OPTIMIZATION AND CHARACTERIZATION CELLULOSE CONTENT OF COCOA POD HUSK FROM COCOA FERMENTATION CENTER IN GUNUNG KIDUL REGENCY, INDONESIA THROUGH THE EXTRACTION PROCESS

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ABSTRACT

Lignocellulose comprises three biopolymers, cellulose, hemicellulose, and lignin that form a complex composite structure. Cellulose, as a biopolymer possesses notable properties including biocompatibility, biodegradability and economic viability. Gunung Kidul, one of the areas in Indonesia, precisely in Yogyakarta, where the population is known to live as cocoa farmers, so the potential for cocoa husks production is quite large. One way to obtain cellulose is by extracting the cacao husks from Gunung Kidul Cocoa Fermentation Center. Cellulose extraction were carried out through two main stages: alkaline delignification and bleaching. Based on the FTIR characterization results, the cocoa skins from Gunung Kidul Fermentation Center have the potential to separate cellulose from lignin and hemicellulose, which is shown in the presence of the function groups O-H, CH_2, and C=O. So, the study aimed to get the optimum condition of the cellulose through several variations of the alkaline (NaOH and KOH), and the type of bleaching reagent (NaOCl and H_2O_2). The concentration of H_2O_2 (10%, 20%, 30% and 40%). The potential for cellulose can be developed and modified into nanocellulose in the future perspective.

INTRODUCTION

Cocoa is one of the leading strategic plantation commodities that has a vital role in the Indonesian economy, namely as a foreign exchange earner, source of income for farmers, job creation, driver of domestic agribusiness and agro-industry, environmental preservation, and regional development. The World Cocoa Foundation reveals an increase in demand for cocoa of 3% per year in the last 100 years. Thus, it is estimated that the rise in world demand for cocoa in the coming years is expected to increase at the same rate. The development of State Large Plantation cocoa production from 2016 to 2017 has increased, where 2016 cocoa production was 12,362 tons, rising to 12,612 tons in 2017 or an increase of 2.02 percent (Directorate General of Estates, 2019). Processing cocoa pods produces 70-75% of the cocoa pod shell (Cruz, 2012).

The use of cocoa pod shells is essential considering Indonesians are experiencing a decline in economic capacity due to the Covid-19 pandemic. Maximizing the use of waste can be an effort so that people can increase the use value of waste into products with higher economic value, one of which is by isolating cellulose. One of the most extensive cocoa plantations in Indonesia is the Gunung Kidul Cocoa Fermentation Center, which produces several cocoa products, such as chocolate through fermentation. However, its use is still limited to cocoa fruit and beans, so a lot of biomass waste is produced in the form of cocoa pod shells at this location. Therefore, this waste needs to be managed so that it does not become waste that is detrimental to the surrounding environment; one way is by taking the fiber.

In general, synthetic fibers harm the environment and human health, worsening the global energy crisis. This is because synthetic fibers require high energy in the production process, so they will produce relatively high greenhouse gas emissions, which will cause heat to be trapped and cause respiratory diseases due to smog and pollution (Amrillah et al., 2022). The biodegradability of natural cellulose from plant fibers is considered the most important and remarkable aspect of its use as a polymer filler due

to its abundance, relatively low energy consumption, and non-toxicity (Zhu et al., 2021).

The lignocellulose component is composed of cellulose, lignin, and hemicellulose. In complex lignocellulosic structures, cellulose maintains its crystalline structure and its fibers (amorf) and it seems that cellulose is the center of complex lignocellulose. Hemicellulose is located between the micro and macro fibers of cellulose. Lignin and hemicellulose act as a matrix structure or framework in which cellulose is embedded as the primary reinforcement of lignocellulose. The hemicellulose and lignin network protect cellulose from biotic attack and abiotic stress (Park et al., 2019; Smith, 2019; Vermaas et al., 2019). After decomposing lignocellulose into its constituent parts, biopolymers can be considered starting materials for low-cost production of innovative biomaterials, chemicals, and high-value fuels. Although lignocellulose has been used by humans for thousands of years, little is known regarding the precise relationship between its chemical structure and physical characteristics (Smith, 2019).

