Microcrystalline cellulose production by acid hydrolysis of hydrotropic rice straw pulp

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Abstract. Rice straw is a by-product of the production of rice. Most of this material is considered as agricultural waste, used only for fodder, left or burned on the fields after harvest. Rice straw contains 35-50% cellulose, making it promising as raw material for microcrystalline cellulose production. The production of microcrystalline cellulose consists of three stages, namely delignification, bleaching, and hydrolysis. Those processes can be conducted at relatively high temperatures by both oven or microwave irradiation. The objective of this study was to compare the performances of those processes (oven and microwave irradiation) by evaluating the cellulose content and the crystallinity index of the microcrystal cellulose. The rice straw pulp was successively pre-treated with hydrotropic delignification and was bleached. Those pretreatments are intended to significantly remove unwanted substances such as lignin, coloring materials, and hemicellulose before the hydrolysis. The hydrotropic delignification was performed using a 20% urea solution with a liquid solids ratio of 1:10 at 80°C for 60 minutes. The bleaching process was carried out at 70°C for 60 minutes using hydrogen peroxide, an environmentally friendly bleaching agent. The hydrolysis was performed using hydrochloric acid and sulphuric acid in an oven for 10 to 50 minutes. The product was analyzed using Chesson-Datta method and FTIR. The FTIR analysis result shows that the highest crystalline cellulose fraction was 63.23%, which was obtained from hydrolysis using 1.5 M sulfuric acid for 50 minutes, the yield content is 55.91%. The same condition using hydrochloric acid resulted in a crystalline cellulose fraction of 60.70% with 59.16% yield content.

1. Introduction
Rice is the largest grain crop in the world after wheat and corn [1]. The amount of rice straw per harvest reaches at least 50% of the total harvest [2]. As an agricultural waste, the commonly used method to deal with rice straw waste is to burn it on the field. This causes environmental pollution because of the smoke that contains carbon dioxide [3]. Rice straw is lignocellulosic biomass consisting of three main components: cellulose, hemicellulose, and lignin. Lignocellulosic biomass has become a promising alternative source for industrial applications because of its main components [4]. They are also non-hazardous, renewable and available at a relatively lower cost [5].

One of the most studied conversions for lignocellulosic materials is the production of microcrystalline cellulose. This material is widely used in pharmaceutical, cosmetic and other industries. Microcrystalline cellulose can be produced by enzymatic processes and acid hydrolysis [5].
Rice straw conversion is a complicated process due to the presence of lignin and hemicellulose. Delignification and bleaching are needed as pretreatment to remove a certain amount of lignin, hemicellulose, and other constituent substances [6]. Various methods have been developed as an effort to use environmentally friendly chemicals, one of them is the use of the hydrotropic solution for delignification [7]. Urea is considered a safe chemical for the lignin removal process due to its non-corrosive and non-toxic nature to human health. Bleaching is a process to remove dirt from the material using chemicals [8]. One chemical widely used for the bleaching process is hydrogen peroxide, it considered an environmentally friendly bleaching agent [9][10].

2. Materials and method

2.1. Materials

The rice straw used in this research was obtained from a local farm around Yogyakarta (Yogyakarta, Indonesia). Other reagents were purchased from Merck Chemicals and Life Sciences (Jakarta, Indonesia); they were analytically pure and applied without pretreatment. The obtained rice straw was washed with water to remove any excess dirt. It was cut into pieces around the size of 1-2 cm, then dried under sunlight for two days. The dried rice straw is then ground into powder. The powder was sieved through 60 mesh sieves; the undersize powder was dried in an oven at 60°C until fully dried.

| Component | Value (%) |
|-----------|-----------|
| Cellulose | 37.5      |
| Hemicellulose | 35.4   |
| Lignin    | 14.5      |
| Ash       | 11.3      |

2.2. Cellulose isolation from rice straw

2.2.1 Hydrotropic delignification of rice straw. 40 gr of dried rice straw powder and 400 ml of 20% urea solution were put into a 1000 ml flask, then heated at 80°C in an oven. After 60 minutes, the mixture was separated using a filter pouch. The solid residue collected was washed thoroughly with distilled water until the water became clear, then dried at 80°C for 6 hours. The yield was determined by weighting the hydrotropic rice straw pulp and dried rice straw powder.

