ORIGINAL ARTICLE

Isolation of Crystalline Microcellulose Fibers Based on Sorghum (*Sorghum bicolor*) Stalks for Biomedical Applications

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ABSTRACT

Introduction: Cellulose nanocrystals are modified through integration with various polymers for a wide range of applications, one of which is for biomedical applications. **Materials and Methods:** The process involves alkalization, bleaching, varying the duration (3, 4, or 5 hours), temperature (50°C, 70°C, or 90°C) and, acid hydrolysis. **Results.:**The results show that the crystallinity index of the cellulose nanocrystal increases with the stages of alkalinization, bleaching and acid hydrolysis. Untreated fibers had a relatively high impurity content, including lignin, hemicellulose, and wax, a crystallinity index of 41.25% and a degradation temperature of 290 °C. Heating temperature lowers the crystallinity index of cellulose. **Conclusion:** The isolation method with the stages of alkalinization process, bleaching for 4 hours, heating temperature of 70 °C and acid hydrolysis is the optimal method in producing crystalline cellulose with low impurity level, high crystallinity (80.25), fine fiber morphology, and degradation temperature 310°C.
Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP8):68-75.doi:10.47836/mjmhs.21.s8.11

Keywords: Nano crystalline cellulose, Shorgum bicolor, Fiber, Chemical method, acyd hydrolysis

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INTRODUCTION

In recent decades, nanocellulose has gained significant attention in nanotechnology due to its beneficial physicochemical properties, high aspect ratio, and exceptional strength and modulus [1]. Noornai et al. (2006, 2007) successfully produced cellulose nanocrystals (CNXLs) from cotton and incorporated them into polysulfone films for ultrafiltration purposes. Li et al. (2011) reported that nanocrystalline cellulose (NCC) was derived from wood and processed using a high-pressure homogenizer to reduce acid concentration during hydrolysis, thereby retaining the natural cellulose properties. Cellulose derived from Pandanus tectorius fibers can be used to reinforce biocomposites, providing a sustainable alternative to synthetic fibers for environmental conservation [2]. Agricultural waste creates environmental issues, but converting it into nano-reinforcements for biopolymers offers a sustainable solution [3]. Considering the medical application of dialysis, there has been research on integrating NCC into polysulfone as porous membranes. The incorporation of NCC significantly improved the hydrophilicity of the dialysis composite membrane due to its large surface area and numerous exposed hydroxyl groups. As a result, when blood comes into contact with the membrane surface, these hydrophilic groups absorb more water, facilitating faster material filtration by the membrane [4].

One previous study attempted to isolate cellulose by chemically treating Helicteres isora plant [5], wheat straw[6], sweet sorghum bagasse [7] with alkali tretment and acid hidrolisis. However, in plants, there are other substances present alongside cellulose in the plant organs, namely lignin and hemicellulose. Both of these substances, along with cellulose, make up the plant cell walls and contribute to the structure of the plant organs [8], [9]. Hemicellulose and lignin are common compounds that make up the plant cell walls [10]. Hemicellulose is also a polysaccharide but has a different carbon chain structure compared to cellulose and has a shorter chain length due to its lower degree

of polymerization [10], [11], [12]. Lignin is an aromatic phenolic compound that provides rigidity to the plant structure [10]. Typically, in woody plants, lignin content is higher than cellulose, giving wood its rigid characteristics [11].

The presence of these two compounds in cellulose reduces its modification compatibility and hampers the influence of hydroxyl groups present in cellulose fibers. These hydroxyl groups can be modified through chemical treatment to produce a hydrophobic and compatible surface with thermoplastic polymers that are hydrophobic [12], [13]. Therefore, to obtain cellulose with high content and good compatibility when combined with other substances to fabricate composites, it is essential to minimize or remove these compounds as much as possible. Active hydroxyl groups can also enhance compatibility with other slightly hydrophilic polymers (which have undergone surface modification) due to Van der Waals interactions on their polar groups. Thus, by reducing the content of these compounds, it is expected that Van der Waals forces and hydroxyl group interactions will be more active, leading to increased compatibility with modified hydrophilic polymers and providing the desired mechanical strength [12].

