SYNTHESIS AND CHARACTERIZATION OF MICROCRYSTALLINE CELLULOSE FROM *Rhizophora apiculata*

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ABSTRACT

This study attempts to extract the microcrystalline cellulose (MCC) from mangrove (Rhizophora apiculata) wood. The wood particles were hydrolyzed with 64 % sulfuric acid (H_2SO_4) consecutively at 45 °C for an h. The chemical composition of the mangrove particles were analyzed using TAPPI standards for their extractives, holocellulose, α-cellulose, and lignin contents. The morphological structure of the fiber was viewed under the scanning electron microscopy (SEM) and light microscopy. The Fourier transform infrared (FTIR) was used to see the changes of any functional group of raw particles and microcrystalline particles after acid hydrolysis. The removal of the impurities along with defibrillation were also shown in the surface of microcrystalline cellulose. The removal of lignin and other extractives during chemical extraction and acid hydrolysis can be seen by the absence of certain band in the FTIR spectra.

Keywords: Chemical properties; mangrove; microcrystalline cellulose; morphological; raw particles.

INTRODUCTION

In Malaysia, mangrove forests are among the most productive and biologically important ecosystems. It provides an important and unique ecosystem for goods and services to human society, coastal and marine systems. Among its functions are as a stabilizer for the shorelines and reducing the devastating impact of natural disasters, providing breeding and nursery grounds for marine species, and food, medicine, fuel and building materials [1, 2]. Besides that, mangrove based products can be found in the form of timber and poles for the construction industry as scaffolding, charcoal, pulpwood, tannin and dyes. *Rhizophora* species are the best producers of tannins of all the mangroves with 20-25% of its bark weight [3].

As one of the lignocellulosic materials, the main component of mangrove is cellulose. Cellulose is a renewable, biodegradable and the most abundant natural biopolymer which can be processed into whisker-like micro fibrils [4, 5]. Cellulose is a high molecular weight linear homopolymer, comprising β-D-glucopyranosyl repeating units joined by (1 → 4) glycosidic linkages [6]. The molecules are completely linear and have a strong tendency to form intra- and intermolecular hydrogen bonds. This leads to a bundling of cellulose molecules into microfibrils, which in turn form fibrils and finally cellulose fibers. Recently, many researchers and manufacturers use natural fibers to replace man-made fibers to make environmentally safe products due to its advantages such as renewable, low cost, low density, high specific strength and stiffness [7, 8]. One of the examples is the microcrystalline cellulose (MCC). The MCC potentially can be used as a filler and binder for the extrusion/spheronization process and consists of aggregated bundles of crystallites with different particles. The properties of the MCC can be influenced by the origin of the raw materials and the isolation method [9].

In this study, the MCC from *Rhizophora apiculata* was extracted by acid hydrolysis using sulfuric acid. The main objectives of this study were to produce and characterize the morphological, chemical compositions and the functional group of the microcrystalline cellulose from *Rhizophora apiculata*. The morphological properties was examined through a scanning electron microscope (SEM) and light microscope (LM). The TAPPI method has been used for chemical composition determination and Fourier transform infrared (FTIR) was used to see the changes of the functional group before and after hydrolysis was conducted.

EXPERIMENTAL

The mangrove (*Rhizophora apiculata*) used was obtained from Larut Mangrove Forest Reserve, Malaysia. The samples were chopped and cut into small pieces and air dried. The samples were then grounded to powder form using Wiley mill grinder machine before being sieved to pass through a 40 mesh sieve.

Preparation of microcrystalline cellulose

The preparation of microcrystalline cellulose were mentioned in our previous work [10] following Fahma et al. (2010) [11] with a slight modification. Briefly, the raw *Rhizophora apiculata* fibers were weighed and the extractives were removed by Soxhlet extraction for 4 hr using ethanol/toluene (v/v 2:1). Then, the bleaching were done four times in sodium chlorite ($NaClO_2$) solution under acidic conditions (pH 4 to 5) at 70°C for 1 h. The fibers were then washed with deionized water before soaked with 6 wt % potassium hydroxide (KOH) solution at 20°C for 24 h. The fibers were then washed with deionized water until neutral. Then, 210 mL of sulfuric acid (H_2SO_4) solution (64%) were added to the cellulose fibers with stirring at 45°C for 1 h before the hydrolysis was terminated with 400 mL of cold water. The precipitate was resuspended in water with strong agitation, centrifuged for 3 days until the pH became constant. It was then homogenized, sonicated, and freeze dried.

Morphological

Surface morphological properties were examined with scanning electron microscope model LEO Supra 50 Vp field emission scanning microscope (FESEM) with ultra-high resolution. The sample was gold coated using a sputter gold coater before viewing process were conducted. The visual morphology of *Rhizophora apiculata* was also investigated by light microscope (LM) Olympus BX 41-CCD.

