Characterization of Microcrystalline Cellulose from Cassava Stems Through Acid Hydrolysis Process Using H_2SO_4 with Variation Concentration

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ABSTRACT

Cassava stems have a large enough lignocellulose content, which consists of 56.82% α cellulose, 21.72% lignin, 21.45% Acid Detergent Fiber (ADF), and 0.05 - 0.5 cm long fiber. cassava stems have the potential to be used as raw material for the manufacture of Microcrystalline Cellulose (MCC). It can be isolated by pre-hydrolysis, delignification, and bleaching stages. Then hydrolysis is carried out using H_2SO_4 acid. the hydrolysis stage using variations of 1.5N, 2N, 2.5N, 3N, and 3.5N. Then the yield, identification, and characterization with FT-IR were tested. The optimum concentration was obtained at 3N with a yield of 35.29%. MCC obtained from cassava stems has similarities with commercial MCC which is indicated by the appearance of the main peak in the IR spectrum at wavelengths of O-H groups, hydrogen bonds, C-H alkane, C-O ether and alcohol bonds. The identification results showed positive results marked by a change in color to violet purple.

Keywords: Cassava Stem, Microcrystalline Cellulose, Hydrolysis, H_2SO_4, Concentration

1. Introduction

Cassava (Manihot utillisima) or cassava is the third staple food after rice and corn for Indonesians. This plant can grow throughout the year in the tropics and has high adaptability to various soil conditions. This plant has a fairly complete nutritional content. National economic growth increased by 2.19% in 2020 from the agricultural sector [1]

Indonesia occupies the fifth position as the world's largest cassava producer. Indonesia is recorded to be able to produce as much as 18.3 million cassava in 2020. In Indonesia, cassava production centers are spread across 13 provinces. The top five cassava producing provinces are Lampung, Central Java, East Java, West Java and DIY Yogyakarta [2]
The increase in production will also lead to an increase in cassava waste that is also produced. Utilization of cassava stem waste is not optimal because only 10% of the stem height can be used for replanting and the remaining 90% is waste. Some processed products that have been successfully developed from cassava waste include feed, food, industry and energy [3]. From the amount of cassava stems that have not been utilized, it can be used as raw material for making excipient materials for the pharmaceutical industry, namely Microcrystalline Cellulose (MCC), which can be done by using cellulose extracts in cassava stems for the manufacture of Microcrystalline Cellulose (MCC). for pharmaceutical preparation raw materials as a binder in the pharmaceutical industry. Tablets as additional fiber and volume enhancers in the food industry, fillers in the field of biosensors, in the field of tissue engineering and magnetic paper.

Microcrystalline Cellulose (MCC) is the crystalline part of micro-sized cellulose obtained through the acid hydrolysis process of alpha-cellulose, which is the alpha fraction of cellulose that is insoluble in NaOH solution. To meet domestic needs and reduce import dependence, it is necessary to develop microcrystalline cellulose manufacturing in Indonesia.

In this paper, MCC will be extracted from cassava stems by chemical method. The stages include pre-hydrolysis, delignification,

bleaching and drying to obtain alpha cellulose. Then proceed with the hydrolysis stage with variations in H_2SO_4 concentration to obtain MCC. The concentration variation used to hydrolyze was chosen to compare the MCC results obtained with previous research conducted using HCl with the same concentration variation. Analysis of MCC obtained was determined based on the percentage of yield, identification test, and analysis with Fourier Transform Infrared Spectroscopy (FTIR).

2. Material and Methods

2.1. Materials

The materials used in this research are cassava stems, water, 25% solution of NaOH, 3% solution of hydrogen peroxide, 0.1N acetic acid solution, $ZnCl_2$, microcrystalline cellulose (HiCell®) and sulfuric acid with various concentrations (1.5, 2N, 2.5N, 3N and 3.5N). and the equipment used are hotplate, oven, filter paper, magnetic stirrer, digital balance, beaker glass, erlenmeyer, pH meter or universal pH indicator, sieve no.100, aluminum foil, nylon filter, grinder, and FT-IR spectroscopy.

2.2. Method

Microcrystalline Cellulose characterization from cassava stems is a quantitative experimental method, using maceration method with alkaline solution and followed by hydrolysis process with H_2SO_4 with variation of concentration needed for hydrolysis to obtain microcrystalline cellulose. This method is used because of the woody characteristics of the material with high lignin and hemicellulose content, so a delignification process is needed to isolate alpha cellulose from cassava stems. the following stages of the research procedure:

2.2.1 Cassava stem preparation

Cassava stem samples were cut into small pieces with a thickness of 1cm. After cutting, the sample is dried in an oven at 150^{o}C for 4 hours. then the sample is crushed / crushed with a grinder and sieved with mesh no.100.

