G-Tech : Jurnal Teknologi Terapan
Vol. 9, No. 3, July 2025, p. 1503-1512
E-ISSN: 2623-064X | P-ISSN: 2580-8737

Study on Microcrystalline Cellulose Derived from Cassava Stems with Variables of Sulfuric Acid Concentration and Hydrolysis Temperature

Rafitrah Rizqullah [1]✉, Muhammad Fadilah [2], Caecilia Pujiastuti [3]

[1, 2, 3] Department of Chemical Engineering, Faculty of Engineering and Science, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Indonesia

✉ **Corresponding Author :** Rafitrah Rizqullah (e-mail: 21031010253@student.upnjatim.ac.id)

Article Information

Article History

Received : June 20, 2025
Revised : June 30, 2025
Published : July 12, 2025

***Keywords*:**
Microcrystalline cellulose;
Cassava stem;
Hydrolysis;
Crystallinity;
Sulfuric acid.

ABSTRACT

This study develops a method for producing microcrystalline cellulose (MCC) from cassava stem waste, aiming to reduce dependence on imported cellulose and promote the utilization of agricultural residues. Cassava stems contain a high cellulose fraction and hold potential as a raw material for pharmaceutical-grade MCC. This study investigates the effects of sulfuric acid concentration and hydrolysis temperature on the yield and quality of the resulting MCC. The production process consists of three main stages: delignification using 25% sodium hydroxide, bleaching with 2% hydrogen peroxide, and acid hydrolysis using sulfuric acid at concentrations ranging from 1.5 N to 3.5 N and temperatures between 60°C and 100°C. The resulting MCC was characterized using Particle Size Analysis (PSA), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and organoleptic testing. The best results were obtained at 1.5 N sulfuric acid and 60°C, yielding 68.24% MCC with a cellulose content of 63.07%. PSA results showed particle sizes of approximately 25.3 µm, which comply with pharmacopeial standards. XRD and SEM analyses confirmed good crystallinity and appropriate morphology. This study demonstrates that cassava stems can be processed into MCC that meets key specifications of the British Pharmacopeia, offering a low-cost and sustainable raw material for industrial and pharmaceutical applications.

INTRODUCTION

Indonesia is one of the largest producers of cassava (*Manihot esculenta*) in the world. However, the utilization of cassava stems as agricultural waste remains very limited. In fact, cassava stems have a high lignocellulosic content—comprising cellulose, hemicellulose, and lignin—which makes them a highly promising raw material for industrial applications. According to a study by Lismeri in 2016, cassava stems contain 39.29% cellulose, 24.34% hemicellulose, and 13.42% lignin. Currently, the demand for microcrystalline cellulose (MCC) in Indonesia is still largely met through imports. Data from the Central Bureau of Statistics (BPS) reported that MCC imports reached 4,359,762 kg in 2019.

Previous studies have explored the potential of cassava stems as a raw material for the production of microcrystalline cellulose (MCC). Research conducted by Widodo in 2013 reported that optimal delignification conditions were achieved using 25% NaOH at 120°C for 60 minutes, resulting in an α-cellulose content of 67.69%. As explained in a study conducted by Wulandari in

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2016, alkaline delignification using NaOH is known to be effective in reducing lignin content from various lignocellulosic materials. The bleaching process using 2% H_2O_2 at 60°C for 120 minutes, as reported by Sumada in 2011, showed that the use of 2% H_2O_2 increased α-cellulose content by 1.69%. Furthermore, according to a study by Octavianan in 2017, hydrogen peroxide has a strong oxygen-releasing capability, does not produce precipitates, yields a stable white product, reduces lignin content, and improves color. This process was then followed by acid hydrolysis to produce MCC. A study by Raja in 2021 found that hydrolysis using 2.5N H_2SO_4 at 80°C for 30 minutes produced MCC with a yield of 87.9%. The resulting product also met pharmaceutical-grade specifications, making it suitable for industrial applications.

