cellulose recovery, hemicelluloses removal, and lignin removal from papyrus after the pretreatments and pulping and bleaching processes

Parameter (%)	Raw papyrus	Pretreatment								
		Hydrothermal (H)	HN2	HF2	N1	F1	PHN2	PHF2	PN1	PF1
Solid recovery	100.00	98.59 ± 0.38	46.36 ± 3.66	37.09 ± 2.82	48.29 ± 4.11	37.91 ± 1.49	28.65 ± 3.71	27.46 ± 1.46	18.69 ± 2.74	26.87 ± 4.71
Cellulose recovery	100.00	1.50 ± 0.42	62.32 ± 5.05	40.47 ± 2.77	62.85 ± 5.46	38.19 ± 1.31	37.87 ± 4.84	33.43 ± 1.58	23.78 ± 3.44	32.36 ± 5.47
Hemicelluloses removal	0.00	1.30 ± 0.37	6.93 ± 0.80	29.17 ± 1.70	11.33 ± 2.00	36.71 ± 2.20	9.63 ± 0.43	12.43 ± 0.90	5.92 ± 0.85	14.19 ± 3.00
Lignin removal	0.00	1.32 ± 0.34	15.28 ± 1.28	19.48 ± 1.59	18.45 ± 0.44	20.86 ± 1.69	7.44 ± 0.60	10.51 ± 1.46	5.77 ± 1.23	12.65 ± 2.15

Also, it was found that NaOH pretreatment yielded a higher solid recovery than $FeCl_3$ pretreatment, as evidenced by the approximately 60% cellulose remaining after each process. Furthermore, the two-stage pretreatment exhibited similar effects on the chemical composition changes of the materials compared to the one-stage method. However, the two-stage pretreatment demonstrated significant improvements in both cellulose recovery and lignin removal, compared to the single-stage pretreatment approach. The two-stage pretreatment employing NaOH solution displayed the highest solid recovery and the most efficient chemical composition alteration. Both $FeCl_3$ and NaOH are chemicals suitable for pretreating lignocellulosic biomass, a process that facilitates the breakdown of plant material and renders it more amenable to further processing. Solid recovery after the pretreatment refers to the amount of solid material remaining after completing the pretreatment process. Various factors can influence the solid recovery of a pretreatment method, including the biomass type, the pretreatment chemicals and their concentrations, the temperature and duration of the pretreatment, and the equipment used. Based on the results, NaOH pretreatment generally exhibited a higher solid recovery than $FeCl_3$ pretreatment due to its less aggressive and less selective action on the plant material. In contrast, $FeCl_3$ could be more aggressive and selective in its attack, potentially leading to a lower solid recovery. When examining the removal of hemicelluloses and lignin, $FeCl_3$ demonstrated superior efficiency in removing both components. For example, Tang *et al.*[25] reported a higher hemicelluloses removal efficiency for $FeCl_3$ (3.2% concentration), compared to NaOH (1% concentration), although at higher concentration. Moodley *et al.*[26] reported a significant removal of hemicelluloses from corn stover of about 100% by pretreating the sample with 0.1 M of $FeCl_3$ at a temperature ranging from 140 to 200 °C for 5-30 min. Generally, it has been reported that cellulose is more resistant to treatments than hemicelluloses due to the existence of crystalline areas inside the microfibrils of cellulose.[27]

Characterization of samples

Untreated and pretreated papyrus samples by all the methods studied and both one-stage and two-stage pretreatments were observed in terms of their morphology and chemical structure. SEM images of the papyrus samples were observed to investigate their surface morphology changes caused by the one-stage and the two-stage pretreatments. X-ray diffraction analysis (XRD) was used to obtain data on the crystallographic structure of the material before and after the treatments, and ATR-FTIR spectra were examined for determining the changes occurring in the functional groups and chemical constituents of the samples.