In addition to improving compatibility, increasing the crystallinity of the obtained fibers is also important to be studied since the chemical resistance, thermal properties, and physical properties of a polymer are influenced by the degree of crystallinity. Reducing the degree of crystallinity will affect the thermal stability, mechanical properties, and chemical properties of a polymer. Therefore, the desired fibers in this study are those with high crystallinity.

Indonesia grows a lot of sorghum plants. Sorghum is one of the potential alternative sources of cellulose in Indonesia [14]. In Indonesia, sorghum is suitable for cultivation due to its ability to withstand extremely dry weather conditions, especially during long drought seasons. The use of sorghum as a raw material in this study aims to find alternative methods to enhance the agronomic value of post-harvest sorghum waste, particularly for the development of materials with easily biodegradable raw materials. Cellulose extraction is one of the utilizations of agricultural waste, minimizing environmental impacts resulting from the increasing agricultural production[15]. The easy biodegradability of cellulose makes it highly suitable for applications in biomedical implants, where materials must naturally decompose without harming the body or environment. The durability of using agro-waste fibers involves several aspects, including mechanical properties, thermal properties, and bioactivity. Bioactivity of hydrogel CNC showed its effectiveness in inhibiting cancer cells (IC50: 34.714 µg/mL) and bacterial growth, highlighting its biomedical potential [16]. CNC extracted from corn has thermal stability above 400 °C, and the addition of 4%-8% CNC into the corn starch polymer matrix significantly improves the mechanical properties [17].
Based on the above explanations, this research needs to be conducted to find effective methods to isolate cellulose with low impurity content, high crystallinity, and relatively high degradation temperature. Cellulose with high crystallinity will provide good mechanical and chemical properties. Very small cellulose size will provide a large interfacial area when combined with other materials in the production of natural fiber-based composites because a larger surface area will facilitate more interactions between the matrix and the reinforcement, resulting in efficient load transfer and increased composite strength [10], [12]. Dialysis membranes require improved hydrophilicity and performance, yet untreated crystalline cellulose fibers suffer from high impurities, low crystallinity, and poor thermal stability. Effective chemical treatments are needed to optimize cellulose properties for advanced applications. This research focuses on the effects of chemical treatments including alkalization, bleaching, and acid hydrolysis. These various treatments aim to observe their effects on the size, crystallinity, changes in chemical groups, and thermal properties of the resulting cellulose.

MATERIALS AND METHODS

Materials

Sorghum fibers (SF) were obtained from a local traditional market, while sodium hydroxide in pellet form, acetic acid, sodium chlorat, and sulfuric acid solution were purchased from Merck (Germany).

Sample Isolation

Preparation

The preparation process began with selecting clean, impurity-free SF, followed by crushing them in a blender and sieving through a 40-mesh sieve.

Sodium Hydroxide treatment

Alkalization was performed by dissolving the SF in a 10% sodium hydroxide solution and stirring for 2 hours at a temperature of 70°C [18], [19], [20]. The alkalized SF were washed with deionized water to remove any soluble substances from the alkalization treatment and optimize the lignin dissolution reaction.

Sodium chlorite treatment

The bleaching treatment was carried out by dissolving the alkalized fibers in a 1.7% sodium chlorite solution, to which a buffer solution consisting of 0.2M acetic acid and sodium hydroxide was added. The buffer solution was used to maintain acidity levels, as sodium chlorate dissociation produces hypochlorous acid, impacting pH stability. This process was performed for 4 hours at a processing temperature of approximately 80°C [19], [21]. Variations were also made in the duration of bleaching, for 3, 4, and 5 hours, at the same temperature

of 70°C.

Sulfuric acid treatment
The bleached SF were dissolved in a 25% sulfuric acid solution with a soaking time of 1 hour at room temperature. The acid hydrolysis process for extraction, the mass ratio between the fibers and the sulfuric acid solution used was 1:16 [19], [22]. The characterizations were performed on a dry basis using an oven drying process.