Generally, MCC production consists of three main stages: delignification, bleaching, and acid hydrolysis. Process parameters such as acid concentration and reaction temperature are known to significantly affect the quality and yield of the final product. In a study conducted by Saenuddin in 2019, it was reported that increasing acid concentration could reduce yield due to excessive degradation of cellulose into glucose monomers. This is supported by Ardyagarin's 2021 study, which also noted that while higher temperatures accelerate reaction rates, they also increase the risk of excessive cellulose degradation. Such conditions can decrease MCC production efficiency and compromise the quality of the final product. Therefore, precise control of process parameters is essential to achieve optimal results.

This study offers a novel approach by systematically combining variations in sulfuric acid concentration and hydrolysis temperature using cassava stems, which are a largely underutilized biomass source. While previous studies focused primarily on optimizing single variables or using other lignocellulosic materials such as wood pulp or cotton, this research highlights the potential of cassava stems as a low-cost, local raw material for MCC production. The integrated evaluation of both acid concentration and temperature aims to provide a more comprehensive understanding of their interaction effects on MCC yield and quality. This dual-variable approach contributes to the optimization of environmentally friendly and economically viable MCC production processes, specifically tailored to Indonesia's abundant agricultural waste.

Based on this background, the present study aims to evaluate the effect of varying sulfuric acid concentrations and hydrolysis temperatures on the characteristics of microcrystalline cellulose produced from cassava stems. Utilizing cassava stems as a local raw material is expected to add value to underutilized agricultural waste. Furthermore, this research aims to reduce Indonesia's dependence on imported MCC. The results of this study are expected to open new opportunities for the development of MCC from more economical and sustainable local resources. This study also contributes to strengthening self-reliance in raw materials for the national pharmaceutical and food industries. Thus, this research serves as an initial step in optimizing the industrial potential of local biomass.

RESEARCH METHODS

Preparation of Research Materials

Raw Material Preparation Cassava stems were collected as agricultural waste from the Gresik region, East Java. The outer bark of the stems was peeled, and the stems were cut into small pieces. These pieces were washed with distilled water and dried in an oven at 100 °C until a constant weight was achieved. Once dried, the stems were ground using a knife mill and sieved to obtain powder (100 mesh). This powder was then analyzed and found to contain 35.69% cellulose, 23.85% lignin, and 15.79% hemicellulose.

Cellulose Isolation and MCC Synthesis

To obtain microcrystalline cellulose (MCC), cassava stem powder was first subjected to a pre-hydrolysis stage using hot water to remove soluble impurities. The resulting solid residue was then immersed in a 25% sodium hydroxide (NaOH) solution at elevated temperatures to remove lignin and produce α-cellulose. Subsequently, the α-cellulose was bleached with a 2% hydrogen peroxide (H_2O_2) solution to improve its purity. The purified α-cellulose was then hydrolyzed using

sulfuric acid (H_2SO_4), with variations in acid concentration (1.5 N, 2 N, 2.5 N, 3 N, and 3.5 N) and hydrolysis temperature (60°C, 70°C, 80°C, 90°C, and 100°C). The main parameter observed in this stage was MCC yield, accompanied by further analyses including cellulose content, particle size, crystallinity, and surface morphology. The hydrolyzed product was subsequently neutralized, filtered, and dried to obtain MCC as the final product. The MCC yield was calculated as the percentage of MCC mass relative to the initial α-cellulose mass, serving as an indicator of synthesis efficiency.

Analysis Methods

Analytical Methods The process output was determined based on the percentage of final product mass relative to the input mass from the previous stage. To evaluate the chemical composition of the materials and products, the contents of cellulose, lignin, and hemicellulose were analyzed. Organoleptic tests were conducted to visually assess color, odor, and solubility. Particle size distribution was analyzed using a Particle Size Analyzer (PSA), while surface morphology was examined using a Scanning Electron Microscope (SEM). Additionally, the crystal structure and degree of crystallinity of MCC were analyzed using X-ray Diffraction (XRD).

