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Isolation and Characterization of Cellulose from Cocoa Shell Waste : A Green Chemical Implementation Approach

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This study analysed the effects of strong acid and green acid usage on the physical and chemical properties of cellulose isolated from cocoa shell waste. The results showed that employing pro analysis and commercial citric acid as a green acid as well as strong acids such as hydrochloric, nitric and sulfuric acids, resulted in the presence of typical functional groups of cellulose, i.e., O-H, C-H and C-O bonds. The presence of lignin as an impurity was detected by FTIR at 1500 cm^{-1} (aromatic C=C bonds) in the GM and HA5 samples which used 5 % NaOH in the delignification process. Employing 17.5 % NaOH and both strong and green acids produced no lignin peaks in the FTIR spectra. Samples were characterized using XRD, and the crystallinity index showed that using pro analysis and commercial citric acid on the isolated cellulose contributed to a poor crystal form compared to the use of strong acid. It also showed that 17.5 % NaOH and a strong acid were more effective than a green acid in terms of crystallinity. However, hydrolysis with nitric acid caused one of the main functional groups of cellulose to be lost and destroyed the main structure. It was also observed that green acids produced a dark brown powder, while strong acids tended to produce powders that were dull brown to yellow. This work demonstrates that optimized cellulose isolation using green chemicals is possible for specific applications.

Keywords: Isolation; waste; cocoa; characterization; cellulose

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Cellulose (chemical formula $(C_6H_{10}O_5)_n$) is a natural biopolymer abundantly available in plant cell walls [1], where it provides structural support and acts as a reinforcing element along with hemicellulose and lignin. Cellulose has gained attention due to the increasing demand for developing environmentally friendly materials. Proper utilization of cellulose promises to contribute to sustainable green growth. Among the various materials developed from natural resources, cellulose fibre is considered the most popular due to its versatile properties [2]. Cellulose has the advantage of being biodegradable, durable, non-toxic, and thermally and mechanically stable. Cellulose can also be used to produce high-value chemicals, biofuels, polymer composites, and many other products [3]. Many studies have been carried out on cellulose derived from biomass sources such as cassava stems, banana peels, empty oil palm fruit bunches, etc.

Cocoa (*Theobroma cacao L.*) is a well-known commodity throughout the world. The cocoa fruit consists of 74 % skin, 24 % cocoa beans and 2 % placenta. The large cocoa shell cannot be utilized properly and ends up as waste which amounts to around 2,000,000 tons every year [4]. The availability of cocoa shell waste is quite high because the cocoa plant is not affected by the seasons, so it can be harvested every day [5]. Cocoa shells contain crude fibres in the form of lignocellulose, which consists of hemicellulose (48.64 %), cellulose (31.25 %), and lignin (20.11 %) [6]. Utilization of lignocellulosic fractions from cocoa shell waste can lead to the development of profitable commodity products. As a result, cocoa shells can generate income for farmers and encourage economic development [7]. The natural organic polymer structure of cellulose makes it an excellent basic ingredient for the production of soft clothing, paper and cosmetic products [8].

Cellulose extraction is a process that separates cellulose from lignin and hemicellulose. Lignin can inhibit the hydrolysis process so this component needs to be removed [9]. The cellulose extraction process involves stages such as delignification, bleaching and hydrolysis [10]. One of the common methods of cellulose hydrolysis uses concentrated mineral acids such as hydrochloric, phosphoric, nitric or sulfuric acid. Although the use of strong mineral acids to hydrolyse the amorphous fraction of cellulose is considered very efficient, concentrated mineral acids also have a number of problems related to environmental and operational risks, which results in an increase in handling costs. For example, strong mineral acids can cause corrosion of equipment, excessive cellulose degradation, high water usage and produce large amounts of acidic waste that is difficult to recycle. The presence of toxic acid residues in cellulose can pose a major risk when cellulose is used to produce other materials, especially in the food, cosmetics, pharmaceutical and biomaterials industries. To overcome this problem, safe weak acids such as citric acid have begun to be widely researched and developed [11].