According to Daud et al. (2013), cocoa pod skin contains lignocellulosic components in the form of 35.4% cellulose, 37% hemicellulose, and 14.7% lignin (Daud et al., 2013). Several studies state that cocoa pod husk cellulose can be used as a material for making carboxymethyl cellulose (CMC) (Ogunneye et al., 2020) and as raw material for making paper (Daud et al., 2013). Cellulose is a biopolymer that has biocompatibility, is biodegradable and is quite economical. This biopolymer is available in nature in quite abundant quantities (Macías-Almazán et al., 2020). Cellulose has properties that make it easy to form into packaging films. Cellulose content can come from several sources such as bacteria, algae, annual plants, agricultural waste, and wood. Along with the development of science and technology, cellulose is modified into materials with higher economic value such as nanocellulose and Microcrystalline Cellulose (MCC) (Hanum et al., 2023; Singh et al., 2019). Cellulose can be extracted from the source using the alkaline delignification method and bleaching process.

Delignification of lignocellulosic biomass with alkali such as Sodium Hydroxide can reduce the lignin content by breaking the ester bonds that bind xylan and lignin to form cross-links thereby increasing lignocellulose porosity. In addition, the use of NaOH can also eliminate the amorphous part of lignocellulose by increasing the inner surface area and degree of crystallinity (Zhao et al., 2017). Akinjokun (2021) has conducted research related to the characterization of cellulose from cocoa pod skin using base delignification, where the percentage of cellulose in cocoa pods reached 67.6% (Akinjokun et al., 2021). Sun (2004) researched base delignification using NaOH and KOH. The results showed that the highest lignin loss among various delignification variations occurred after NaOH delignification (Sun et al., 2004).

In general, alkaline delignification will produce a dark color, so cellulose must be treated with a bleaching process. Bleaching is a whitening process to degrade residual lignin in lignocellulosic materials. Chemicals commonly used in bleaching are oxidizing agents such as sodium hypochlorite (Hutomo et al., 2012) and hydrogen peroxide (Silitonga et al., 2019) in alkaline conditions. The oxidizer in the bleaching process functions to degrade lignin from the chromophore group.

Cellulose fiber as a polymer matrix has developed rapidly in the last decade. This is due to its advantages such as good mechanical properties, low density, environmentally friendly, abundant, cheap, non-toxic, easily degraded, and included in renewable natural resources. Therefore, this research aims to exploit the potential of cocoa pod hulls to produce the best cellulose content under optimum conditions based on Chesson Datta's characterization.

MATERIALS AND METHODS

Material and Apparatus

The primary material was Cocoa pod husk from Gunung Kidul Cocoa Fermentation Center (Yogyakarta, Indonesia), NaOH Merck (CAS 1310-73-2), KOH Merck (Potassium hydroxide CAS 1310-58-3), NaOCl 12%, H_2O_2 50%, Aquadest and H_2SO_4 Merck PA. The sequence of equipment employed is illustrated in Figure 1.