2.2.2 Alpha Cellulose Isolation Process

A total of ±15 g of fine cassava stem sample was added to 0.1N Acetic Acid solution which was previously dissolved in water in a ratio of 1:20. At 105^{o}C the de-hemicellulose process was carried out by heating and stir the sample on a hotplate for 1 - 2 hours using magnetic stirer. After that the sample is separated from the solvent by filtering and squeezing, then Alpha Cellulose is rinsed with distilled water until the pH is neutral, and that's where Alpha Cellulose isolation occurs.

No	Sample Weight (g)	Alpha Cellulose Obtained Weight (g)	% Yield
1	16.40	10.20	62.20 %

2.2.3. Delignification Process

Alpha Cellulose samples that have been rinsed are dissolved with 25% solution of NaOH in a ratio of 1:20 and heated at 105^{o}C for 1 - 2 hours to obtain a dense brown pulp or slurry that settles in the NaOH solution. The fibers are separated from the solvent by screening, squeezing and washing the pulp with water until a neutral pH is obtained.

2.2.2. Bleaching

The samples were soaked overnight in 3% H_2O_2 solution in a ratio of 1:8 at 80^{o}C. The samples were then filtered using filter paper and rinsed until the pH was neutral. The pulp was dried in an oven at 50^{o}C for 12-24 hours. The dried pulp is Alpha Cellulose.

2.2.3. Hydrolysis

A sample of 1g Alpha cellulose was put in a beaker, then hydrolyzed with 25 mL of H_2SO_4 with concentrations of 1.5, 2N, 2.5N, 3N and 3.5N heated to boiling for 15 minutes. then filtered using filter paper and rinsed with purified water until the pH was neutral. Dried the pulp, the dried pulp is MCC.

2.2.4. MCC Characteristic Analyze

The microcrystalline cellulose powder obtained from cassava stems was weighed and the percentage yield of each variation of H_2SO_4 (1.5, 2N, 2.5N, 3N and 3.5N) concentration used was calculated. Then the identification test, Identification test is a chemical reaction used to determine the presence of a substance either ion or groups in a sample. It was carried out using an iodinated zinc chloride solution. Place a sample of 10 mg on the watch glass and disperse 2 mL of

No	H_2SO_4 Conc (N)	Sample Weight (g)	MCC Obtained Weight (g)	MCC's Yield (%)
1	1.5	1.13	0.38	33.62
2	2	1.18	0.40	33.90
3	2.5	1.12	0.38	34.46
4	3	1.02	0.36	35.29
5	3.5	1.00	0.32	32.00

solution of iodinated zinc chloride. Positive results are characterized by the formation of violet blue color. In addition, the MCC obtained was characterized by FT-IR spectroscopy to determine the similarity of the structure and elemental composition of the sample based on the spectrum of the MCC Infra Red band obtained with commercial MCC as a comparison.

3. Results and Discussions

The following are the results of the analysis of MCC from cassava stems

3.1 Yield Result

Table 3.1 Alpha Cellose Yield

Before the isolation process of alpha cellulose from cassava stems, it was crushed and sieved with the intention of increasing the surface area at the next stage. The alpha cellulose isolation process was carried out with 0.1N acetic acid as a reagent to break the hemicellulose and liginin bonds from the sample, and obtained light brown cassava stem powder. Followed by a delignification process with 25% solution of NaOH to degrade the lignin polymer which will dissolve in water, leaving a dense brown pulp that is insoluble in strong bases. The pulp residue is alpha cellulose. The pulp was washed until neutral and dried, and continued the bleaching stage by soaking the sample with 3% solution of Hydrogen Peroxide in a ratio of 1:20 to the sample in an acidic atmosphere, washed and dried. In the bleaching stage, physical changes occur, namely the powder becomes softer and the color of the pulp becomes yellowish brown. At this stage of alpha cellulose isolation, the percentage yield of the sample was 62.20%, with a reduction in mass of 4.20g, 10.20g alpha cellulose was obtained. Alpha cellulose obtained will then continue the hydrolysis process with variations in H_2SO_4 concentration.