This study examined the use of cocoa shells as a source of cellulose which is extracted through delignification, whitewashing and acid hydrolysis processes using concentrated strong acids and a green acid. The evolution of the chemical structure and physical properties of cellulose with different acids was comprehensively studied. The isolated cellulose was characterized using Fourier Transform infrared spectroscopy (FTIR), X-Ray diffractometry (XRD) and scanning electron microscopy (SEM).

EXPERIMENTAL

Chemicals and Materials

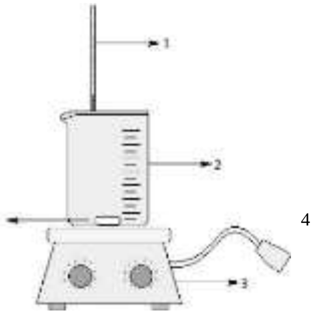


Figure 1. Extraction tool circuit. Note: (1) Thermometer, (2) Beaker, (3) Hot plate, (4) Magnetic stirrer

Cocoa shell waste was obtained from the Indonesian Cocoa and Coffee Research Center, Jember, Indonesia. Sodium hydroxide (NaOH, 40 gr/mol, Merck – Germany), hydrogen peroxide (H_2O_2, 30 %, Merck – Germany), hydrochloric acid (HCl, 37 %, 36.46 gr/mol, Smart Lab – Indonesia), citric acid monohydrate ($C_6H_8O_7.H_2O$, 210.16 gr/mol, Smart Lab – Indonesia), commercial citric acid ($C_6H_8O_7$) obtained from grocery stores, sulfuric acid (H_2SO_4, 95-97 %, Merck – Germany), nitric acid (HNO_3, 65 %, Merck – Germany), ethanol (C_2H_6O, 46.07 gr/mol, Merck – Germany), and Aquades (distilled water) were obtained from the Laboratory of the Faculty of Science and Data Analytics, Sepuluh Nopember Institute of Technology.

The equipment used included a hot plate, magnetic stirrer, 1 L measuring flask, thermometer, stand and clamp, analytical balance, 2 L glass beaker, 100 ml measuring cup, dropper pipette, vacuum pump, Buchner funnel, 1 L filtering flask, Whatman filter paper no. 42, oven, pH meter, and a mortar and pestle.

Material Preparation

The cocoa shells were first washed with Aquades to remove impurities, then dried for 24 hours at 80 °C in an oven to remove the remaining impurities and water. The dry cocoa shells were ground using a mortar and pestle and then sieved to 100 mesh size as noted elsewhere [12].

Cellulose Extraction

In this study, cellulose was extracted using two methods. Cellulose extraction Method 1 was a modification of the method reported by Susilowati et al [13] and employed a green acid. The ground cocoa shell powder was mixed with citric acid in a ratio of 1:16, heated at 80 °C for 175 minutes and then filtered. The precipitate obtained was mixed with 96 % ethanol in a 1:1 ratio and left for 24 hours. After filtering, the precipitate obtained was washed using distilled water and heated for 24 hours at 80 °C. Method 2 was carried out by modifying a previously published method [10]. To 100 g of cocoa shell powder was added a 5 %: 17.5 % (w/v) NaOH solution with a ratio of 1:20, then stirred at a temperature of 90 °C for 2 hours and filtered. The precipitate was washed with distilled water until a pH of 7 was obtained. Next, NaOH 5%; 17.5% (w/v) and H_2O_2 3% (v/v) was added to the precipitate in a ratio of 1:1 so that the ratio of sample to total solvent was 1:40 for each sample. Stirring was carried out at 55 °C for 1.5 hours and the solution was then filtered. This step was carried out 3 times, then the precipitate was washed with distilled water until pH 6 was obtained. After that, the hydrolysis process was continued using hydrochloric acid (HCl), citric acid monohydrate ($C_6H_8O_7.H_2O$), commercial citric acid ($C_6H_8O_7$), sulfuric acid (H_2SO_4), and nitric acid (C_2H_6O), each with the same concentration of 3.5 M and a precipitate to solvent ratio of 1:20. Stirring was carried out at 70 °C for 1.5 hours and the solution was filtered and washed with distilled water to pH 5. The cellulose samples were dried at a temperature of 60 °C for 24 hours in an oven.