Methods
Sample Preparation

Cacao Pod Husk (CP) was processed at the Cacao Fermentation Center in Gunung Kidul, DIY. The husks were separated from the colored skin, thoroughly washed, and sun-dried. After reaching the desired dryness, the material was finely ground into a powder using a grinder. After that, CP will be treated by alkaline delignification.
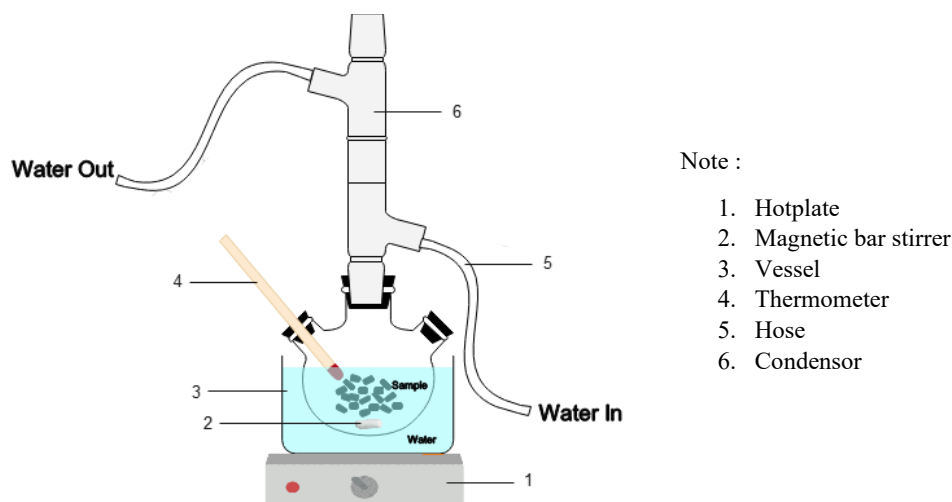
Figure 1. Cellulose Extraction Apparatus Series

Note :

1. Hotplate
2. Magnetic bar stirrer
3. Vessel
4. Thermometer
5. Hose
6. Condensor

Alkaline Delignification

A quantity of 10 grams of CP was subjected to reflux using 100 mL of a 12% alkaline solution (NaOH and KOH) at 100°C for 2 hours. Subsequently, CP was filtered to eliminate any residue and rinsed with distilled water until the pH level became neutral and the washing residue displayed no colour. The filtered CP was then dried in an oven until a constant weight was achieved. After that, filtered CP will be treated by a bleaching process.

Bleaching

A quantity of 5 grams of filtered CP from the alkaline delignification process, will undergo reflux with 100 mL of bleaching reagent (NaOCl and H_2O_2) at a temperature of 100°C for 1 hour. Following this, the bleached CP is filtered to eliminate any residue and is rinsed with distilled water until the pH reaches a neutral level. The filtered bleached material is then dried in an oven until a constant weight is achieved. The result of the bleaching process is cellulose, characterized by Chesson datta.

Chesson Datta Characteristic

Analysis was carried out using the Chesson-Datta method to determine the content of cellulose, lignin, and hemicellulose. Firstly, 1 g of bleached CP (a) was mixed with 150 mL of aquadest and heated at 100 °C for 1 hour. Then, the mixture was filtered, washed with hot aquadest, and dried in the oven until the weight was constant (b). The concrete was mixed with 150 ml of H_2SO_4 (1 N) and heated at 100 °C for 1 hour. The solids were filtered and washed with distilled water. After that, the solids were dried in the oven until the weight was constant (c). The dried solid was soaked in 10 ml of H_2SO_4 (72%) at room temperature for 4 hours. After that, 150 ml of H_2SO_4 (1 N) was added to the mixture and refluxed for 1 hour. Then the solid was washed with 400 ml of distilled water and heated in an oven. Heating is carried out until the weight is constant (d). The solid of weight d is heated to ash using a furnace at 450 °C for 1 hour, then heated at 700 °C for 2 hours to get an ash (e). The percentage of water-soluble, hemicellulose, cellulose, lignin and ash can be calculated by Equation (1), (2), and (3), respectively (Chesson, 1981).

$$\% \ Hemicellulose = \frac{b-c}{a} \times 100\% \quad …(1)$$

$$\% \ Cellulose = \frac{c-d}{a} \times 100\% \quad …(2)$$

$$\% \ Lignin = \frac{d-e}{a} \times 100\% \quad …(3)$$

RESULT AND DISCUSSION

Chemical Composition of Cacao Pod Husk Powder

Cacao Pod Husk Powder obtained from the Gunung Kidul Cocoa Fermentation Center was analyzed using the Chesson Datta method to determine the Chemical Composition contained therein. Based on this analysis, Cacao Pod Husk Powder is 36.0% water soluble, 16.3% hemicellulose, 26.7% cellulose, 20.1% Lignin, and 0.9% ash. The potential for cellulose in Cacao Pod Husk Powder is also supported by the results of FTIR characterization as in Figure 2.