SEM analysis

Morphological structure changes in the pretreated samples, compared to the untreated one, were examined using scanning electron microscopy (SEM). The SEM images illustrated in Figure 2 reveal that the raw papyrus sample exhibited a rigid surface structure. Also, the papyrus structure after the two-stage pretreatment (hydrothermal and chemical pretreatment) had a smaller particle size than the sample after one-stage pretreatment (chemical pretreatment only). Furthermore, the two-stage pretreated samples displayed a rough surface with visible cracks. These findings suggest that the hydrothermal pretreatment at high temperatures resulted in a more disordered morphology. The sample cracks were likely caused by the separation and increased exposure of fibers, as well as a loosening of the fibrous network. Moreover, hemicelluloses underwent hydrolysis, releasing acetyl groups and other acids. Concurrently, lignin experienced depolymerization, and cellulose binding was diminished.[28] Compared to NaOH, the impact of the $FeCl_3$ solution on pretreated samples demonstrated a reduction in particle size and a greater presence of holes and cracks on the surface. Both $FeCl_3$ and NaOH effectively removed lignin and hemicelluloses components, disrupting the original dense structure of the biomass. However, it was only the $FeCl_3$ solution pretreatment that significantly altered the fibrillar structure.

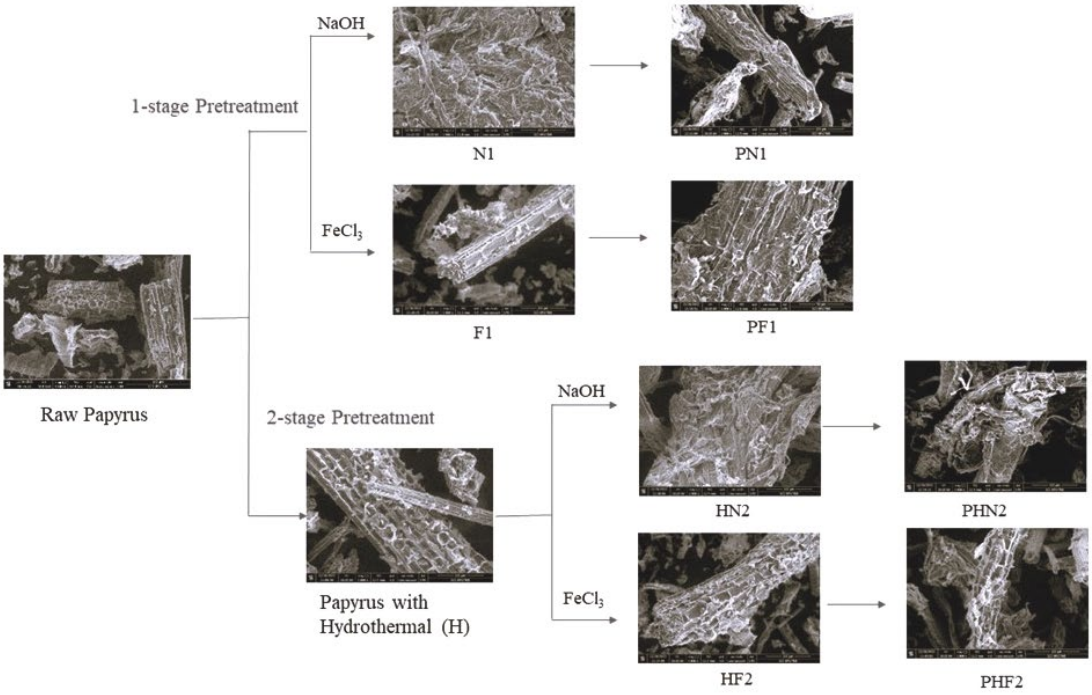

Figure 2: Morphology changes after each stage of pretreatment, including pulping and bleaching processes

XRD analysis

The X-ray diffraction (XRD) patterns of untreated papyrus and papyrus cellulose fiber are depicted in Figure 3. The spectra predominantly featured three peaks at 2θ diffraction angles of approximately 15°, 22°, and 34°, corresponding to the 101, 002, and 004 lattice planes, respectively.[29] Moreover, the peak at 22° 2θ constituted the crystalline phase of cellulose. In contrast, the other two peaks, at 15° and 34° 2θ, represented the amorphous phases of cellulose[12] as non-cellulose materials (*e.g.*, hemicelluloses and lignin) in the fibers.[30] The XRD patterns displayed peaks for both cellulose I and cellulose II polymorphs. Chemical pretreatment has the potential to eliminate impurities and amorphous regions within cellulose chains, while also converting some crystalline structures from cellulose I to cellulose II.[31] In the case of the two-stage pretreatment, the hydrothermal stage did not affect the cellulose crystals, and after it, the cellulose still exhibited the crystalline structure of cellulose I.[31] Still, the two-stage pretreatment reduced the content of amorphous components in the papyrus fibers, such as hemicelluloses, lignin and some other non-cellulose material, resulting in sharper XRD peaks. As reported previously, the large crystal size of the two-stage treated fiber could reduce the chemical reactivity and capacity for water absorption.[32]