Characterization Methods

Fourier-transform Infrared Spectroscopy (FTIR)

FTIR analysis (Thermo Scientific Nicolet iS10) was used to evaluate the chemical composition of the cellulose fibre samples obtained from cocoa shell waste that had undergone chemical treatment using Method 1 and Method 2. This is a qualitative analysis.

X-Ray Diffraction (XRD)

XRD (X'Pert PRO PANalytical) was performed to determine the crystal structure of cellulose extracted from cocoa shells. The crystallinity index (CI) was used to quantitatively measure the degree of crystallinity in the cellulose structure. This value provides information regarding the crystalline and amorphous regions of the cellulose structure. The crystallinity index (%) can be calculated using the Saegal equation (1).

$$I_{Cr}(\%) = \left(\frac{I_{200}-I_{am}}{I_{200}}\right) x\ 100\%\ldots \qquad (1)$$

I_{200} represents both crystalline and amorphous regions of the material while I_{am} represents only the amorphous region [15].

Scanning Electron Microscopy (SEM)

SEM (Zeiss EVO MA 10) was performed to identify the morphology and determine physical changes in the sample due to the cellulose extraction process using Method 1 or Method 2. The SEM images revealed differences in the morphology, shape, and particle size of the samples due to the different methods used to extract cellulose [15].

RESULTS AND DISCUSSION

Characterization

Figure 2 displays the results obtained from the extraction process using the two methods. In this study, to make it easier to read the data, each sample was given a sample code as detailed in Table 1 (employing green acids) and Table 2 (employing strong acids), while the raw materials for the cocoa shell powder samples were labelled RM.

Table 1. Samples that used green acids in the hydrolysis process.

Sample Code	Delignification	Bleaching	Hydrolysis
GM	-	-	$C_6H_8O_7.H_2O$ 1M (Pro Analysis)
CAM17.5	NaOH 17.5%	NaOH 17.5% + H_2O_2 3%	$C_6H_8O_7.H_2O$ 3.5M (Pro Analysis)
CA17.5			$C_6H_8O_7$ 3.5M (Commercial)

Table 2. Samples that used strong acids in the hydrolysis process.

Sample Code	Delignification	Bleaching	Hydrolysis
D5	NaOH 5%	-	-
BM		NaOH 5% + H_2O_2 3%	-
HA5			HCl 3.5M
HA17.5	NaOH 17.5%	NaOH 17.5% + H_2O_2 3%	
SA17.5			H_2SO_4 3.5M
NA17.5			HNO_3 3.5M


(a) (b) (c) (d) (e) (f) (g) (h)

Figure 2. Cocoa shell powder samples: (a) RM (b) GM (c) HA5 (d) HA17.5 (e) CAM17.5 (f) CA17.5 (g) SA17.5 (h) NA17.5.