2.2.2 Bleaching process. 20 gr of hydrotropic rice straw pulp was mixed with 5% hydrogen peroxide solution with a solid to liquid ratio of 1:10 in a flask. The bleaching process was carried out in alkaline condition of pH 11 by adding 2% sodium hydroxide solution. The mixture was heated for 60 minutes at a temperature of 70°C. After that, the mixture was separated and washed with distilled water. The solid obtained from the mixture was dried in an oven until a constant weight was achieved.

2.2.3 Acid Hydrolysis process. 10 gr of bleached hydrotropic pulp and 200 ml of 2M acid solution (sulphuric and hydrochloric acid) was mixed in a flask. This process was carried out in an oven at the temperature of 80°C for 10, 20, 30, 40, and 50 minutes with mild agitation. The reactor was equipped with a reflux system to prevent water loss by evaporation. The mixture was then separated and washed with distilled water until the water became clear. The residue was dried at 80°C until fully dried for further analysis.

2.3. Analysis

The yield of microcrystalline cellulose from rice straw was calculated according to equation (Eq. 1) as follows:
Yield(%) = \frac{m_i}{m_0} \quad (1)

Where \( m_i \) is the mass of microcrystalline and \( m_0 \) is the mass of raw bleached hydrotropic pulp.

The cellulose, hemicellulose, lignin, and ash content in rice straw were determined using the Chesson-Datta analysis methods. A mixture of 1 gr dried sample (a) and 150 ml distilled water was refluxed in a water bath at 100°C for 1 hour. The mixture was filtered. The residue was washed with 300 ml hot water and dried in an oven until the weight was constant (b). The dried residue was mixed with 150 ml of 1 N H\(_2\)SO\(_4\), then refluxed in a water bath for an hour at 100°C temperature. The mixture was filtered and washed with 300 ml of distilled water. The obtained solid was dried and weighed (c). The dried solid was added to 10 ml of 72% H\(_2\)SO\(_4\) at room temperature for 4 hours, then mixed 1 N H\(_2\)SO\(_4\) in to the mixture and refluxed in a water bath for an hour. The solid was filtered and washed with 400 ml distilled water, then heated in an oven at a temperature of 105°C until the weight was constant (d). Lastly, the dried solid was heated until it became ash and was weighted (e). The pulp component was calculated by:

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Hemicellulose(\%) = \frac{b-c}{a} \times 100 \quad (2)
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Cellulose(\%) = \frac{c-d}{a} \times 100 \quad (3)
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Lignin(\%) = \frac{d-e}{a} \times 100 \quad (4)
\]
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Ash(\%) = \frac{e}{a} \times 100 \quad (5)
\]

Some methods to determine the crystallinity degree of cellulose are X-ray Diffraction (XRD), Nuclear Magnetic Resonance (NMR) Spectroscopy, and Fourier Transform Infrared Spectroscopy (FT-IR). The most commonly used method to identify different functional groups consist in a compound is FT-IR analysis. FT-IR is a non-destructive analysis technique to determine the qualitative and quantitative biomass components in the middle IR region [11]. FT-IR provides information such as molecular fragments, the presence of specific functional groups and even gives a deeper insight into the sample structure. FTIR with Attenuated Total Reflectance (ATR) unit allows attenuation of the incident radiation, also provides IR spectra without the water background absorbance [12]. Infrared spectroscopy combined with the ATR technique is perfect for investigating the IR of solids without the need to do any sample preparation. ATR spectroscopy is a mobile and non-damaging technique to measure the IR spectrum. To perform the analysis, the sample is placed in contact with the surface of an IR transmitting crystal. Although the IR light is reflected from the inside surface of the crystal, it penetrates a small distance and is partially absorbed into the sample. No sample preparation is needed; the only requirement is that the sample is in contact with the crystal surface [13].