Chemical composition

Sample preparation for chemical composition was carried out according to TAPPI T 257 cm-02 [12]. The preparation of extractive free wood was done according to T 264 cm-97 [13]. Extractives free sawdust from ethanol-toluene extraction was used for the holocellulose determination [14]. Determination of alpha cellulose was conducted based on TAPPI T203 cm-99 [15] and determination of lignin was referred to T 222 0m-02 [16]. All samples were done in 3 replications.

Fourier transform infra-red analysis

The FT-IR Spectroscopy was used to measure the functional groups that were present in the samples. A pellet of 1 mm in thickness was made by mixing approximately 5 mg of powder of each sample with 95 mg of finely ground potassium bromide (KBr). Perkin Elmer 1600 infrared Spectrometer machine were used in order to view the spectrum. All spectra produced were collected over a range of wavenumbers of 4000 cm^{-1} and 500 cm^{-1} with a resolution of 4 cm^{-1}.

RESULT AND DISCUSSION

Morphological studies

In this study, the microstructures of the samples were examined in order to understand the changes in fiber structure, shape, and particle size affected by acid hydrolysis. Figure. 1 shows the SEM micrographs of raw particles and microcrystalline cellulose from *Rhizophora apiculata* after acid hydrolysis. A definite change in the morphological structure of the whole cellulose fibers occurred after acid hydrolysis. From the SEM micrograph of the raw particles of *Rhizophora apiculata* (Figure. 1a, b and c), a pit (indicated by an arrow) can be seen clearly. The presence of pit in the radial wall will cause deviation of microfibrils and affects the shrinkage and swelling properties of wood [17]. Besides that, Figures. 1 b and c shows a particle still in a bundle form and are still bound together by lignin. After the chemical treatment and acid hydrolysis, most of the lignin and hemicellulose were removed, thus producing microcrystalline cellulose. The chemical treatment separated and reduced the size of the bundles into a single fiber as can be seen in Figure. 1d. The surface of the microcrystalline cellulose was clean and free from impurities as observed in Figures. 1e and f.

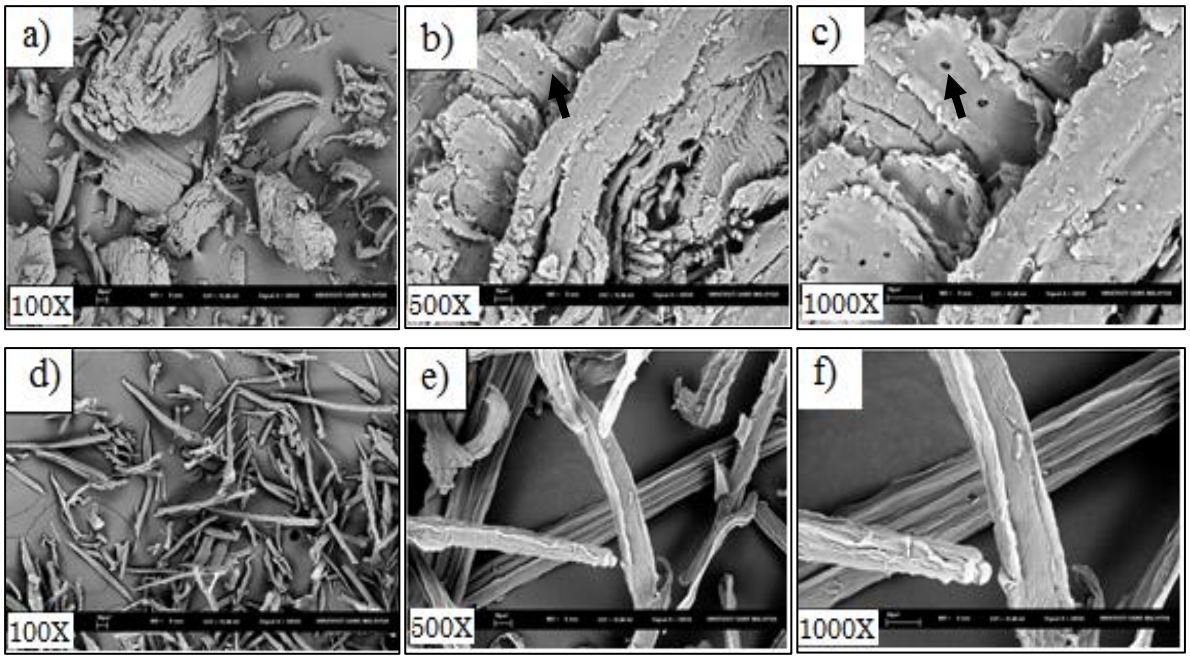

Figure 1. SEM micrographs of (a), (b), (c) raw *Rhizophora apiculata* and (d), (e), (f) microcrystalline cellulose after acid hydrolysis

The morphological properties of *Rhizophora apiculata* was also viewed by the light microscope. Figure. 2a and b show the morphological of both raw particles before and after acid hydrolysis in the form of microcrystalline cellulose. It can be seen clearly that after raw particles underwent chemical treatment, the size of the raw particles was reduced and turned into an individual fiber.