RESULTS AND DISCUSSION

Analysis Method of the Effect of Cooking Temperature on the Yield (%) Obtained Based on the Variation of Sulfuric Acid Concentration

The content of cassava stems was analyzed prior to the cellulose isolation and microcrystalline cellulose (MCC) synthesis process to determine the influence of the material's components, including cellulose, lignin, and hemicellulose. Understanding the composition is crucial because each component plays a significant role in the efficiency and quality of cellulose extraction and subsequent MCC production. For instance, a higher cellulose content generally leads to a better yield of MCC, while the presence of lignin and hemicellulose can affect the purity and properties of the final product. The results of the cassava stem composition analysis are presented in the following table, providing a detailed breakdown of each component's percentage within the raw material.

Tabel 1. The Results of The Cassava Stem Composition Analysis

Element	Content (%)
Cellulose	35,69
Lignin	23,85
Hemicellulose	15,79

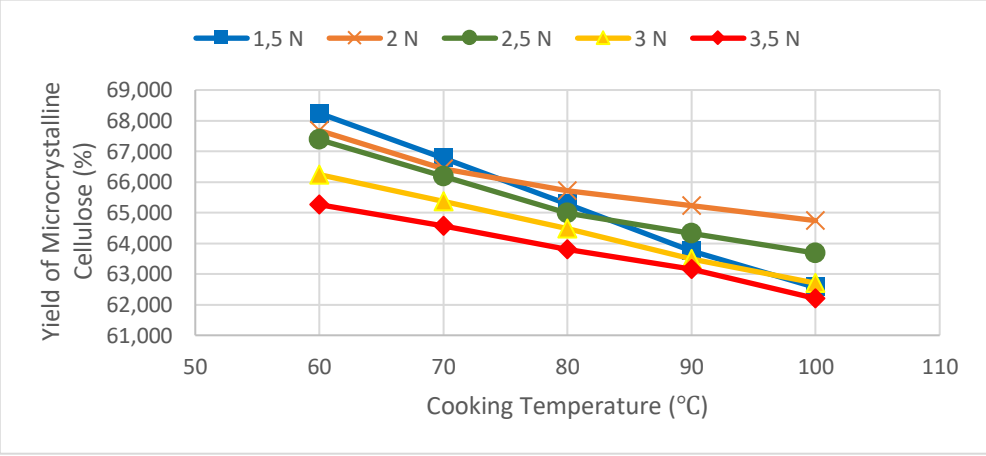


Figure 1. The Relationship Between Yield (%) and Cooking Temperature Based on Variations in Sulfuric Acid Concentration

Based on Figure 1, increasing the cooking temperature results in a gradual decrease in the yield of microcrystalline cellulose (MCC). The weighing results of each sample consistently show a decline in production yield as the temperature increases. A similar pattern is also observed with increasing sulfuric acid concentration. For instance, at a cooking temperature of 60°C, the highest yield was obtained at a sulfuric acid concentration of 1.5 N, reaching 68.240%. This yield then gradually decreased to 67.680% (2 N), 67.380% (2.5 N), 66.240% (3 N), and 65.260% (3.5 N). This trend clearly indicates that the simultaneous increase in temperature and acid concentration has a significantly negative impact on MCC yield. Figure 1 supports this observation, showing curves that consistently decline with increasing cooking temperature and sulfuric acid concentration. Specifically, at a 1.5 N acid concentration, the MCC yield gradually decreased from 68.240% at 60°C to 62.560% at 100°C. This reduction may be due to the faster hydrolysis rate under high-temperature and high-acid conditions, which leads to excessive degradation of cellulose into monomeric sugars and unwanted by-products.

These findings are consistent with studies conducted by Saenuddin in 2019 and Priatna in 2021, which emphasized that overly high acid concentrations and temperatures accelerate hydrolysis and cause significant damage to the cellulose structure. Research by Raja in 2021 and Edison in 2015 also confirmed that high acid concentrations can damage the crystalline structure of cellulose and negatively affect the physical properties of MCC, including discoloration and the loss of structural integrity. Moreover, increasing temperature raises the kinetic energy of particles, thereby accelerating the reactions that lead to glucose formation. As a result, excessive heating can promote the formation of undesirable by-products such as furfural and hydroxymethylfurfural, ultimately reducing both the purity and yield of MCC.