Characterization of samples
The characterizations were conducted at the Metallurgy and Materials Engineering, Universitas Indonesia, morphology using FE-SEM (Inspect F50, FEI USA), Structure and functional groups using FTIR (Perkin Elmer, USA), and crystallinity index using XRD (XRD-7000, Shimadzu, Japan), thermal behaviour using STA (Perkin Elmer, USA)

RESULT

Morphology
Each treatment will have a different effect on the morphology of the resulting fibers, as influenced by the chemical reactions that occur with the constituents of the natural fibers. From the SEM test results, the morphology of the fibers obtained in the study is shown in the following image.

From the image, it can be seen that the fibers are still intact, and the cellulose threads are not clearly visible. The fibers are still composed of large diameter sizes, approximately 400 mm. It can be observed that the fibers are still arranged in thick thread-like structures consisting of hemicellulose and lignin, indicated by the dark color of the thick threads, which represents a mixture of lignin and hemicellulose. The arrangement of these thick threads appears irregular and rough. This may be a result of the process of cutting sorghum stems into fine fibers. After the sorghum stems are cut into fine fibers, the fibers are subjected to an alkalinization treatment. The morphology test results using SEM are shown in Figure 1.

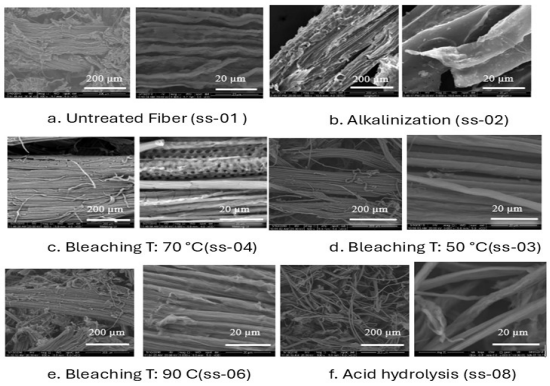

FIGURE 1. Morphology of untreated fiber and various chemically treated

Significant changes can be observed in the fibers after the alkalinization treatment, where the resulting fibers have a smoother morphology compared to the untreated fibers. Cellulose fibers start to become visible as fine threads, although their arrangement is still dense and interconnected. However, at a magnification of 5000x, the morphology of cellulose fibers in the form of threads becomes clearer

FTIR
The obtained results show an increase in cellulose content, indicated by a decrease in the transmission peaks at 3337, 2900, 1640, 1030, and 890 cm^{-1}. These peaks correspond to the vibrations of -OH groups, $-CH_2$ groups, absorbed water -OH, carbonyl groups, and -CH in cellulose. The peak at 2923–2925 cm^{-1} corresponds to C-H stretching [23]. The decrease in transmission indicates a higher energy absorption due to an increased content of functional groups that absorb energy at those specific wavelengths. Therefore, an increase in transmission level at a specific wavelength indicates a decrease in the corresponding substance. If there is no transmission peak at a specific wavelength, it indicates no energy absorption and a decrease in the corresponding substance. For the hemicellulose and pectin content, a decrease is observed, as evidenced by the disappearance of peaks at 1734 and 1514 cm^{-1} in the alkalization-treated fibers, as shown more clearly in Figure 2. These peaks correspond to the carbonyl groups of acetyl groups and the CH vibration in hemicellulose.

Changes in lignin composition are indicated by changes in peaks at 1734, 1598, and 1244 cm^{-1} in the alkalization-treated fibers. These peaks correspond to the vibrations of carbonyl groups, aromatic groups, and carbonyl groups in lignin. The disappearance of peaks at 1598 cm^{-1} and 1244 cm^{-1} indicates a decrease in lignin content. The analysis of these peaks indicates a decrease in lignin content, which can also be attributed to the alkalization treatment. This suggests that alkalization is suitable as an initial step in cellulose fiber isolation. The chemical treatment enhanced fiber roughness while reducing impurities, lignin, and hemicellulose [23].

The bleaching treatment aims to remove lignin content without damaging the cellulose fibers. Lignin is oxidized and dissolved, resulting in brighter-colored fibers. This process is also known as delignification. The reagent used in this step is $NaClO_2$. Previous studies have shown that lignin is destroyed and dissolved through oxidation reactions by ClO_{2-} ions, facilitating the isolation of cellulose fibers.

From the comparison of the infrared spectra of alkalization-bleaching-treated fibers with untreated fibers (ss-01) and alkalization-treated fibers (ss-02), cellulose content is detected at peaks 3337, 2900, 1640, 1033, and 896 cm^{-1}.