Table 3.2. MCC's Yield

Alpha cellulose yield is continued to the final stage, namely alpha cellulose hydrolysis with varying concentrations of H_2SO_4 with a constant reaction time of 15 minutes. This stage aims to remove the amorphous part of cellulose and leave the crystalline part of cellulose [4]. From these data, the optimum concentration in terms of percentage yield of MCC can be determined, and the optimum H_2SO_4 concentration is obtained at concentration 3N with a percent yield of 35.29%. The 3N concentration can be said to be optimum because of an increase from a concentration of 2.5N by 0.83% and a decrease in yield of 3.29% at a concentration of 3.5N. The magnitude of this yield can be influenced by the acid hydrolysis process, it will be faster if the acid concentration used is higher The hydrolysis process using sulfuric acid can produce a larger product because this acid has a greater number of hydronium ions than other strong acids such as hydrochloric acid. This can cause the breaking of monomers in the glucose polymer chain to take place perfectly [5]. Like the starch, cellulose is a glucose polymer that can be hydrolyzed with strong acids to form glucose monomers that can dissolve in water, so it will escape in filtration and reduce yield. Moreover, errors in sample treatment in the washing and filtering process also affect the yield of MCC.

At the hydrolysis stage, variations in H_2SO_4 concentration also produce MCC with colors that are quite significantly different. The same research has been conducted by [6] using HCl as a reagent in the hydrolysis stage, showing results that are not significantly different. The higher concentration produces a darker MCC colors. According to Safitri et al., 2018 in his research, this can be influenced by the concentration of acid so that the strength of hydrolysis will increase and produce a further degradation to form carbon.

3.2 MCC Identification Result

Identification test can be used as a characteristic of the presence of a substance in a sample from other samples, from the five variations showed positif MCC results which were indicated by the formation of violet purple color on the addition of iodinated zinc chloride [7]. Based on the test results, the positive results of MCC are shown in all sample variants which are characterized by the forming of violet purple color.

3.3 MCC FT-IR Analysis Results

The following is the measurement result of FT-IR spectrum of MCC samples

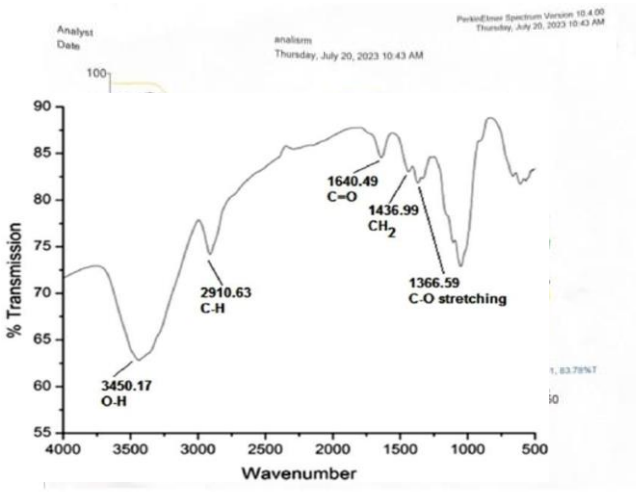

Figure 3.1. Spectrum and Functional Group of MCC (Researchgate.net, 2016)

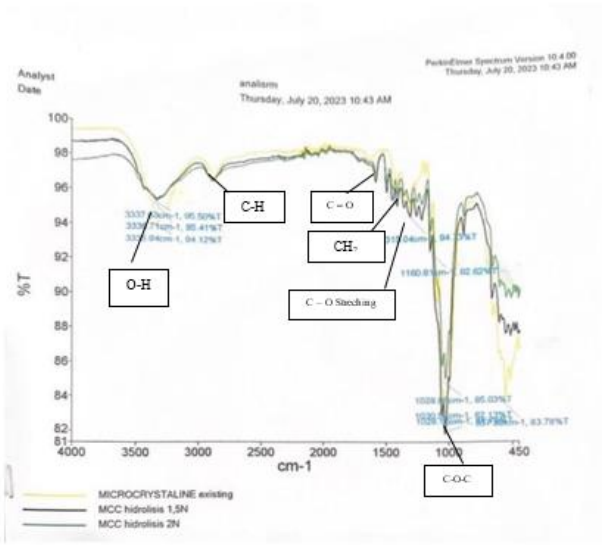

Figure 3.2. Spectrum and Functional Group of MCC Varian Concentration 1.5N and 2N

