# Isolation and Characterization of Microcrystalline Cellulose from Cotton Stalk Waste

Ming Li, Beihai He, and Lihong Zhao *

An effective method for microcrystalline cellulose (MCC) isolation from cotton stalk is reported. Cotton stalk was subjected to pretreatment and hydrolysis to determine the optimum conditions for isolating cotton stalk MCC (S-MCC). The main purpose of pretreating the cotton stalk with acetic acid was to remove ash. As a result, the ash content was reduced from 5.70% to 1.10%. After acid hydrolysis, the remaining ash was removed. Based on the single factor and orthogonal experiments, the optimum hydrolysis conditions were 85 °C, 1 mol/L HCl, 90 min, and a solid-to-liquid ratio of 1:10. The results showed that the reaction temperature and time strongly influenced the yield and size of the S-MCC particles. In addition to the yield, D90 was shown to be a good parameter to represent the degree of cellulose hydrolysis. The S-MCC had an ash content of 0.06%, *α*-cellulose content of 98.6%, moisture content of 4.64%, degree of polymerization of 146, and crystallinity index of 80.3%. These chemical and physical properties were comparable to those of commercial MCC. Other structural characterizations were determined by Fourier-transform infrared spectroscopy, thermogravimetric analysis, and scanning electron microscopy, and compared with commercial MCC.

*Keywords: Cotton stalk; Acetic acid pretreatment; Microcrystalline cellulose; Hydrochloric acid hydrolysis*

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## INTRODUCTION

Cellulose is a renewable, natural biopolymer derived from biomass (Wan Daud *et al.* 2011; Adel and El-shinnawy 2012). Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$ with a *n* of 1500 to 5000. Specifically, cellulose is a polysaccharide with long chains of *β*-(1-4)-linked D-anhydroglucopyranose repeating units (Merci *et al.* 2015; Zhao *et al.* 2018). Currently, cotton is an important commercial product and an abundant crop globally, particularly in China, the United States, India, and Brazil (Silverstein *et al.* 2007). Presently, cotton stalk left over after harvesting is subjected to field burning or landfilling. Growing concerns over environmental protection and utilization of agricultural waste have led to intense research to develop high-value applications for cotton stalk. Cotton stalk residue contains three major components, which are cellulose, hemicellulose (including xylan, arabinan, and galactan), and lignin (Table 1). Based on its main chemical components, cotton stalk has the potential to serve as a raw material for producing dissolving pulp, microcrystalline cellulose (MCC), cellulose derivatives, biofuel, and other useful products. However, the high ash content of cotton stalk can hinder its application.

**Table 1.** Chemical Composition of the Cotton Stalk

| Study | Component (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Glucan | Xylan | Arabinan | Galactan | Acid-insoluble Lignin | Acid-soluble Lignin | Ash |
| Silverstein *et al.* (2007) | 31.1 | 8.3 | 1.3 | 1.1 | 27.9 | 2.2 | 6 |
| Kaur *et al.* (2012) | 30.6 | 14.6 | 1.2 | 1.1 | 27.9 | 2.1 | 3.6 |

Microcrystalline cellulose is a natural cellulose obtained from partially depolymerized and purified cellulose (Nada *et al.* 2009; Kaur *et al.* 2012; Xiang *et al.* 2016; Kambli *et al.* 2017). Because MCC has unique properties, such as a high strength, low density, biodegradability, and favorable mechanical properties, its potential utilization for producing nanocellulose and bio-composites has generated attention and interest in both academic and industrial fields. In the pharmaceutical industry, MCC is often used as an excipient and disintegrant in the manufacturing of pharmaceutical tablets.

Inorganic matter contained in plant resources is commonly termed as ash. It is well known that cotton stalks have a higher ash than the traditional raw material used for producing MCC (cotton, softwood, and hardwood). Although agricultural residues such as cotton stalk and sisal hemp could be used to prepare MCC, through the traditional acid hydrolysis process, a higher level of ash content generally remains in the prepared MCC products. Most of the inorganic matter in plant tissues is in an adsorbed state or present in combination with organic or inorganic acids. It is widely believed that ash-forming elements in biomass include aluminum, calcium, chlorine, iron, potassium, magnesium, phosphorus, sodium, sulfur, manganese, silicon, and titanium, and these give rise to different technical and environmental problems in the process of biomass utilization. Especially for pharmaceutical application, minerals or some ash elements (Mondal *et al.* 2015) in MCC could cause health problems. In order to solve the problem, besides the lignin removal, the key technique is to minimize the content of ash, thereby meeting the requirements of the US Food and Drug Administration. Acid pretreatment is commonly used to remove ash content. Turn *et al.* (1997) treated bana grass by mechanical dewatering and leaching. In addition, pyrolysis acid leaching was used to remove insoluble inorganic elements (Oudenhoven *et al.* 2016). So far, there are relatively few studies on ash removal from cotton stalk.