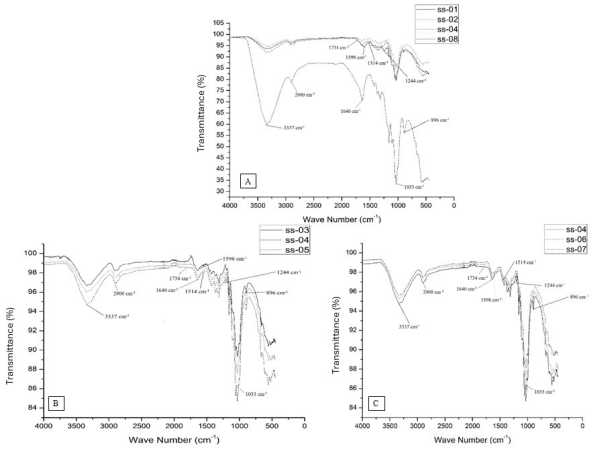

FIGURE 2. Infrared spectra of untreated fibers (ss-01), alkalization (ss-02), bleaching (ss-04), and acid hydrolysis (ss-08) (A), alkalization-bleaching-treated fibers with temperature variations: a) 50°C (ss-03), b) 70°C (ss-04), c) 90°C (ss-05) (B); and alkalization-bleaching-treated cellulose fibers with time variations: a) 3 hours (ss-06), b) 4 hours (ss-04), c) 5 hours (ss-07) (C),

The decrease in lignin content in the bleached fibers (ss-04) is indicated by the continued absence of peaks at 1734, 1598, and 1244 cm^{-1}, as shown in Figure 2A. Temperature is one of the important variables in reaction kinetics. The effect of temperature on the changes in various constituents, particularly lignin, in the bleaching-treated fibers is of interest. The temperatures used in this study were 50, 70, and 90°C for 4 hours. All the fibers underwent alkalization treatment before bleaching.

From the graph in Figure 2, differences in detected peak intensities at certain wavelengths indicate changes in the content. Cellulose is detected at peaks 3337, 2900, 1640, 1033, and 896 cm^{-1}. For these peaks, there are differences in transmission levels for each temperature variation. The order of transmission levels for these peaks, from highest to lowest, is observed in the alkalization-bleaching-treated fibers for 4 hours at 50°C, followed by 90°C and 70°C (Fig.2B). In all three temperature variations, a decrease in hemicellulose content is observed, indicated by the absence of peaks at 1734 and 1514 cm^{-1}. Similarly, a decrease in lignin content is observed in all three variations, indicated by the absence of peaks at 1598 and 1244 cm^{-1}.

Time is another important variable, along with temperature, in reaction kinetics. The effect of time variations on changes in fiber constituents, particularly lignin, is of interest. The time variations tested were 3, 4, and 5 hours at a treatment temperature of 70°C (Fig. 2C). Each fiber underwent alkalization treatment before bleaching. The spectra show that cellulose content is detected at peaks 3337, 2900, 1640, 1033, and 896 cm-1. There are differences in transmission levels for each time variation. The highest transmission levels for these peaks are observed in the alkalization-bleaching-treated fibers for 4, 5, and 3 hours, respectively.

The acid hydrolysis process aims to increase the crystallinity of the resulting fibers by dissolving the amorphous parts of cellulose fibers. This results in an increase in cellulose content and sharper peaks. The FTIR analysis of fibers treated with acid hydrolysis shows a significant increase in cellulose content, as indicated by the drastic decrease in the transmission peaks corresponding to cellulose functional groups at 3337, 2900, 1640, 1033, and 896 cm^{-1}.

Crystallinity

Diffractometer analysis resulted in testing spectra as shown in Figure 2. The spectrum shows two peaks identified as diffraction from the crystalline and amorphous parts of cellulose fibers. The intensity level of crystalline parts, known as I002, is detected at a peak with 2θ = 22°, while the intensity level of amorphous parts, known as Iam, is detected at a peak with 2θ = 16°.