The highest MCC yield in this study was 68.24%, obtained at a sulfuric acid concentration of 1.5 N and a temperature of 60°C. This result is lower than the 87.9% yield reported by Raja in 2021, who used 2.5 N H_2SO_4 at 80°C on oil palm midrib. However, the higher yield in that study may be attributed to differences in the composition of raw materials and a shorter hydrolysis duration. In comparison, Saenuddin's study in 2019 reported a yield of 65.3% from tapioca waste using 2 N H_2SO_4, which is slightly lower than the result obtained in the present study. This suggests that cassava stems could be a competitive alternative raw material with better α-cellulose retention under milder conditions. This comparison indicates that lower acid concentrations and cooking temperatures may be more effective in cassava-based MCC synthesis to prevent cellulose degradation. Therefore, careful optimization of both cooking temperature and sulfuric acid concentration is crucial to maximize MCC yield while maintaining the quality and structural integrity of the final product.

Analysis of the Effect of Cooking Temperature on the Cellulose Content Produced Based on Variations in Sulfuric Acid Concentration

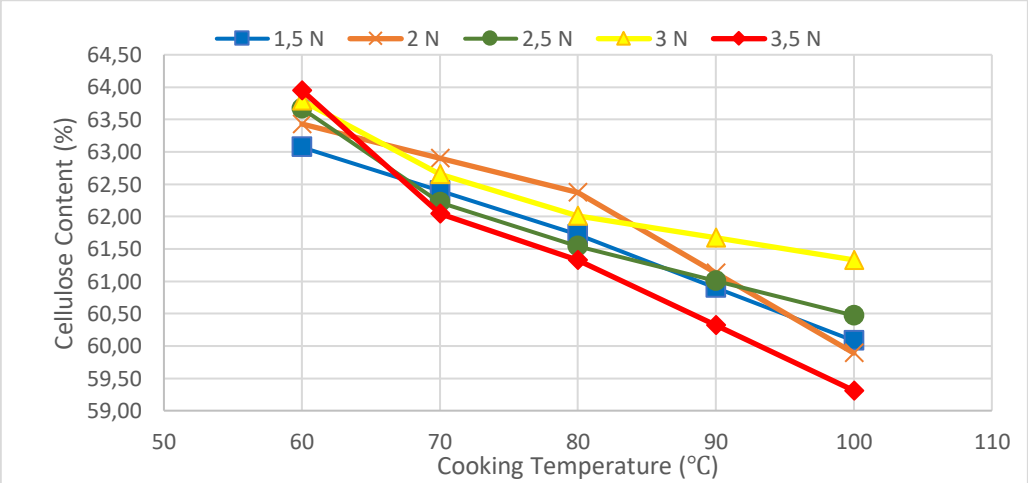


Figure 2. The Relationship Between Cellulose Content and Cooking Temperature Based on Variations in Sulfuric Acid Concentration in Microcrystalline Cellulose

Based on Figure 2, the analysis of microcrystalline cellulose (MCC) indicates that increasing sulfuric acid concentration and cooking temperature significantly reduces the cellulose content. This finding supports the theory proposed by Artati in 2012, which states that high acid concentrations and elevated temperatures accelerate the hydrolysis process and the degradation of cellulose. Under such conditions, not only the amorphous regions of cellulose are broken down, but the more resistant crystalline regions can also be damaged. This leads to a reduction in cellulose yield and the formation of degradation by-products such as hydroxymethylfurfural (HMF), humin, and hydrochar, which also cause the MCC to darken in color. Studies conducted by Edison in 2015 and Ardyagarini in 2021 support these findings, showing that excessively harsh hydrolysis conditions negatively impact both the quantity and quality of MCC.

Under optimal conditions, the cellulose content in MCC obtained from this study reached 63.07%, which is comparable to the 62.3% reported by Edison in 2015 for seaweed-based MCC. However, this value is slightly lower than the 67.69% α-cellulose content reported by Widodo in 2013 from cassava stems without acid hydrolysis. This suggests that although purification through hydrolysis improves selectivity, the process may also cause partial degradation of cellulose chains, especially under severe conditions. Therefore, optimizing acid concentration and cooking temperature is a critical factor not only for maximizing yield but also for preserving the structural integrity of cellulose during MCC synthesis from cassava biomass.