3. Result and discussion

Cellulose content is the main quality aspect of microcrystalline cellulose production. The hydrolysis process in the production of microcrystalline cellulose aims to release crystalline cellulose from the amorphous part. The first hydrolysis was carried out using sulfuric and hydrochloric acid solution with concentrations of 0.5, 1, and 1.5 M at 80°C for 20 minutes. This stage was to determine the acid concentration to use at second hydrolysis with variations of time and temperature. The initial hydrolysis results were analyzed using the Chesson-Datta method to determine the content of lignin, cellulose, and hemicellulose in microcrystalline cellulose. The content in microcrystalline cellulose with various acid concentration are shown in table 2.
Table 2. Hemicellulose, cellulose, and lignin content in microcrystalline cellulose with various acid concentration.

| Acid            | Acid Concentration | Microcrystalline Cellulose Content (%) | Hemicellulose | Cellulose | Lignin |
|-----------------|--------------------|---------------------------------------|---------------|-----------|--------|
| Sulphuric Acid  | 0.5 M              | 17.6646                               | 53.8538       | 5.0943    |        |
|                 | 1 M                | 14.2110                               | 54.7207       | 3.2342    |        |
|                 | 1.5 M              | 12.7729                               | 54.7735       | 2.8022    |        |
| Hydrochloric Acid| 0.5 M              | 16.7768                               | 52.0145       | 5.7133    |        |
|                 | 1 M                | 15.8596                               | 54.4538       | 2.4707    |        |
|                 | 1.5 M              | 13.2231                               | 57.6299       | 1.7544    |        |

From table 2, it can be seen that in the first hydrolysis using various acid concentrations, is that hydrolysis using a concentration of 1.5 M in sulfuric acid and hydrochloric acid produces microcrystalline cellulose with the highest cellulose content. Hydrolysis using sulfuric acid resulted in 54.77% cellulose content and 57.62% for the one hydrolyzed with hydrochloric acid. Both were the highest cellulose content obtained. Also, the content of hemicellulose and lignin in microcrystalline cellulose using 1.5 M concentration with both acids is the lowest. Based on these data, further hydrolysis was carried out using sulfuric and hydrochloric acid solution with a concentration of 1.5 M with time variation. The temperature of the process was 80°C. The reaction time was varied from 10 to 50 minutes with 10 minutes intervals. The yield of microcrystalline cellulose was calculated using equation (1). The yield of microcrystalline cellulose with time variation is shown in table 3.

Table 3. The yield of microcrystalline cellulose hydrolysed with time variations.

| Entry | Time (minute) | Sulphuric Acid | Hydrochloric Acid |
|-------|---------------|----------------|-------------------|
| 1     | 0             | 100            | 100               |
| 2     | 10            | 61.079         | 64.616            |
| 3     | 20            | 59.721         | 63.824            |
| 4     | 30            | 59.197         | 61.552            |
| 5     | 40            | 57.504         | 60.694            |
| 6     | 50            | 55.912         | 59.153            |

Based on table 3, the yield of microcrystal cellulose hydrolyzed with both sulphuric and hydrochloric acid slightly decreased as the reaction time increased. For the sulfuric acid, the yield decreased from 61.079% at 10 minutes time reaction to 55.912% at 50 minutes. As for hydrochloric acid, the yield decreased from 64.616% at 10 minutes to 50.153% at 50 minutes. The decrease of yield probably happened due to the excessive hydrolysis of cellulose. Cellulose and lignin content from microcrystalline cellulose hydrolyzed with a sulfuric and hydrochloric acid concentration of 1.5 M at various times are shown in table 4.

Table 4 shows that hydrolysis with sulphuric acid has the lowest cellulose content and highest lignin content, among others. Hydrolysis for 10 minutes with sulphuric acid increased cellulose content by 7.15% and decreased lignin content by only 0.25%. Microcrystal cellulose with the highest cellulose content is the 40 minutes of hydrolysis, the cellulose content increased by 8.94%, and the lignin decreased by 3.20%. Meanwhile, for hydrochloric acid, the highest cellulose content is 10 minutes of hydrolysis. The cellulose content is 47.239%, but the lignin content is 13.441% that still relatively high. This study was compared to the microwave irradiation by Damayanti [14], using the same kind of acid, same temperature, and hydrolysis time. With identical operation condition, microcrystalline obtained with oven heating contain lower cellulose content and higher lignin content.
Table 4. Lignin and cellulose content of microcrystal cellulose hydrolysed with time variations.

| Acid           | Time (minutes) | Lignin (%) | Cellulose (%) |
|----------------|----------------|------------|---------------|
| Sulphuric Acid | 10             | 14,275     | 44,658        |
|                | 20             | 12,129     | 44,575        |
|                | 30             | 12,176     | 45,875        |
|                | 40             | 11,360     | 46,449        |
|                | 50             | 11,199     | 45,909        |
| Hydrochloric Acid | 10    | 13,441     | 47,239        |
|                | 20             | 12,782     | 46,383        |
|                | 30             | 12,614     | 46,265        |
|                | 40             | 12,159     | 46,473        |
|                | 50             | 11,551     | 46,683        |

FTIR analysis was carried out to determine the functional groups contained in microcrystalline cellulose. Infrared spectra were used to determine the structure of cellulose, hemicellulose, and lignin of the microcrystal cellulose. FTIR analysis could also be used to determine the crystallinity of microcrystalline cellulose. The crystallinity of microcrystalline cellulose hydrolyzed with time variations using FTIR is shown in table 5.