Figure 2 shows two spectra between Cacao Pod Husk Powder before the extraction process. The results of FTIR analysis show that the wave intensity peak at 3200-3600 cm^{-1} indicates the O-H stretch functional group which represents the bond in cellulose. Then the peak with an intensity of 2000-1650 cm^{-1} shows the presence of the CH$_2$ functional group which symbolizes the presence of bonds in lignin and the peak with an intensity of 1110-1360 cm^{-1} shows the C=O functional group which symbolizes hemicellulose bonds (Lismeri et al., 2019). The presence of a quite sharp CH$_2$ functional group peak indicates that the lignin content in CP is quite dominant, while the O-H and C=O peaks are not too sharp. Therefore, CP has the potential to be isolated from cellulose by extraction.
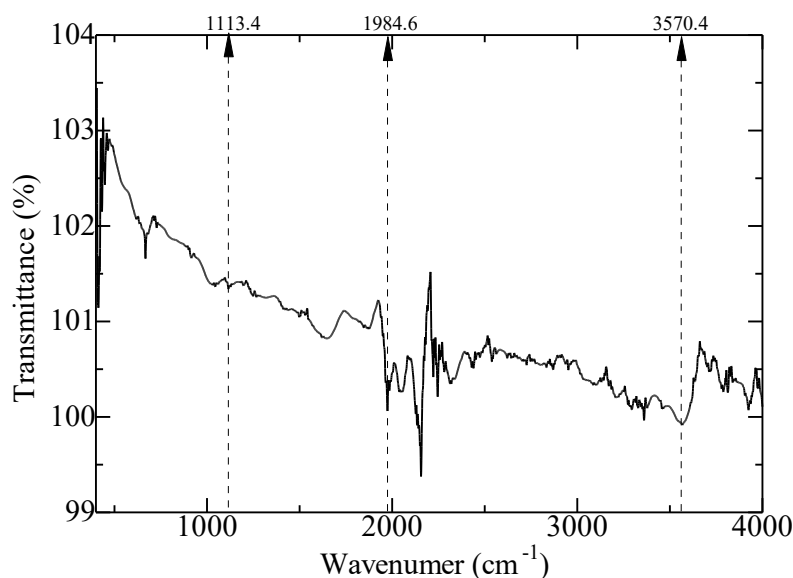

Figure 2. FTIR Analysis for Cacao Pod Husk Powder
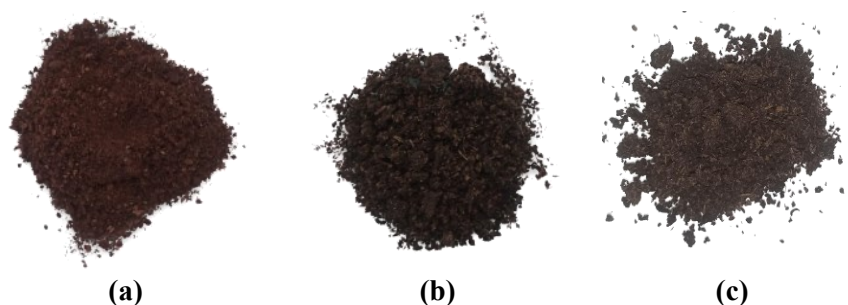