A variety of physical, chemical, physico-chemical, and biological technologies have been reported to isolate MCC, such as hot water hydrolysis, acid hydrolysis, alkali hydrolysis, gamma irradiation (Swantomo *et al.* 2017), and steam explosion. Hou *et al.* (2019) prepared microcrystalline cellulose from waste cotton fabrics by using phosphotungstic acid. Huang *et al.* (2011) obtained MCC from bagasse by degradation of bagasse cellulose in subcritical water/$CO_2$. Owolabi *et al.* (2017) isolated MCC from oil palm fronds by pre-hydrolysis of alkaline hydrogen peroxide ($H_2O_2$). Kian *et al.* (2017) reported the isolation of MCC from roselle fibers by hydrochloric acid (HCl) hydrolysis. Jahan *et al.* (2011) prepared MCC from jute fibers by hydrolysis with sulfuric acid. Regarding the mild reaction conditions, acid hydrolysis is considered to be a common process for MCC separation. Haafiz *et al.* (2013) isolated MCC from empty fruit bunch fiber-total chlorine free (TCF) pulp by HCl acid hydrolysis. Shi *et al.* (2018) isolated microcrystalline cellulose from waste cotton fabrics under hydrothermal conditions (solid-liquid ratio 1:30, HCl concentration 0.6 mol/L, 150 °C, 100 min).

The novelty and objectives of recent studies are to produce MCC from various lignocellulosic materials, such as cotton fabrics, soybean hulls (Merci *et al.* 2015), kenaf fibers (Wang *et al.* 2010), corn husk fibres (Kambli *et al.* 2017), and define the optimum hydrolysis conditions for producing MCC. In general, the acid hydrolysis step could reduce a certain amount of ash, which is suitable for cotton and cotton fabrics. But for raw materials with high ash content, it is obviously not enough to remove that amount of ash by acid hydrolysis alone. Compared with the previous studies, the aim of this study is not only to define the optimum hydrolysis conditions of cotton stalk fiber, but also to decrease the high ash content in order to meet the requirements of pharmacopoeia. Moreover, it can provide a new direction for the utilization of high ash agricultural and forestry wastes, which have been seldom used in pharmaceutical field.

On characterizing and determining the extent of cellulose hydrolysis, previous methods primarily measured the yield and degree of polymerization (DP) of MCC (Zhao *et al.* 2018), which is time-consuming. Additionally, the degree of cellulose hydrolysis by acid cannot be readily controlled. In the acid hydrolysis process (Fig. S1), both the cellulose yield and particle size decreased with the breaking and shortening of the cotton stalk cellulosic molecules. Therefore, it is worth exploring the use of the instant D values (D10, D50, and D90) of the particle size and the intercepts for 10%, 50%, and 90% of the cumulative mass to describe and control the phases of depolymerization during the acid hydrolysis process, instead of the DP method. An investigation into this new method was involved in this work, and the correlations between the yield, particle size, and extent of cellulose hydrolysis are discussed.

Although there have been many reports on the method of isolating MCC from agricultural residues, there are few studies on isolating MCC from cotton stalk waste, particularly when it comes to removing a large amount of ash to meet the requirements of the United States Pharmacopoeia (2012). Considering the lack of research on ash reduction and MCC isolation from cotton stalk, the aim of this study was to (1) reduce the ash content remarkably from cotton stalk by acetic acid pretreatment; (2) reveal the dynamic depolymerization of cellulose with the change in the yield and particle size during acid hydrolysis; (3) analyze the optimum acid hydrolysis conditions by a single-factor experiment and an orthogonal experimental design; and (4) isolate MCC from cotton stalk and characterize its properties to provide a scientific basis for high value-added utilization of cotton stalk.

## EXPERIMENTAL

### Materials

Cotton stalk was collected from Xinjiang, China and chipped with a cutting type grinding apparatus (CM 100, Beijing Grinder Instrument Co., Beijing, China). The powdered cotton stalk was sieved through a 40-mesh screen and stored in sealed plastic bags at room temperature. Stabilized chlorine dioxide ($ClO_2$) was purchased from Huashi Pharmaceutical Company (Weifang, China). Commercial MCC (C-MCC) was purchased from Sinopharm (Beijing, China) as a reference. Hydrogen peroxide (30%) was purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). All of the other chemicals were of analytical grade and from commercial sources.

## Methods

### *Acetic acid pretreatment and kraft cooking process*

Acetic acid ($CH_3COOH$) was used to pretreat the cotton stalk powder samples at a solid-to-liquid ratio of 1:1 (w/v). Pretreatment was performed in a boiling water bath for 1 h. After pretreatment, the samples were washed with water until the samples were neutral. Kraft cooking was performed in a laboratory rotary digester under the following conditions: solid-to-liquid ratio of 1:15 (w/v), 23% active alkali, 25% sulfidity, 160 °C, and 3 h. The pulp obtained was then washed and screened before undergoing the next process.