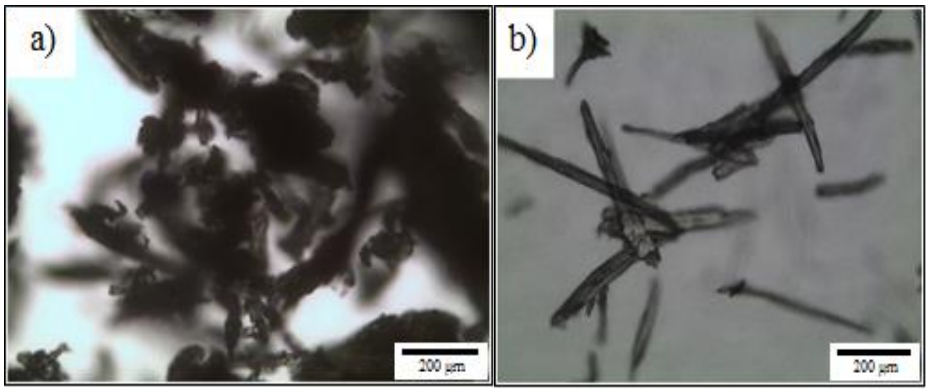

Figure 2. Light microscope of a) raw particles and b) microcrystalline cellulose

Chemical composition analysis

The extractives, holocellulose, alpha-cellulose and lignin content in *Rhizophora apiculata* were investigated and the results were depicted in Table 1. The raw particles having a low extractive content of 2 % which is lower than other extractive content of some wood fibers. However, the value was in the range of wood fiber extractive content (0.1-8.5%) reported by other researcher [17]. The percent of extractive content can be in the range of between 1 % to 20 % in regards to the plant species or the section part of the plant [18].

Table 1. Chemical composition of raw particles and microcrystalline cellulose of *Rhizophora apiculata*

R. apiculata	Extractive (%)	α-cellulose (%)	Hemicellulose (%)	Holocellulose (%)	Lignin (%)
Raw	2.00	48.65	30.97	79.62	18.38
MCC	-	67.89	23.12	91.01	-

Native cellulose is generally known to be fibril and crystalline. It plays a significant role in contributing to the high strength and stability of plant cell walls [19, 20]. Alpha cellulose content of raw particles was calculated to be 48.64 %. After the acid hydrolysis, the result showed an increase in α-cellulose content (67.89 %). The fiber that contained high cellulose level and low microfibril angle when being pulled under a density will create a higher tensile strength [21].

Softwood has more lignin content than hardwood. The lignin content is found high in *Rhizophora apiculata* that is 18.38 %. After chemical treatment and acid hydrolysis, the lignin was removed from the cellulose fibers. High lignin content in the sample is known to impede the acid hydrolysis process [22], therefore low lignin composition is desirable for the production of cellulose microfiber using acid hydrolysis. The elimination of lignin is desirable in obtaining pure cellulose.

Fourier transform infra-red analysis

The changes that occurred to raw particles of *Rhizophora apiculata* before and after acid hydrolysis were depicted in Figure. 3. The stretching vibration of –OH groups was shown at the absorption peaks between 3200-3600 cm^{-1}. The peaks are dominant in both spectra of raw particles and microcrystalline cellulose at 3410 cm^{-1} and 3351 cm^{-1}, respectively. The existence of O-H containing hydroxyl is found in cellulose, hemicelluloses and lignin. The peaks at the 2901 cm^{-1} and 1430 cm^{-1} are characteristics of C-H stretching and bending of CH_2 groups respectively [23].