(a) **(b)** **(c)**

Figure 3. Physical of the sample (a) Cacao Pod Husk Powder (b) NaOH Delignification Results (c) KOH Delignification Results
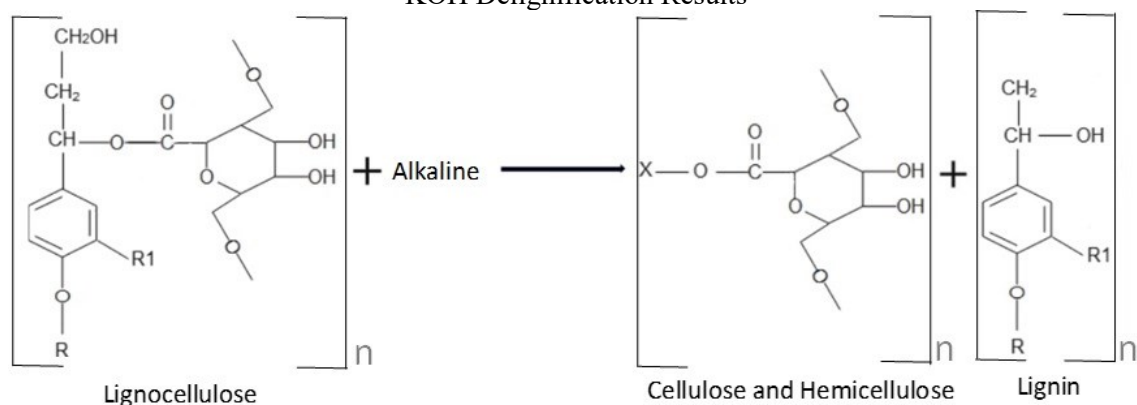

Figure 4. Breaking down reaction from lignin and cellulose from lignocellulose

Based on the FTIR characterization results in Figure 2, cellulose from Cacao Pod Husk will be characterized using Chesson Datta to determine the best optimum conditions based on variations in the type of alkali reagent, bleaching reagent, and bleaching reagent concentration.

Optimization of Alkaline Delignification Reagent

The alkaline delignification process works by opening the lignocellulose structure so that cellulose is more easily obtained using an alkaline solution. This process will dissolve the lignin in the cocoa shell, making it easier for the process of releasing lignin from the fibers which causes damage to the lignin structure (Moniruzzaman & Ono, 2013). This process will produce a different physical shape for the Cacao Pod Husk as in Figure 3.

Figure 3(a) is an initial CP sample that has not been treated, the color is still the same as Cocoa pod husk in general, namely brown. Meanwhile, Figures 3(b) and 3(c) are the results of the alkaline delignification process using NaOH and KOH. The main difference that can be seen directly is the color of the sample. The color of the sample tends to be blacker and darker after being given alkaline delignification. This has been previously explained by research by Geng et al (2019), where delignification produces a darker color due to the higher lignin content (Geng et al., 2019). This is due to the breakdown reaction of lignin by alkali, where the solution can destroy the lignin structure in the crystalline and amorphous parts and separate some of the hemicellulose (Bacha & Demsash, 2021) as in Figure 4.

Figure 4 shows that lignocellulosic materials consisting of cellulose, hemicellulose, and lignin can be separated by reacting with alkali. Cellulose and hemicellulose are two types of polymers that are included in the polysaccharide type. The main difference between cellulose and hemicellulose is that cellulose is a straight-chain polymer and hemicellulose is a cross-linked polymer. Meanwhile, lignin is a constituent of lignocellulose which occupies the space in the cell walls between cellulose and hemicellulose, so it must be removed because it can hinder the formation of cellulose and hemicellulose bonds in the formation of fiber bonds (Jonasson et al., 2021). The base in delignification functions to dissolve lignin, where lignin molecules can degrade esters and glycosidic chains which results in changes in the structure of lignin, swelling of

cellulose, and partial decrystallization of cellulose and dissolution of some hemicellulose (Brodeur et al., 2011). The presence of lignin is indicated by the presence of a blackish-brown solution as seen in Figure 3.

The alkaline delignification method is the main process in cellulose extraction which greatly influences whether or not the cellulose is separated from the other components. There are 3 lignocellulose components that can be analyzed through Chesson Datta characterization, namely Hemicellulose, Cellulose, and Lignin. In the sense of physical structure, the lignin is located in the outer cell wall of biomass. In general, cellulose is located within a lignin shell while the hemicellulose, with a random and amorphous structure, is located within the cellulose and between the cellulose and lignin. From a chemical perspective, hydrogen bonding exists between cellulose and lignin as well as cellulose and hemicellulose. Additionally, covalent linkages, mainly ether bonds, have been proposed to be present between cellulose and lignin (Zhang et al., 2015).