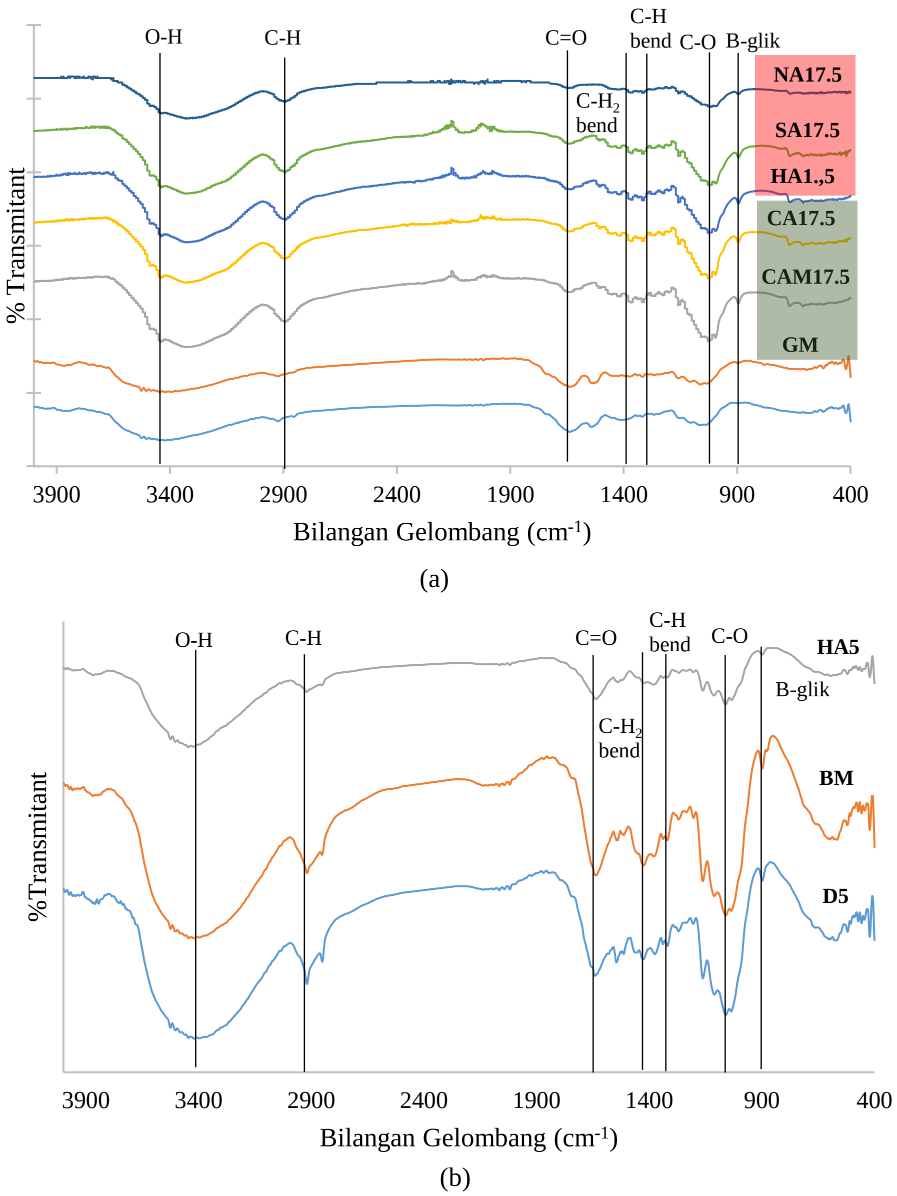
In Figure 2, the cellulose produced from Method 1 with the sample code GM was still dark brown like cocoa shell waste before extraction. Cellulose formed using Method 2 with different hydrolysis solvents produced cellulose powders that were dull brown to yellow in colour. Physically, the cellulose obtained from extraction was in the form of a soft powder. In terms of colour, samples HA17.5, CAM17.5 and SA17.5 tended to be whitish or faded brown in colour. However, CA17.5 was brown, while NA17.5 was yellow. According to Kunusa et al [16], cellulose is a white fibre which possesses the characteristic of being unable to dissolve in water, acid, or alkaline solutions, as well as organic solvents such as benzene, alcohol, ether, and chloroform. In Method 1, citric acid was used to separate polyvalent ions, namely ions that have more than one valence. The use of citric acid as the best extraction agent for pectin is based on its properties as a weak acid. When hydrolysed using strong acids, pectin particles decrease in size due to partial hydrolysis. Citric acid is used as an extraction agent resulting in depolymerization and de-esterification of pectin [17]. Pectin is a methylated form of polygalacturonic acid, which is a complex mixture of polysaccharides consisting of about one-third of the dry substance in the cell walls of higher plants [18].