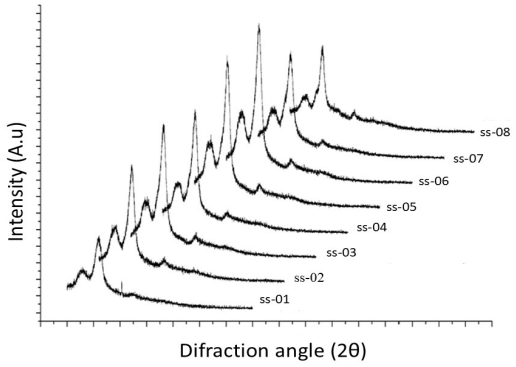

FIGURE 3. XRD Test Spectra of all fibers of untreated fibers (ss-01), alkalization-treated fibers (ss-02), and alkalization-bleaching-treated fibers with temperature variations: 50°C (ss-03), 70°C (ss-04), 90°C (ss-05), alkalization-bleaching-treated cellulose fibers with time variations: 3 hours (ss-06), 4 hours (ss-04), 5 hours (ss-07) and acid hydrolysis (ss-08).

The lowest level of crystallinity is observed in untreated fibers (41.25%). This value is lower than the crystallinity index of other natural fibers, including *Muntingia calabura* (69.30%) [23] and rice straw (61.8%) [24]. The highest level of crystallinity is found in fibers treated with acid hydrolysis (80.25%). The variations in chemical treatments result in an increase in the crystallinity of the produced fibers, as shown in the following graph (Fig. 4).

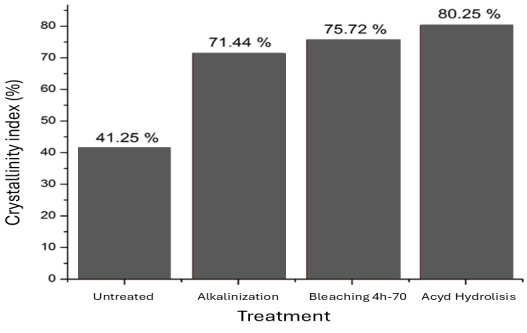

FIGURE 4. Crystallinity level for each treatment.

This indicates that there is an increase in the crystalline portion of cellulose fibers at each stage of the chemical treatment reactions. Alkalinization has been proven to remove hemicellulose and pectin content. Bleaching has been shown to significantly reduce lignin content. The effects of these two reactions clearly increase the level of crystallinity as a result of removing the amorphous part of the natural fibers. Acid hydrolysis has successfully hydrolyzed the amorphous part of the produced cellulose fibers, resulting in the highest level of fiber crystallinity. This is also consistent with the results of the infrared spectrum test, which showed that the highest cellulose content is found in fibers treated with alkalinization-bleaching-acid hydrolysis.

There is a decrease in crystallinity with increasing treatment temperature from 50 to 90°C. This is more clearly illustrated in the Figure 5.

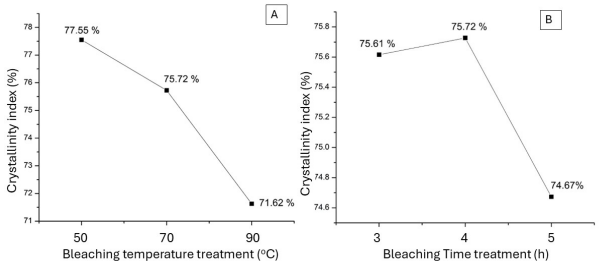

FIGURE 5. Crystallinity index against bleaching temperature (A) and bleaching time variations (B).

This is because higher temperatures result in excessive oxidation reactions, which also affect the cellulose fiber content [43]. The highest cellulose content is found in the alkalinization-bleaching treatment at 70°C. However, this does not necessarily determine that this treatment has the highest crystallinity. With increasing temperature, there is a possibility that the reagent used will degrade both amorphous and crystalline cellulose due to the relatively high treatment temperature. This leads to a downward trend in crystallinity due to the temperature increase.