Table 5. Crystallinity of microcrystal cellulose using FTIR.

| Acids          | Time (minutes) | Crystallinity Fraction (%) |
|----------------|----------------|----------------------------|
| Sulfuric Acid  | 10             | 52.20                      |
|                | 20             | 55.47                      |
|                | 30             | 55.75                      |
|                | 40             | 59.85                      |
|                | 50             | 63.23                      |
| Hydrochloric Acid | 10  | 51.78                      |
|                | 20             | 53.21                      |
|                | 30             | 56.50                      |
|                | 40             | 59.24                      |
|                | 50             | 60.70                      |

Table 5 shows that the crystallinity of fraction microcrystalline cellulose increased as the reaction time increased. For hydrolysis with sulfuric acid, the crystallinity increased from 52.20% at 10 minutes to 63.23% at 50 minutes. As for hydrochloric acid, the crystallinity increased from 51.78% at 10 minutes to 60.70% at 50 minutes. The structure of microcrystalline cellulose with the highest crystallinity fraction using both acid, sulfuric acid and hydrochloric acid, were compared with rice straw as the raw material. The FTIR spectra of rice straw and microcrystal cellulose hydrolyzed with sulphuric acid and hydrochloric acid are illustrated in figure 1.
Peaks around 3410 cm$^{-1}$, 3413 cm$^{-1}$, and 3411 cm$^{-1}$ in the figure show the presence of cellulose because the region between 3800 cm$^{-1}$ and 3000 cm$^{-1}$ indicates the crystalline structure of cellulose. Peaks between this range describe the vibration of valence bands of the hydrogen bond of the O-H group and the bands of infra-molecular and intermolecular hydrogen bonds [13]. The range between wavenumber 1200 cm$^{-1}$ and 1100 cm$^{-1}$ is the area of hemicellulose and cellulose, which reach a maximum value at around 1063 cm$^{-1}$ because of the stretching of C-O. A band around wavenumber 1457 cm$^{-1}$ is the deformation of lignin CH$_2$ and CH$_3$. The peak at 1630 cm$^{-1}$ is reported to be stretching of the C=C and C=O lignin aromatic rings [15]. The region around wavenumber 1247 cm$^{-1}$ indicates the removal of hemicellulose. It can be seen that the region around wavenumber 1734 cm$^{-1}$ is affected after hydrolysis, which denotes the decrease in hemicellulose content. A band around 1735 cm$^{-1}$ is the familiar characteristic of C=O stretching of unconjugated hemicellulose. Meanwhile, the peak at 2918 cm$^{-1}$ is due to the unsymmetrical stretching of CH$_2$ and CH, indicating the characteristics of cellulose. The area of wavenumber 1630 cm$^{-1}$, which is described as lignin macromolecule, also changes after hydrolysis[16]. The region between 2918 cm$^{-1}$ and 2851 cm$^{-1}$ is also affected. The band between these two peaks indicates that the cellulose chain is being affected, the increase in line width and curves unsymmetrical within 3800 cm$^{-1}$ to 3000 cm$^{-1}$ proves the disturbance in the crystalline structure of cellulose. The changes are due to the inference of intramolecular hydrogen bonding in cellulose [17].

4. Conclusion
The highest crystallinity fraction from microcrystalline cellulose hydrolyzed with time variations was 63.23%, which was obtained from hydrolysis using 1.5 M sulfuric acid for 50 minutes, the yield content is 55.91%. For hydrochloric acid, the highest crystallinity fraction was 60.70% with 59.16% yield content, this was also obtained from hydrolysis for 50 minutes.

5. Reference
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Acknowledgments
The authors are grateful to The Ministry of Research, Technology and Higher Education of Republic of Indonesia that support this work through PDUPT research grant of 2020, with contract number of 2845/UN1/DITLIT/DITLIT/LT/2020.