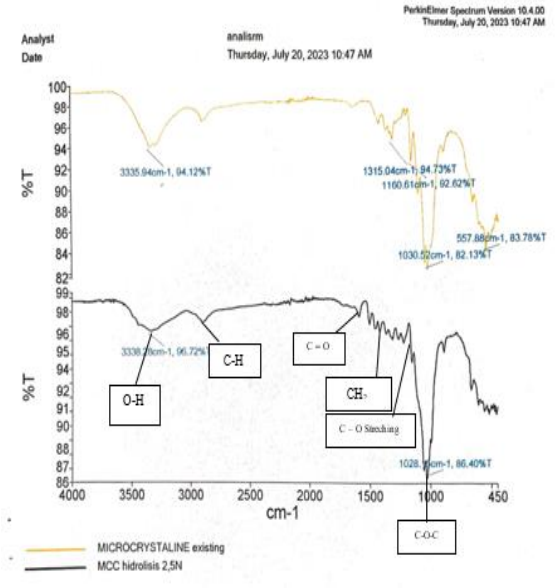

Figure 3.2. Spectrum and Functional Group of MCC Varian Concentration 2.5N

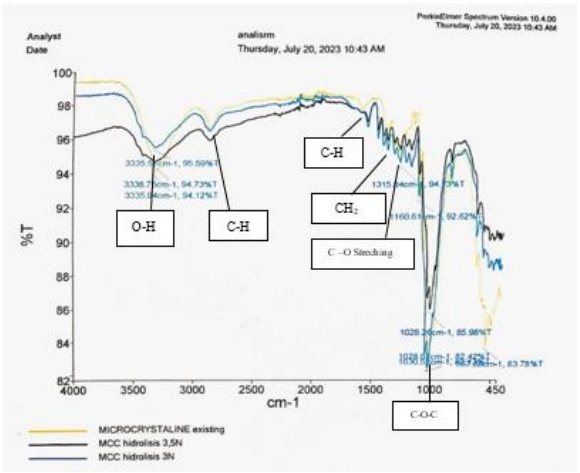

Figure 3.2. Spectrum and Functional Group of MCC Varian Concentration 3 and 3.5N

FT-IR testing is intended to determine the functional groups in MCC obtained from cassava stems, and compare it with the spectrum band of pharmaceutical grade commercial MCC with the trademark HiCel product from PT Sigachi as a comparison raw material. Measurements were carried out with FT-IR PerkinElmer UATR Two at wave

numbers 500-4000 cm-1 Based on the figure 3.1 measurement results of MCC samples from variations of 1.5N, 2N, 2.5N, 3N and 3.5N IR Spectum results of MCC samples indicated peaks appear respectively at wave numbers 3337, 3336, 3338, 3335, and 3338 cm-1 where the peak between 3400cm-1 - 3500cm-1 indicates the presence of O-H stretch, then indicated peaks at wave numbers 2800 cm^{-1} - 2900 cm^{-1} with insignificant differences in intensity, indicating the presence of C-H stretching, at 1160 cm^{-1} identified peaks in all spectra of MCC sample variations, identifying the presence of C-O-C stretching. Then at wave numbers 1035 cm-1-1060 cm-1, peaks were also identified indicating the presence of C-O stretching. All spectra of cassava stem samples indicated to have the same functional groups as commercial MCC. In the fingerprint region cellulose gives a peak around 1300 cm-1 indicating the presence of C-H bending and around 1400 cm-1 indicating the presence of CH_2 bending [8]. Good microcrystalline cellulose will show the main absorption at wave numbers 3344, 2884, 1316 and 1024 cm-1 which indicates the presence of O-H groups, hIdrogen bonds, C-H alkane, C-O ether and alcohol bonds [9]. From the measurement results with FT-IR, MCC samples from cassava stems successfully produced MCC which is quite good, and has similarities with the IR spectrum of commercial MCC.

4. Conclusion

The conclusions of this research:
Based on the hydrolysis process of alpha cellulose from cassava stems carried out on 5 variants of H_2SO_4 concentration, the optimum H_2SO_4 concentration was found to be 3N H_2SO_4 with a yield of 35.29%, and the concentration of H_2SO_4 at the hydrolysis stage affects the amount of yield and color of the MCC obtained.

MCC obtained from cassava stems has similarities with pharmaceutical grade commercial MCC with the trademark HiCell which is indicated by the appearance of the main peak in the IR spectrum at wavelengths of 3344, 2884, 1316 and 1024 cm^{-1} which indicates the presence of O-H groups, hydrogen bonds, C-H alkanes, C-O ether and alcohol bonds. And the identification results showed positive results marked by the formation of violet purple color.

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