Thermal Properties

The fibers will have different thermal properties, specifically their degradation temperature (Td). The difference in Td arises from variations in their different levels of crystallinity. The fibers tested for their thermal properties include untreated fibers, fibers treated with alkalization, fibers treated with alkalization-bleaching for 4 hours at 50 °C, fibers treated with alkalization-bleaching for 4 hours at 90 °C, and fibers treated with alkalization-bleaching for 4 hours at 70 °C followed by acid hydrolysis. These fibers were selected based on their different levels of crystallinity resulting from various chemical treatments. Particularly for the bleaching treatment, fibers with the highest and lowest levels of crystallinity were chosen. Termogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) shown in Figure 6A.

In the untreated fiber, there was a drastic decrease in weight at the beginning of the temperature increase. According to Johar et al., this occurred because the fiber contained a high moisture content, which evaporated completely as the temperature increased [6]. Therefore, the steeper the curve's decline during heating, the higher the moisture content. The decrease caused by vaporization absorbed heat, indicated by fluctuations in the DSC curve within the temperature range of evaporation, which was observed in all fibers.

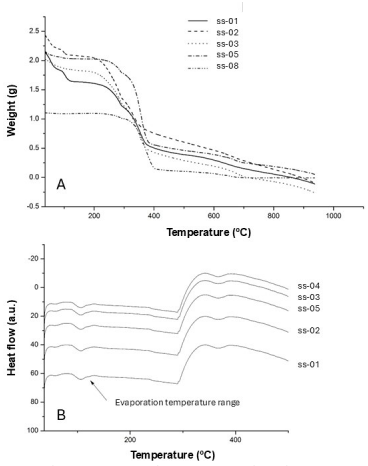

FIGURE 6. Thermal property data of each fiber: A) DSC curve graph; B) TGA curve graph.

This moisture reduction trend occurred due to differences in other content among the fibers. Untreated fibers typically consist of cells containing a significant amount of water. With chemical treatments that degrade other components such as lignin and hemicellulose, the stored water evaporates more easily in the absence of barriers (lignin), resulting in chemically treated fibers being drier than untreated fibers. Chemically treated fibers were isolated from the ambient air with the presence of silica gel, which has higher water absorbency, thereby keeping the fibers dry.

When examining the TGA curves (Figure 6B), each fiber experienced a significant reduction in mass. The temperature range in which this reduction occurred represented the degradation temperature for each fiber. It was observed that the positions of these significant mass reductions varied among the TGA curves of each fiber, indicating different degradation temperatures. As the position of the mass reduction shifts further to the right, the degradation temperature increases. Thus, the order of degradation temperatures from lowest to highest was: untreated fiber >> alkalization-treated fiber >> alkalization-bleaching for 4 hours at 90 °C fiber >> alkalization-bleaching for 4 hours at 50 °C fiber >> alkalization-bleaching for 4 hours at 70 °C followed by acid hydrolysis fiber.

DISCUSSION

The resulting fibers have a size of 400 mm, and there are regular line patterns indicating the successful isolation of microfibril cellulose fibers. The black-colored part of the fibers has disappeared, indicating the removal of hemicellulose, pectin, and some lignin, as indicated by the brighter morphological appearance.

From the image, it is also evident that the cellulose fibers have a porous morphology. According to the previous study conducted by Chalid, this is caused by the alkalinization reaction that occurs on the fibers, resulting in a rough surface [25]. In the bleaching treatment at 70 °C, it can be observed that the fibers start to separate from each other. Microfibril cellulose threads become visible, indicating the removal of the binding matrix, which consists of lignin and hemicellulose, due to the oxidation reaction from the $NaClO_2$ solution. However, these threads appear to be damaged at their ends, suggesting that cellulose may have experienced excessive degradation from oxidation with $NaClO_2$.

The fiber size becomes smaller compared to untreated fibers and fibers treated only with alkalinization. The fiber size is approximately 164 mm. The best fiber morphology is shown by the bleaching treatment at 50 °C for 4 hours. The fibers appear separate and undamaged, unlike in the bleaching treatment at 70 °C. This indicates that the oxidation reaction of lignin occurs optimum without causing damage to the cellulose fibers, thereby maintaining a high level of crystallinity. These separate fibers have a very small size of 6 mm, clearly indicating that the resulting crystalline cellulose fibers are microfibrils [26]. In the bleaching treatment with varying times, it is found that the lowest level of crystallinity is exhibited by the fibers bleached for 5 hours at 70 °C. The observations indicate that the fibers have a rough appearance and experience damage at their edges and ends. This suggests that the 5-hour bleaching treatment results in fiber degradation, which in turn damages the cellulose fibers and reduces their crystallinity. The fiber size measures 150 mm, smaller than the bleaching treatment at 70 °C. This indicates that increasing the bleaching temperature could lead to an additional reduction in fiber size. The crystallite size of CNCs is smaller than that of cellulose, as sulfuric acid hydrolysis selectively removes the amorphous regions, preserving the crystalline domains[27].