In Method 2, there are three stages of cellulose extraction: delignification, bleaching, and hydrolysis. The lignin structures in the crystalline and amorphous parts can be damaged in the delignification process using NaOH solution. NaOH also plays a role in the process of separating cellulose from lignin and hemicellulose [19]. The concentration of sodium hydroxide was shown to have a significant effect on the aliphatic hydroxyl content. The higher the concentration of NaOH used, the more lignin is separated [20]. The bleaching stage degrades the remaining lignin in the lignocellulosic material. Hydrogen peroxide (H_2O_2) has a strong ability to release oxygen. It is not only effective as an oxidizing agent, but is also considered an environmentally friendly oxidizer, since its by-product is nothing more than water. As such, hydrogen peroxide is increasingly being used as an alternative to oxidants and chlorine bleaching agents [21]. The final stage of cellulose extraction is hydrolysis, which is the decomposition of compounds. During the hydrolysis process, the pH decreases. Acid solvents release an acid group in the form of H^+ ions, which speeds up the reaction because it combines with OH^- ions [22].

Cocoa pods contain lignocellulosic compounds (lignin, hemicellulose and cellulose). Thus, to extract cellulose it is necessary to separate hemicellulose and lignin. The delignification of the alkaline method has the ability to remove lignin and hemicellulose because it causes the lignocellulose to swell. Generally, sodium, potassium, calcium and ammonium hydroxide are used for pre-treatment. However, sodium hydroxide is more widely used and more effective in terms of safety, cost and reusability [23]. Method 1 does not use NaOH to degrade lignin. With Method 1, there is a peak at 2924.18 cm^{-1} that has a smaller intensity than with Method 2, namely 46.218 km/mol, which is likely to be the $-CH_3$ group. Stretching of $-CH_3$ groups are usually observed in the region of 3000-2800 cm^{-1} [24]. This indicates the presence of methyl esters $-CH_3$, which is a characteristic of pectin [25].

The Functional Groups of Cellulose

The FTIR spectra of cellulose samples from cocoa shell waste using Method 1 and Method 2 can be seen in Figure 3. Cellulose is a polymer formed by glucose molecules bonded together by β-1,4-glycosidic bonds. The chemical structure of cellulose molecules is characterized by the presence of many hydroxyl groups attached to carbon atoms. C-O peaks indicate the presence of a carbonyl group [26]. The FTIR spectra in Figure 3 showed a peak at 3396.76 cm^{-1} for Method 1 and 3444.98 cm^{-1} for Method 2 which indicates the presence of an O-H group hydrogen bound to phenolic and aliphatic structures [27]. The peaks at 2924.18 cm^{-1} for Method 1 and 2852.81 cm^{-1} for Method 2 indicate the stretching vibration of CH bonds present in all hydrocarbon components within the polysaccharide [28]. A peak around 1400 cm^{-1} indicates the presence of CH_2 bending. With Method 1 there was a peak at 1433.16 cm^{-1} and this appeared at 1421.58 cm^{-1} with Method 2. The peaks at 1317.43 cm^{-1} and 1319.35 cm^{-} indicate C-H bending. Further, the peak at 1033.88 cm^{-1} indicates a C-O group [29].


(a)

(b)

Figure 3. FTIR spectra of cellulose samples from cocoa shell waste using (a) green acids and some strong acid variations, and (b) functional group evolution of cellulose using 5 % NaOH for delignification, 5 % NaOH: 3 % H_2O_2 for bleaching, and 3.5 M hydrochloric acid for hydrolysis. Method 1 (coloured green) and Method 2 (coloured red).

Vibrations at 1523.82 cm^{-1} and 1537.32 cm^{-1} were C=C stretching vibrations which indicate the presence of lignin compounds. In the spectra of the GM and HA5 samples, there were peaks at 1523.82 cm^{-1} and 1537.32 cm^{-1}. In the spectra of HA17.5, CAM17.5, CA17.5, SA17.5 and NA17.5 there was no peak at 1500 cm^{-1} thus in these samples lignin had been separated from the extracted cellulose. Hemicellulose compounds were also shown at a vibration of 1600 cm^{-1}, there were peaks at this wave number in the GM sample; HA5; HA17.5; CAM17.5; CA17.5; SA17.5; and NA17.5 1635.39 cm^{-1} for GM samples and 1635.69 cm^{-1} for HA5 samples where a peak at 1640.16 cm^{-1} indicated ester formation [30]. The peak at 895 cm^{-1} indicates the presence of glycosidic bonds. These bonds affect insoluble cellulose and are not easily decomposed by chemical solutions. Hemicellulose compounds were also indicated at 1635.39 cm^{-1} for Method 1 and 1635.69 cm^{-1} for Method 2 which are the stretching vibrations of acetyl or ester groups[29].