As illustrated in Table 2, the crystallinity index (CrI) of the fibers increased from 38.79% to a maximum of 65.66% under the conditions of the two-stage pretreatment (hydrothermal and $FeCl_3$ pretreatment). These results suggest that the two-stage pretreatment led to a higher CrI, compared to that achieved after the one-stage pretreatment, indicating that the hydrothermal process effectively broke down the more pliable hemicelluloses and lignin components. Meanwhile, cellulose fibrils might regenerate the inter- and intra- chain hydrogen bonding and increase chemical resistance resulting in reducing water absorption. As mentioned previously, acid treatment easily degrades the disordered regions of cellulose chains, while the crystalline parts are more resistant and left intact.[33] Metal chlorides have been reported to act as Lewis acids, and their main activities involve hemicelluloses solubilization.[7] Pulping and bleaching processes also have an impact on the crystal content, causing more crystallinity and the appearance of fewer amorphous regions. This effect was also discussed in the literature, regarding chemical treatment using sodium hydroxide and sodium hypochlorite in the extraction of cellulose.[34,35] Higher crystallinity means higher capacity to produce nanocellulose.

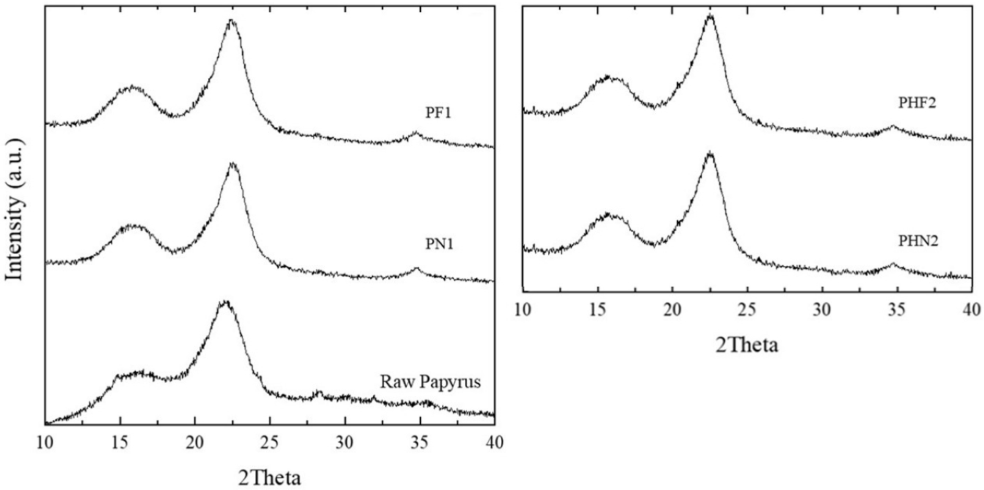

Figure 3: XRD patterns of raw papyrus, and one-stage pretreated (PN1 and PF1 for NaOH and $FeCl_3$, respectively), and two-stage pretreated samples (PHN2 and PHF2) after pulping and bleaching processes

Table 2
Cellulose crystallinity index (CrI) of papyrus before and after different pretreatment steps after pulping and bleaching processes

Parameter	Pretreatment				
	Raw	PN1	PF1	PHN2	PHF2
CrI (%)	38.79	44.45	45.54	51.14	65.66

ATR FT-IR spectroscopy

Attenuated total reflectance–Fourier transform infrared spectroscopy was used to characterize the extracted fibers, based on the characteristic transmittance bands of their constituents. The spectra presented in Figure 4 reveal the presence of characteristic peaks of cellulose at 3340, 2900, 1440, 1314, 1027, and 898 cm^{-1}, which are consistent with those reported in previous literature.[36,37] The broad peaks of cellulose at 3340 and 3320 cm^{-1} refer to O–H stretching due to the presence of α-cellulose. The peaks at 2920 and 2900 cm^{-1} represent the C–H stretching, which occurs due to the vibration of cellulose and hemicelluloses.[38,39] The peaks at 1300–1500 cm^{-1} were attributed to C–H bending, O–H, and C–O stretch of hemicelluloses bonds, cellulose bonds, and alcohol group, respectively.[32] The peak at 898 cm^{-1} represents the C-H deformation of cellulose and hemicelluloses. The band at 1608 cm^{-1} corresponds to the bending mode of naturally absorbed water. The spectra display absorption bands around 1426 and 1314 cm^{-1}, which are attributed to the -CH2 and O–H bending, respectively.