Analysis of Microcrystalline Cellulose Characteristics

Tabel 2. The Results of Microcrystalline Cellulose Characteristics Result (1.5N / 60°C)

Examination	Requirement	Result
Form	Crystalline / Powder	Crystalline / Powder
Color	White	Yellowish brown
Odor	Odorless	Odorless
Taste	Tasteless	Tasteless
pH	5-7	5-7
Solubility	Insoluble in water	Insoluble in water

Based on the data in Table 2, the microcrystalline cellulose (MCC) produced from treatment with 1.5 N sulfuric acid at 60°C meets the physical quality standards established by the British Pharmacopeia in 2002, as cited by Nawangsari in 2018. The resulting MCC appeared as a white crystalline powder, odorless, tasteless, with a pH range between 5 and 7, and was insoluble in water. These physical properties indicate that the production process was carried out properly, allowing the crystalline structure of MCC to be preserved. The formation of this crystalline structure also serves as an indicator of the successful hydrolysis and purification process, which are essential for ensuring the final product's quality.

The average particle size of MCC in this study was approximately 25.3 µm, which falls within the acceptable range for MCC (20–180 µm) and is consistent with the standards of Avicel PH-101. When compared to the results reported by Yuen et al. in 2023, who found particle sizes for cotton-based MCC to be 180–300 nm (volume-based) and less than 200 nm (number-based), the MCC in this study exhibited larger cumulant-based values but showed similar submicron distributions in the SBL-Volume and SBL-Number analyses. This indicates that cassava-based MCC possesses physical characteristics suitable for pharmaceutical applications, particularly as a binder or filler in tablet formulations.

However, under treatment conditions involving higher acid concentrations and cooking temperatures, the MCC tended to exhibit a color change from light yellow to brown. This discoloration is likely due to excessive cellulose degradation, mild carbonization reactions, or residual lignin that was not fully removed. According to Rosli in 2013, the darkening of MCC color is generally associated with the formation of degradation by-products such as humins or hydrochar. Although other parameters such as odor, taste, form, pH, and solubility remained

stable, color remains an important quality control indicator, as it reflects the purity and structural stability of MCC. Therefore, treatment with 1.5 N sulfuric acid at 60°C can be considered the optimal condition for producing MCC with acceptable physical characteristics and high quality.

Analysis and Discussion Of Particle Size Analysis (PSA) Nn Microcrystalline Cellulose