The peaks at 895 cm^{-1} with Method 1 and 898.86 cm^{-1} with Method 2 indicate the presence of glycosidic bonds. This implies that the composition of the monosaccharides had not changed significantly, and that the resulting cellulose was not prone to degradation by chemical solutions [31].

Table 3. FTIR peak data.

Functional groups	Reference [29], [32]	GM	HA5	HA 17.5	CAM 17.5	CA 17.5	SA 17.5	NA 17.5
O-H stretch	3300	3396.7	3444.9	3328.8	3331.1	3440.0	3331.3	3325.8
C-H stretch	2920	2924.1	2852.8	2891.0	2890.9	2891.1	2890.6	2890.9
$C-H_2$ bend	1420	1433.1	1421.5	1419.8	1418.6	1417.8	1417.5	-
C-H bend	1320	1317.4	1319.3	1312.7	1313.8	1313.7	1312.7	1314.1
C-O stretch	1035	1033.8	1033.8	1022.9	1023.6	1024.2	1022.6	1015.6
Glycoside	895	898.8	894.3	893.5	894.31	894.2	893.3	894.4
C=O	1603	1635.6	1636.4	1640.0	1643.4	1631.7	1643.2	1636.4

Cellulose Crystal Structure

Figure 4 shows the XRD spectra of cellulose samples extracted from cocoa shell waste using Method 1 and Method 2. In Figure 4, it can be seen that different treatments produced different degrees of crystallinity. In samples extracted using Method 1 (GM), the main crystal peak occurred around 2θ = 14.74°. The main crystal peak value in this sample was the lowest when compared with other samples extracted using Method 2. The difference in the diffraction patterns of cellulose from these two methods was caused by the bleaching and acid hydrolysis processes. As a result of the bleaching process on the cellulose extracted using Method 2, the lignin fraction disappeared from the fibre so that the crystals formed were narrower and more ordered. In samples HA5, HA17.5, SA17.5, and NA17.5 there was a sharp increase in peak intensity, indicating an increase in nanocrystal purity after acid hydrolysis. The increase in purity was the result of the removal of non-cellulosic materials from nanocellulose [33]. However, CAM17.5 and CA17.5 did not experience a sharp increase in peak intensity. This is because in this study citric acid with a low concentration was used, and the weak acidity of citric acid makes it difficult to hydrolyse the amorphous part of cellulose and results in low cellulose production [34].