To understand the chemical composition of the alkali delignification results, Chesson Datta analysis was carried out as in Figure 5.
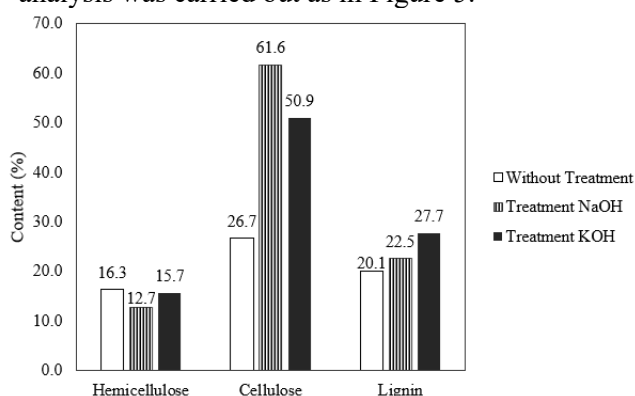

Figure 5. Comparison of the composition of hemicellulose, cellulose, and lignin before and after alkaline delignification

Figure 5 shows that the cellulose composition of Cacao Pod Husk cellulose obtained through alkaline delignification has a higher yield compared to cellulose without delignification. This shows the importance of using alkaline reagents in the initial stages of cellulose extraction. This shows that the cellulose and lignin contained in Cacao Pod Husk are mostly still formed in hemicellulose and have not been completely separated.

(a)   (b)

Figure 6. Bleaching results (a) NaOCl (b) H_2O_2

On the other hand, Figure 5 also shows that the results of NaOH delignification have a composition of 12.7% hemicellulose, 61.6% cellulose, and 22.5% lignin, while KOH delignification has a composition of 15.7% hemicellulose, 50.9% cellulose, and 27.7% lignin. The percentage of each component indicates the effectiveness of the alkali delignification reagent. In KOH, the percentage of cellulose is smaller and lignin and hemicellulose are greater than the results of NaOH, indicating that KOH is still not effective in breaking down lignocellulose in the cellulose extraction process. This is by the results of research conducted by Sun (2004), it was found that NaOH had greater effectiveness in isolating cellulose with a cellulose yield than KOH (Sun et al., 2004).

Optimization of Bleaching Reagent

The bleaching process is an important factor in cellulose extraction. The cellulose obtained from alkaline delignification still looks black as in Figures 3(b) and 3(c), indicating that lignin is still present. Therefore, a bleaching process is carried out using sodium chloride and hydrogen peroxide as in Figure 6

Figure 6 shows that the whitening process is influenced by the bleaching reagent. In Figure 6(a), Cacao Pod Husk which has been bleached using NaOCl has a brighter color compared to the results of delignification alone in Figures 3(b) and 3(c). However, the white color in the bleaching results is not completely white, whereas, in Figure 6(b), the bleaching results using H_2O_2 are completely white. This is supported by the results of Chesson Datta's characterization as in Figure 7.
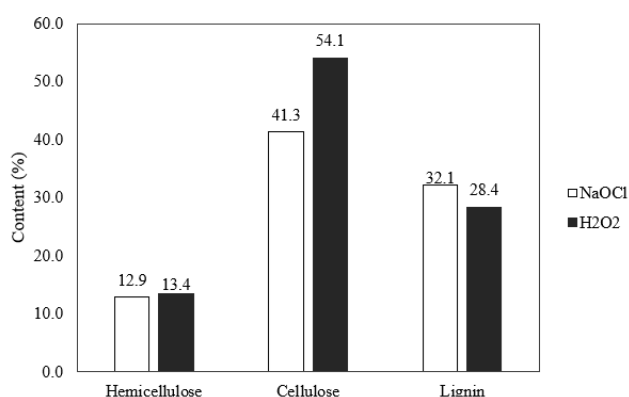

Figure 7. Comparison of the composition of hemicellulose, cellulose, and lignin resulting from NaOCl and H_2O_2 bleaching