In the spectrum of raw papyrus, the vibration at 1640 cm^{-1} is associated with the carbonyl groups of aldehyde and ketone, which are typically present in the lignin component of raw papyrus,[38] the band at 1734 cm^{-1} corresponds to C=O stretching of (carbonyl ester) hemicelluloses and the peak at 1238 cm^{-1} indicates the syringyl ring and C-O stretch of lignin and xylan.[40] The band at 1604 cm^{-1} representing the aromatic C=C ring stretching and C-H deformation in methyl, methylene and methoxyl groups of lignin[41] was decreased in papyrus after the pretreatments and the pulping and bleaching processes, indicating the efficient removal of lignin and hemicelluloses.

The two-stage pretreatment with $FeCl_3$ yields a crystalline structure that is very close to that of commercial cellulose products. Therefore, $FeCl_3$ pretreatment can be utilized effectively to obtain papyrus cellulose fibers with high crystallinity.

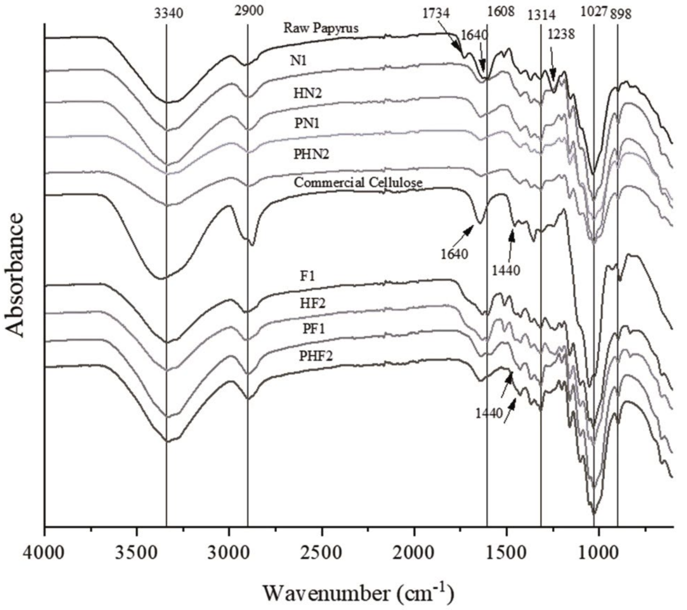

Figure 4: FT-IR spectra of raw papyrus, as well as those of the samples subjected to different pretreatments and pulping and bleaching procedures

CONCLUSION

Out of the procedures examined in this work for extracting cellulose from papyrus, the two-stage pretreatment incorporating a hydrothermal step combined with chemical pretreatment enhanced the potential for cellulose fiber production, eliminating the hindrance of hemicelluloses and lignin, and increasing the cellulose content with enhanced fiber crystallinity. The impact of using NaOH and $FeCl_3$ in chemical pretreatment was examined. For the cellulose fibers produced following the preparation process including pulping and bleaching stages, cellulose recovery after the two-stage pretreatment with NaOH solution (37.87%) was approximately equal to that with $FeCl_3$ solution (33.43%). The highest hemicelluloses and lignin removals were achieved with the two-stage pretreatment using $FeCl_3$ solution, at 12.43% and 10.51%, respectively. Moreover, the crystallinity index of cellulose reached its peak after the two-stage pretreatment with $FeCl_3$ solution, increasing by 26.87% compared to that of native papyrus. This study demonstrates that the two-step process, involving sequential hydrothermal and chemical pretreatment – particularly with $FeCl_3$ solution – offers a promising approach for obtaining high cellulose yields from papyrus. Further optimization of pretreatment conditions could lead to improved cellulose production and pave the way for nanocellulose development in future research.

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