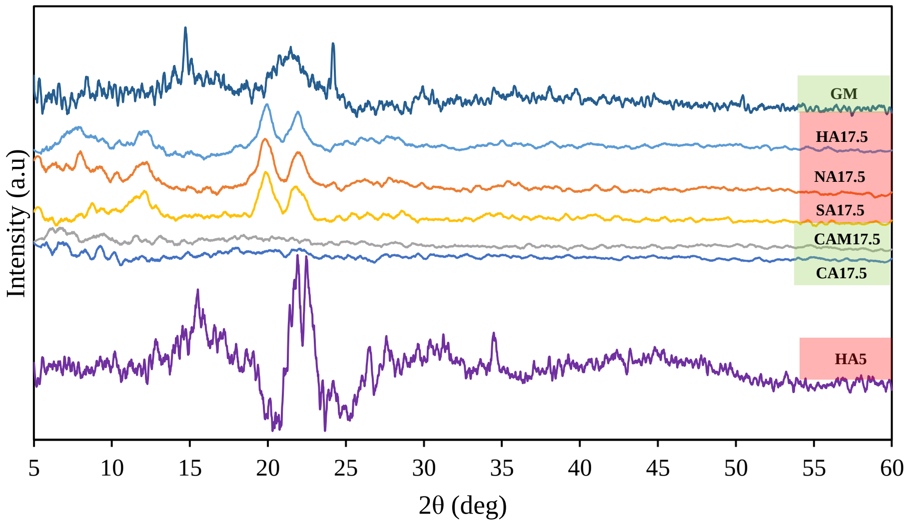


Figure 4. XRD spectra for cellulose samples extracted with Method 1 (highlighted in green) and Method 2 (highlighted in red).

Table 4. Crystallinity index of cellulose samples extracted using Method 1 and Method 2.

Sample Code	Crystal Peak (°)	Crystallinity Index (%)	Total area of crystalline and amorphous peaks
GM	14.74	12.06	1373.28
HA5	22.3	26.74	2851.68
HA17.5	19.93	51.74	660.41
CAM17.5	18.42	31.74	332.98
CA17.5	17.98	33.39	387.37
SA17.5	19.93	54.67	449.25
NA17.5	19.83	51.77	611.26

The crystallinity index (CI) was calculated for different cellulose samples using the Segal formula in equation (1). The Segal formula is one of the most commonly used and easiest techniques to calculate the crystallinity index at the peak height of a crystalline material. Based on these calculations, the crystallinity index obtained for the GM sample was 12.06 %. The crystallinity index value of this sample was lower when compared to the samples extracted using Method 2. The low crystallinity index value indicates that the sample had a poor crystalline form and showed impurities during crystallization. In samples extracted using Method 2, there was an increase in the crystallinity index, where the highest value obtained was for NA17.5, namely 51.77 %. The increase in crystallinity is attributed to the removal of amorphous regions of cellulose during acid hydrolysis and the subsequent hydrolytic cleavage of glycosidic bonds and release of individual crystallites [33].

Cellulose Morphology

Figure 5 shows the SEM images of the raw material (RM) cocoa shell samples which had a morphology dominated by irregular oval particles. The RM sample had an average particle size of 99.98 μm. After the cellulose extraction process using Method 1, the average particle size was 71.4 μm. The resulting particles were mainly oval shaped.

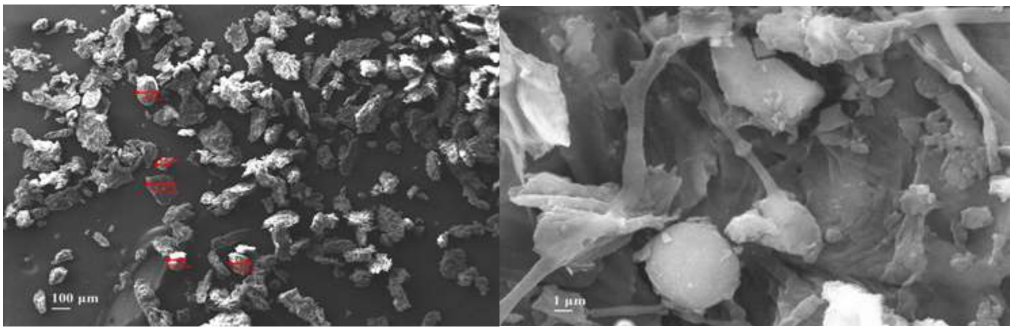


Figure 5. SEM results of raw material cocoa shells (RM).