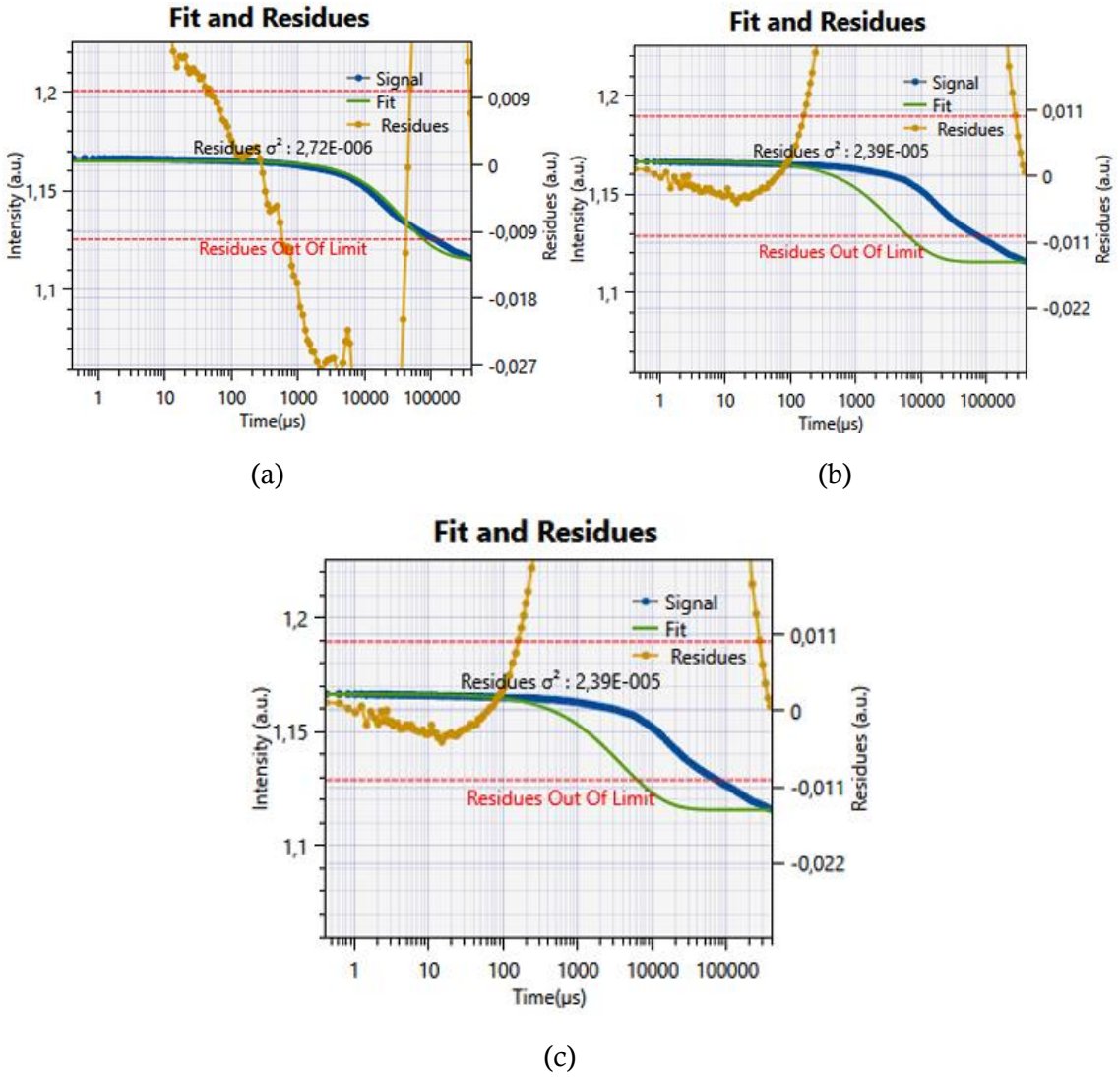


Figure 3. PSA (a) Cumulants, (b) SBL-Volume, (c) SBL-Number Results of Microcrystalline Cellulose Treated with 1.5 N Sulfuric Acid at 60°C

Based on Figure 3, the particle size analysis of microcrystalline cellulose (MCC) treated with 1.5 N sulfuric acid at 60°C showed that the average particle size obtained using the Cumulants method was 25,286.88 nanometers, or approximately 25.3 micrometers. This value falls within the general MCC standard range of 20–180 µm, in line with pharmacopeial specifications such as Avicel PH-101. Measurements using the SBL-Volume and SBL-Number methods yielded particle sizes of 250.39 nm and 138.06 nm, respectively. These values represent the particle contribution by volume and number, respectively, and remain consistent with findings by Yuen et al. in 2023, who reported volume-based distributions of 180–300 nm and number-based distributions below 200 nm for MCC derived from cotton waste. Although slightly lower, the values in this study remain within the acceptable range, indicating the presence of fine particles that support compressibility and flowability, which are crucial in pharmaceutical processing.

The Polydispersity Index (PDI) value obtained in this study was 0.4348, suggesting a moderately broad particle size distribution. This is still within the industrially acceptable range of 0.3 to 0.7 for MCC, as supported by Yuen in 2023, who reported a PDI of 0.304. A PDI within this range indicates a relatively uniform and consistent particle distribution, essential for maintaining the physicochemical stability of MCC. Uniform particle distribution enhances performance in applications such as powder blending, granulation, and direct compression for tablet production. Therefore, the particle size results in this study confirm that cassava stem-derived MCC meets physical quality standards and demonstrates comparable characteristics to MCC derived from other biomass sources, reinforcing its potential as a pharmaceutical excipient.