The cellulose fibers subjected to acid hydrolysis have a cross-sectional diameter of 3.8 mm. This indicates that the acid treatment reduces the fiber size even more due to the breakdown of the amorphous regions of the microfibrillar cellulose fibers.

The attacked region is specifically the amorphous part of the microfibrillar cellulose, as it is less stable and resistant to chemical reactions compared to the crystalline part, attributed to the free volume in the amorphous region of the fibers. The amorphous part undergoes hydrolysis, leaving behind the separate crystalline part, as shown in the SEM morphology test results. The fibers appear separate in the form of fine microfibrils, and at a magnification of 5000 x, the regular line patterns are clearly visible, indicating a high level of crystallinity in the resulting fibers. Therefore, a higher crystallinity index corresponds to greater thermal resistance in the fiber, as an increased crystalline phase enhances thermal stability (Hajiha, Sain, and Mei, 2014).

The alkalization treatment was performed to remove hemicellulose, pectin, and wax content from the fibers. The reduction in hemicellulose content can be identified by changes in the transmission levels of the hydroxyl and ester groups that make up the hemicellulose structure. A decrease, increase, or disappearance of peaks at specific wavelengths indicates changes, which can be observed as a decrease in peak sharpness in certain wavelength ranges.

For the alkalization-bleaching-treated fibers, the peaks appear with higher transmission intensities, indicating a decrease in cellulose content. This is likely due to the degradation of cellulose by sodium chlorate solution. The degraded cellulose is likely to be amorphous cellulose, which has lower chemical resistance. The bleaching process leads to a decrease in lignin content due to the oxidation of ClO_{2-} ions, which breaks down the aromatic chains of lignin, causing it to dissolve in water and increasing the brightness of the fibers. The temperature range of 50-90 °C is suitable for lignin removal, but considering the cellulose content, a temperature of 70 °C is the most optimum in the bleaching process, as it results in the highest cellulose content among the three temperature variations, indicated by transmission levels representing the vibrations of cellulose functional groups.

The elimination of these lignin-associated peaks underscores the effectiveness of the bleaching treatment in isolating cellulose by removing non-cellulosic components. The reduced lignin content is critical for enhancing the purity and functionality of the fibers, especially for applications where high cellulose content is essential, such as in biomedical and composite materials.

This result aligns with the expected outcomes of bleaching treatments, which are designed to selectively degrade and remove lignin and hemicellulose while preserving the cellulose structure.

Hemicellulose content in three time variations also decreases, as evidenced by the absence of peaks at 1734 and 1514 cm^{-1}. Similarly, lignin content decreases in these three variations, as indicated by the absence of peaks at 1734, 1598, and 1244 cm^{-1}. This suggests that

the time range of 3-5 hours is suitable for delignification, but to prevent cellulose degradation, the most optimum time is 4 hours. It also highlights the potential of the processed fibers for further chemical modifications or applications that require minimal lignin interference.

CONCLUSION

Bleaching cellulose for 4 hours at 70 °C followed by acid hydrolysis is the optimum method to produce microfibrillar cellulose fibers with high crystallinity (80.25%), low impurity content, fine morphology, and excellent thermal resistance. The 4-hour bleaching duration yielded the highest crystallinity index, as confirmed by XRD. This process also produced fibers with the lowest moisture content and the highest degradation temperature, indicating that increased crystallinity enhances thermal resistance, as the denser molecular structure requires more energy to degrade.

ACKNOWLEDGMENTS

The authors are grateful for the research grant from the Hibah Publikasi Terindeks Internasional (PUTI) Pascasarjana 2024-2025 Nomor: NKB-119/UN2.RST/HKP.05.00/2024

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