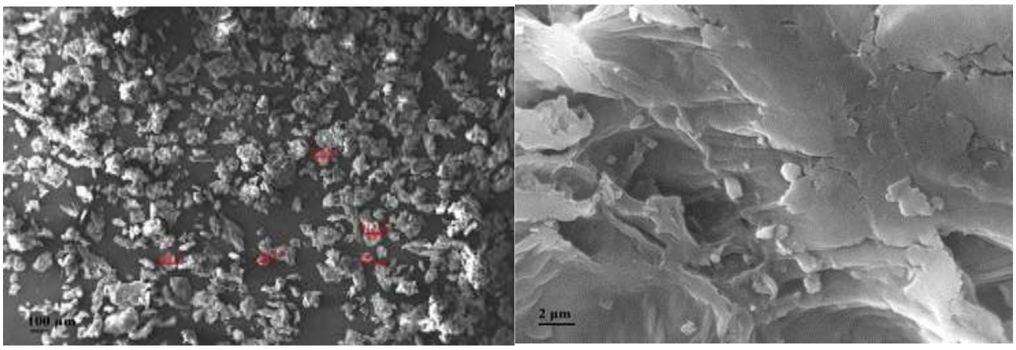


Figure 6. SEM results for cellulose from extraction Method 1 (GM).

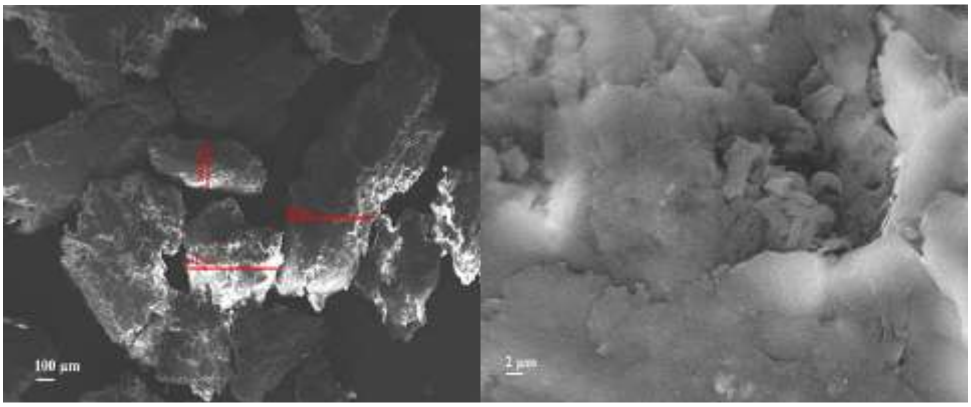


Figure 7. SEM results for cellulose from extraction Method 2 and delignification treatment using 5% NaOH (HA5).

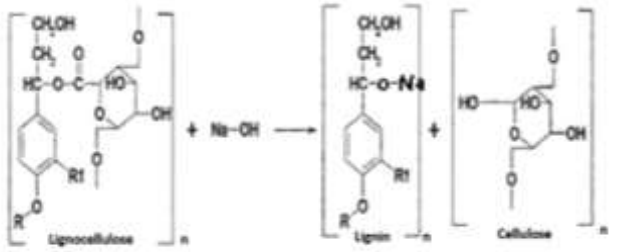


Figure 8. Lignocellulose bond breaking reaction using NaOH in Method 2.

A comparison of the shapes and sizes of the RM and GM samples showed no significant differences; there was only a reduction in particle size with an increasingly regular structure. This is because the composition of the two samples was very similar.

Figure 7 shows SEM images of the sample that was extracted using Method 2 (HA5). It shows that elongated and grouped particles were produced, with an average particle size of 419.03 µm. These results also show that the formation of interparticles or new morphology was due to the lignin and pectin content in the raw material cocoa shells having been lost through extraction Method 2, and this was confirmed by the FT-IR spectra. In Figure 6 there are also more interconnections between particles which can grow to produce new interparticles or morphologies.

CONCLUSION

In this study, the cellulose produced from Method 1 was still dark brown in colour, while the cellulose resulting from Method 2 tended to be a dull brown. Extraction using 17.5 % NaOH was more effective because it removed the lignin content. However, hydrolysis with nitric acid caused one of the main functional groups of cellulose to be lost, namely the CH_2 group at 1400 cm^{-1}. The chemical treatment in extraction Method 2 was more effective in degrading the amorphous region and maintaining the crystalline structure compared to Method 1. SEM characterization found that the particle size of HA5 was larger than that of GM, and HA5 showed new morphology because the lignocellulose content had been removed.

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