Figure 7 shows that the composition for NaOCl bleaching results is 12.9% hemicellulose, 41.3% cellulose, and 16.3% lignin. Meanwhile, the composition for Bleaching H_2O_2 is 13.4% hemicellulose, 54.1% cellulose, and 28.4% lignin. In the bleaching process, lignin will be degraded and dissolved through an oxidative reaction. The fiber has to be treated by bleaching since lignin is what gives it its dark color. By adding bleaching chemicals like NaOCl and H_2O_2, the bleaching procedure breaks down long lignin chains into shorter ones in an attempt to remove remaining lignin compounds and modify the color of the affected areas. The length of time the biomass remains white increases with the amount of lignin lost in it. By increasing the cellulose's purity through the bleaching process, the biomass's cellulose content will be higher and its lignin content will be at its lowest (Rahayu et al., 2022).

Figure 6 and Figure 7 show that Hydrogen Peroxide reacts optimally as in the H_2O_2 bleaching reaction mechanism on cellulose shown in Figure 8.
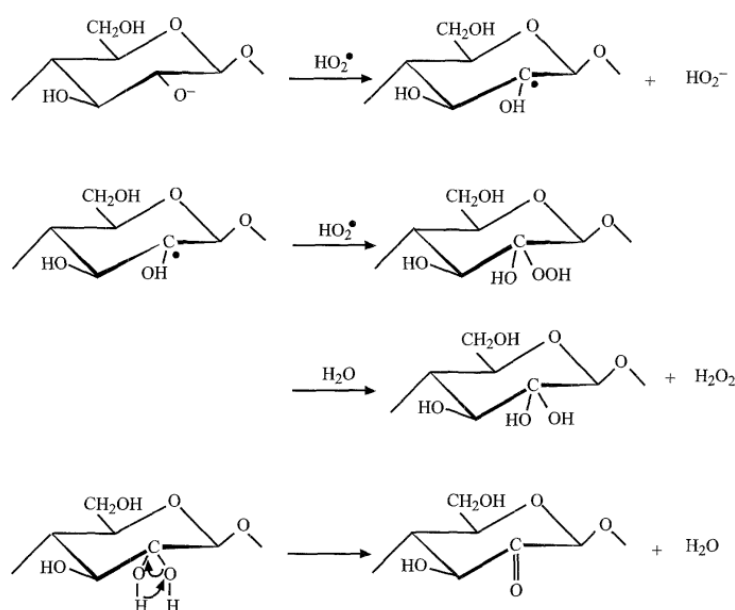
Figure 8. Mechanism of the H_2O_2 bleaching reaction on cellulose (Zeronian & Inglesby, 1995)
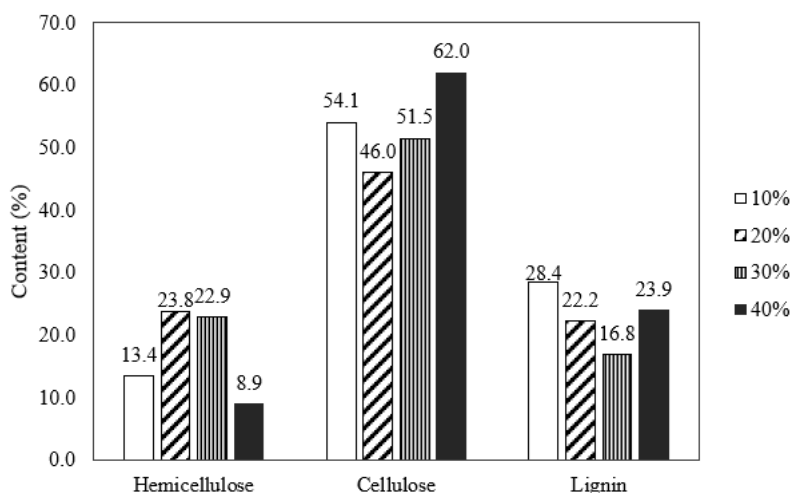

Figure 9. Comparison of the composition of hemicellulose, cellulose, and lignin resulting from H_2O_2 bleaching

Figure 8 shows that Hydrogen Peroxide under alkaline conditions will dissociate into hydroperoxide anions and react with H_2O_2 to produce hydroxyl radicals (OH) and superoxide anions (O_2^-) which are active delignification species as an oxidizer of lignin. This anion will attack the ethylene groups and carbonyl groups of lignin and convert them into non-chromophore groups (Thakur et al., 2021). The final cellulose product obtained is white as seen in Figure 6(b).