Analysis and Discussion Of X-Ray Diffraction (XRD) On Microcrystalline Cellulose

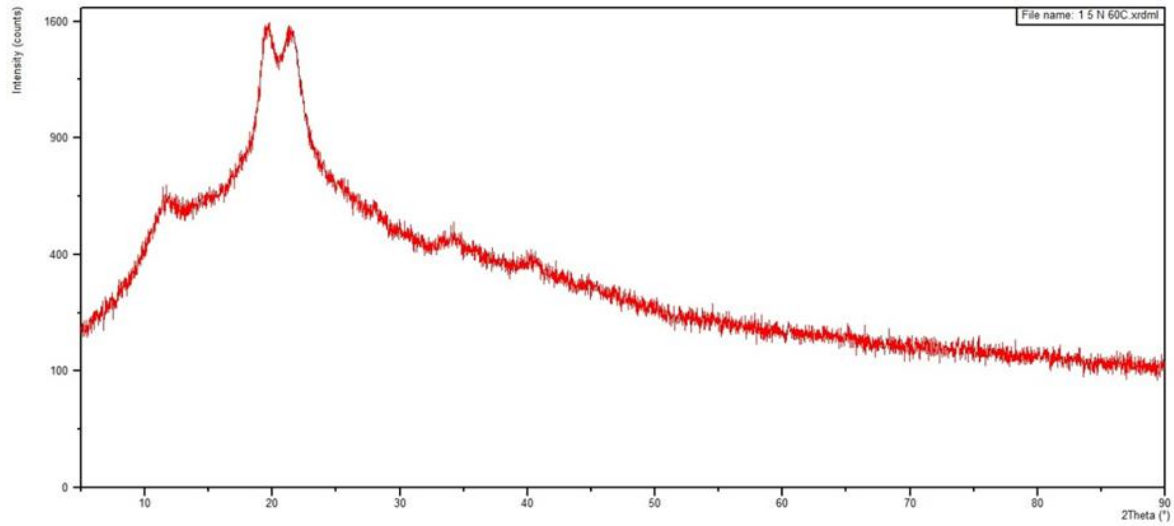
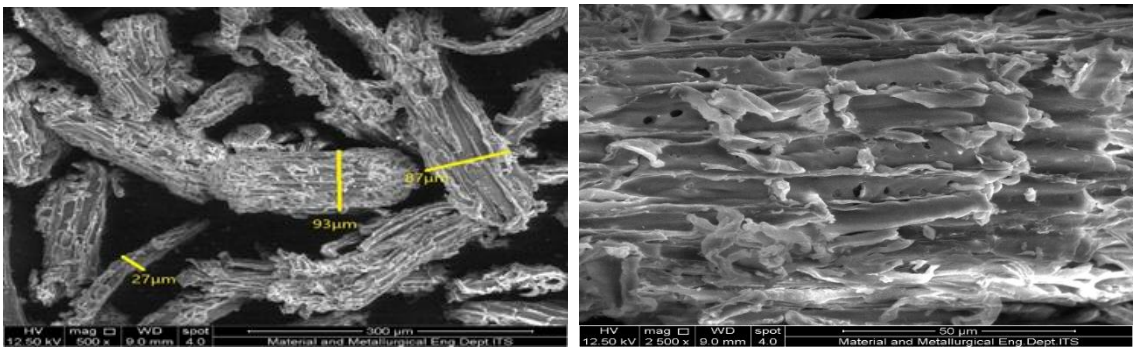


Figure 4. XRD Analysis Results of Microcrystalline Cellulose at 1.5 N Sulfuric Acid Concentration and 60°C Temperature

The XRD pattern of the MCC sample treated with 1.5 N sulfuric acid at 60°C showed diffraction peaks at $2\theta \approx 19.6°$ and 22.4°, which are characteristic of cellulose II, indicating a semicrystalline structure. The appearance of broad peaks also suggests the presence of amorphous regions, a common feature in partially crystalline cellulose. These findings are comparable to the results reported by Santos et al. in 2020, who observed similar diffraction patterns in chitin-based materials, and Yuen et al. in 2023, who identified cellulose II in MCC produced from cotton waste. The similarity in peak positions confirms that the MCC derived from cassava stems exhibits typical structural features associated with high-quality cellulose.