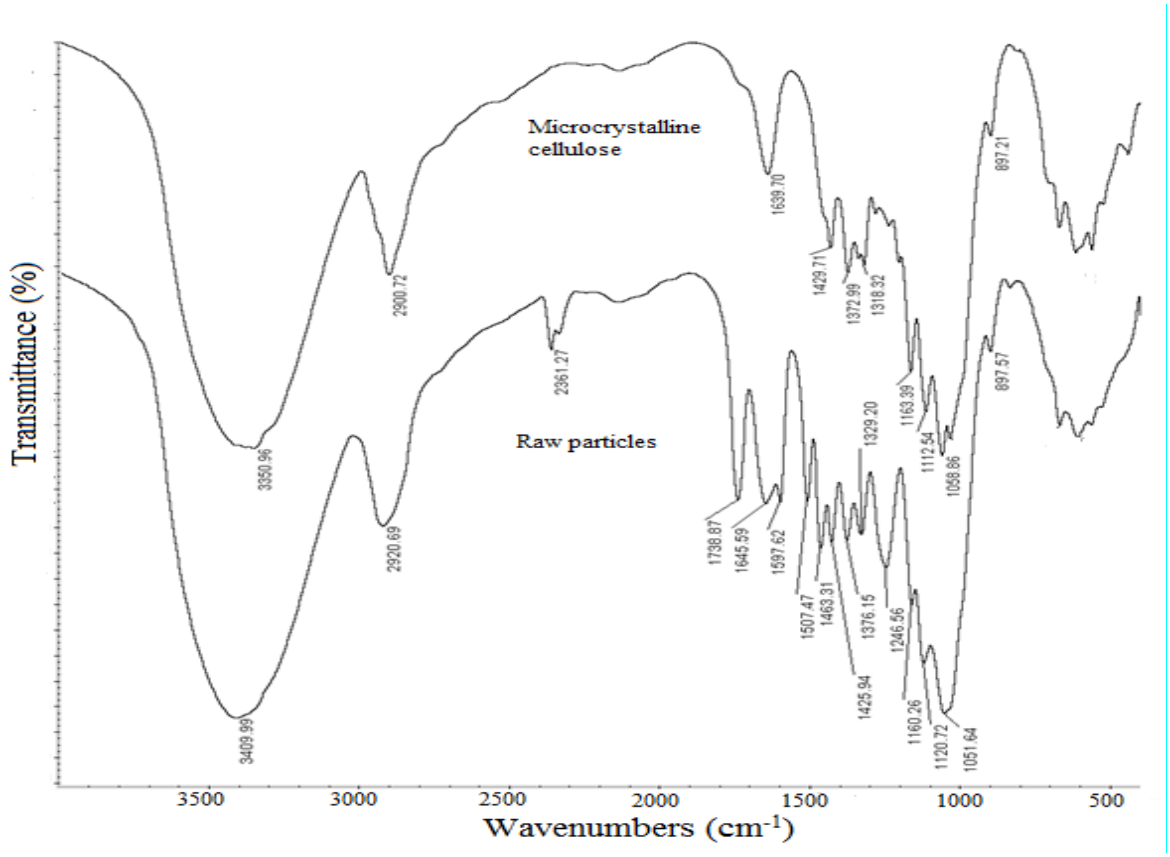

Figure 3. FTIR spectra of raw particles and microcrystalline cellulose of *Rhizophora apiculata*

The peaks at 1645 cm^{-1} and 1639 cm^{-1} in the Figureure represent the stretching of C=C. The peaks between 1045-

1096 cm^{-1} correspond to the –OH bending, which is strongly influenced by the degree of branching. The peaks between 1670-1760 cm^{-1} correspond to the carbonyl (C=O) stretching frequency that exists in the side chains of lignin structural units and is also an important functional group in the polysaccharide units of hemicelluloses chains, which either is in the form of aldehyde groups or keto groups. Usually, for carbonyl stretching (C=O), acetyl groups present in hemicelluloses and aldehyde group present in the lignin, respectively. In the raw particles spectra, the peak at 1738 cm^{-1} indicates the carbonyl (C=O) group stretching and peak at 1507cm^{-1} attributed to C=C stretching vibration in the aromatic lignin [24]. However, the peaks disappear after acid hydrolysis as shown in the microcrystalline cellulose spectra indicating the complete removal of the lignin and also the hemicellulose.

Hemicelluloses are made of a relatively limited number of sugar residues of pentosans and hexosans, respectively. The *β*-glycosidic linkage between the sugar units broadens the OH peak frequency. The peaks between 1120 -1260 cm^{-1} are representative of the antisymmetric bridge stretching of C-O-C groups in cellulose and hemicelluloses. However, in the microcrystalline cellulose spectra, the peaks at 1246 cm^{-1} were absent indicating the removal of hemicellulose. The peaks at 1640 cm^{-1} and 897 cm^{-1} are attributed to the O-H stretching vibration of the absorbed water in the carbohydrates and the C-H deformation vibrations of cellulose, respectively [23].

CONCLUSION

In this study, microcrystalline cellulose was successfully isolated from *Rhizophora apiculate* wood using the acid hydrolysis method. The chemical composition of raw *Rhizophora apiculata* shows a higher content of holocellulose (79.62 %), low extractives content and lignin (18.38%). The result showed an increase in α-cellulose content and decrease in lignin and hemicelluloses after acid hydrolysis. A definite change in the morphological structure of the MCC occurred upon acid hydrolysis was observed using SEM. After the chemical treatment the lignin and hemicellulose were largely removed, and the bundle size of the raw particles was reduced into an individual fiber. For FTIR analysis, there are some changes happened in the spectra after the wood particles underwent chemical treatment and acid hydrolysis indicating the removal of lignin and hemicelluloses.

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