Optimization of Bleaching Concentration

To understand the chemical composition of the results of various bleaching concentrations, Chesson Datta analysis was carried out. From this analysis, the composition obtained for the 10% H_2O_2 bleaching results was 13.4% hemicellulose, 54.1% cellulose, and 28.4% lignin. The composition for 20% Bleaching H_2O_2 is 23.8% hemicellulose, 46.0% cellulose, and 22.2% lignin. The composition for 30% H_2O_2 Bleaching results is 22.9% hemicellulose, 51.5% cellulose, and 16.8% lignin. The composition for 40% H_2O_2 Bleaching results is 8.9% hemicellulose, 62.0% cellulose, and 23.9% lignin. A comparison of bleaching results at various H_2O_2 concentrations is shown in Figure 9.

Table 1. Comparison Study Cellulose Extraction

Title	Author, Year	Description	Cellulose
Corn Plant Stem Cellulose Extraction (*Zea mays*) Alkaline Method	(Asmoro et al., 2018)	The process of extracting cellulose from corn stalk waste using alkaline treatment and a bleaching process (NaOCl)	35,61%
Cellulose nanofibers produced from banana peel by chemical and enzymatic treatment	(Tibolla et al., 2014)	The process of extracting cellulose from banana peels using the Enzymatic (ET) and Chemical Treatment (ET) Methods	10%
Isolation of cellulose from sugarcane bagasse through microwave irradiation heating	(Jufrinaldi, 2018)	The process of extracting cellulose from sugar cane bagasse using a liquefaction process using the microwave heating method	38.57%

This is by research conducted by Akinjokun, et al., (2021) showing that the highest average value of cellulose content of cocoa pod skin from bleaching was obtained using a higher cellulose than a lower concentration (Akinjokun et al., 2021). The higher the concentration of H_2O_2 and the temperature of the bleaching process, the higher the cellulose content in the bleached cocoa pod skin tends to be. This happens because the higher concentration of H_2O_2 and temperature of the bleaching process will provide greater energy to the reaction so that the bond-breaking reaction in the lignin and hemicellulose chains runs better so that more cellulose bonds can be liberated (Singh et al., 2019). Partial degradation of hemicellulose and lignin components during the bleaching process then causes an increase in the percentage of cellulose content (Fitriana et al., 2020).

Comparasion Study

Several studies have been carried out on the extraction of cellulose from waste. The results obtained also vary, this is due to the type of raw material and process used as in Table 1. Biomass waste has the potential to produce cellulose for extraction. The percentage of cellulose from several studies in Table 1 shows that Study Cocoa Pod Husk from Cocoa Fermentation Center in Gunung Kidul Regency, Indonesia more effective in cellulose extraction so it can produce 62% cellulose.

CONCLUSION

Based on the research that has been carried out, it can be concluded that the Cocoa pod husk from Gunung Kidul Cocoa Fermentation Center has the potential to extract cellulose from lignin and hemicellulose based on FTIR characterization. Optimum conditions were obtained through the characterization of Chesson Datta when Cocoa pod husk was treated using the alkaline reagent NaOH and then bleached with 40% H_2O_2 with a resulting composition of 8.9% Hemicellulose, 62.0% Cellulose, and 23.9%. The limitation of this study is that the running and filtration process is quite long, so it is necessary to update the method so that the research runs more effectively. However, the potential for cellulose can be developed and modified into nanocellulose in the future perspective. This is because nanocellulosic nano-sized materials are one of the foundations for developing science and technology. In recent years, nanocellulose has been explored in many applications, such as films, photonics, surface functionalization, nanocomposites, customizable optoelectronics, and medical sciences.

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