The crystallinity index (CrI) obtained in this study was 58.47%, which falls within the standard range for microcrystalline cellulose used in industrial applications, typically between 50% and 70%. This value is slightly lower than the 63.5% CrI reported by Yuen in 2023 for cotton-based MCC but higher than the 52.1% reported by Edison in 2015 for MCC derived from seaweed. These comparisons indicate that cassava stem-based MCC achieves a balanced level of crystallinity that supports both structural stability and functionality. A crystallinity index in this range is desirable in pharmaceutical formulations, as it contributes to enhanced compressibility, controlled hydration, and consistent tablet strength. Therefore, the XRD analysis confirms that the MCC produced in this study meets acceptable crystallographic criteria for pharmaceutical-grade materials.

Analysis and Discussion of Scanning Electron Microscopy (SEM) on Microcrystalline Cellulose



(a) (b)

Figure 5. SEM Analysis Results of Microcrystalline Cellulose at 1.5 N Sulfuric Acid Concentration and 60°C with Magnifications of (a) 500µm and (b) 2500µm

Based Based on Figure 5, SEM analysis observing the surface morphology of the sample treated with 1.5 N sulfuric acid at magnifications of (a) 500x and (b) 2500x showed that the particles had irregular shapes with varying lengths. The particles also exhibited sharp angles, uneven surfaces, and slight porosity. At 500x magnification, the observed particle diameters ranged from 27.14 µm to 93.75 µm. This size range falls within the standard for microcrystalline cellulose according to Avicel PH-101, which is between 20 µm and 180 µm. These results indicate that the produced material possesses morphological characteristics consistent with typical MCC.

A study conducted by Lismeri in 2016 showed that the morphology of cellulose derived from cassava exhibited numerous pores, a loose structure, and a fibrous form, which supports similar findings in the present study. This indicates that the resulting MCC falls into the category of microscale cellulose, formed as a result of acid hydrolysis. Furthermore, according to Nasution in 2020, additional factors influencing MCC synthesis include hydrolysis temperature, reaction duration, and the effectiveness of the sieving process. Lower temperatures may slow down the cleavage of cellulose chains, thus requiring a longer hydrolysis time to achieve optimal results. Similarly, Fitriani in 2022 stated that both acid concentration and temperature critically affect the morphology and crystallinity of the resulting MCC. However, in this study, the use of sulfuric acid as the sole hydrolyzing agent successfully cleaved the cellulose chains, removed the amorphous regions, and produced MCC particles with appropriate size.

CONCLUSION

This study demonstrates that cassava stems can be used as an alternative raw material for producing microcrystalline cellulose (MCC). The best results were obtained at a sulfuric acid concentration of 1.5 N and a hydrolysis temperature of 60°C, yielding 68.24% MCC with 63.07% cellulose content, an average particle size of approximately 25.3 µm, and a crystallinity index of 58.47%. These values fall within the acceptable standards for pharmaceutical-grade MCC and are comparable to those reported in previous studies using raw materials such as cotton, seaweed, and other types of lignocellulosic biomass. Therefore, MCC made from cassava stems offers competitive quality and can be a promising solution to reduce Indonesia's dependence on imported MCC. In addition, using this agricultural waste supports the development of affordable, local, and sustainable raw materials for pharmaceutical and industrial applications.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the Faculty of Engineering and Science, Universitas Pembangunan Nasional "Veteran" Jawa Timur, for providing research facilities and support throughout this study. Special thanks are also extended to Ir. Caecilia

Pujiastuti, M.T., for her invaluable guidance and supervision during the research process. We also acknowledge the assistance from the laboratory staff and all parties who contributed to the completion of this research. This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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