### *Cold alkali isolation and bleaching process*

Cold alkali isolation (Gehmayr *et al.* 2011; Li *et al.* 2015), $ClO_2$ and $H_2O_2$ bleaching process (Nakamata *et al.* 2004; Jahan *et al.* 2011; Kambli *et al.* 2017) were performed as described in detail elsewhere. In preliminary experiments, it was found that (i) the optimal alkali extraction condition should be followed as: solid-to-liquid ratio of 1:10 (w/v), 8% sodium hydroxide, 55 °C, and 1 h. After that, the pulp was washed with hot water until the sample was neutral. (ii) The optimal bleaching process was as follows: firstly, stabilized $ClO_2$ was used to bleach the pulp at 95 °C to 100 °C for 1 h. This process was repeated three times.

The pulp was washed several times with distilled water until the yellowish green filtrate was clear. Secondly, $H_2O_2$ bleaching process was performed at 90 °C for 1 h with 1.2wt% NaOH, 1.2wt% $Na_2SiO_3$, 0.01wt% EDTA, and 5wt% $H_2O_2$ at a ratio of 1:15 (v/v). The pulp was then washed with distilled water until the sample was neutral.

### *Preparation of the S-MCC*

Cellulose was partially polymerized through acid hydrolysis to prepare the MCC. Details of the acid hydrolysis have been published previously (Kian *et al.* 2017; Zhao *et al.* 2018). In order to study the influence of reaction condition in acid hydrolysis, the bleached pulps were hydrolyzed with different HCl acid concentrations (0.5 mol/L to 2.5 mol/L), reaction times (30 to 120 min), temperatures (80 °C to 100 °C), and solid-to-liquid ratios (1:30 to 1:10), which is briefly shown in Table 2. The pulps were hydrolyzed under agitation in a single factor experiment that investigated the effects of the hydrolysis conditions on the yield and D50 of the S-MCC. The reaction was terminated by a large quantity of cold water.

After acid hydrolysis, the samples were filtered and thoroughly washed with distilled water until the MCC became neutral. Finally, the obtained S-MCC was dried by a spray dryer.

### *Analytical methods*

The yields of the cellulose samples were calculated based on the weight over the untreated and pretreated cotton stalk cellulose as a percentage. The particle size analysis (D90, D50, and D10) of the cellulose hydrolysis was performed with a Mastersizer 3000 (Malvern Panalytical, Malvern, UK). Factors and levels of the orthogonal experimental design are shown in Table 2. The orthogonal data was analyzed by SPSS 19 (IBM Corporation, Armonk, NY, USA), and a P-value of 0.05 was used for the significance level.

**Table 2.** Factors and Levels of the Orthogonal Experiment Design

| Level | Factor | | | |
|---|---|---|---|---|
| | (A) HCl Concentration (mol/L) | (B) Temperature (°C) | (C) Hydrolysis Time (min) | (D) Ratio of Acid to Material |
| 1 | 1 | 85 | 60 | 1:20 |
| 2 | 2 | 90 | 90 | 1:15 |
| 3 | 3 | 95 | 120 | 1:10 |

The Fourier-transform infrared (FTIR) spectra of the S-MCC and C-MCC were acquired using 32 scans with a Vector 33 spectrometer (Bruker, Karlsruhe, Germany) over the range of 400 $\text{cm}^{-1}$ to 4000 $\text{cm}^{-1}$. The samples were mixed with potassium bromide (KBr) (Kermel, Tianjin, China) and pressed.

The crystallinity index (CrI) was analyzed with a D8 ADVANCE (Bruker, Karlsruhe, Germany) by X-ray diffraction (XRD) with CuKα radiation ($\lambda$ = 1.54 Å) at 40 mA and 40 kV. The scattering angle ($2\theta$) range was 5° to 60°, the step width was 0.02°, and the scanning speed was 19.2 s/step. The CrI was calculated using Eq. 1 (Wang *et al.* 2010; Kian *et al.* 2017),

$$\boldsymbol{CrI}\ (\%) = \frac{\boldsymbol{I_{002} - I_{\mathrm{am}}}}{\boldsymbol{I_{002}}} \times \mathbf{100}\% \tag{1}$$

where $I_{002}$ is the peak intensity of the (002) lattice plane at a $2\theta$ of 22.5°, and $I_{\mathrm{am}}$ is the peak intensity of the amorphous domain at a $2\theta$ of 18.5°.

A TA Instruments Q500 thermogravimetric analyzer (New Castle, DE, USA) was used to characterize the thermal stability of the samples. Thermogravimetric analysis (TGA) was conducted under a nitrogen flow and at a constant heating rate of 10 °C/min from 30 °C to 800 °C. The morphological characterization was obtained by scanning electron microscopy (SEM) (EVO18; ZEISS, Jena, Germany). The MCC powder was fractured in liquid nitrogen and then coated with gold before being tested.

To detect the heavy metals and other elements, the ash composition was recorded with an energy dispersive spectrometer (EVO18, ZEISS, Jena, Germany). An infrared moisture analyzer (MA35, Sartorius, Göttingen, Germany) was used to analyze the moisture content of the sample at 105 °C. The DP and ash residue were determined according to the United States Pharmacopoeia (2012).

## RESULTS AND DISCUSSION

Dilute acid pretreatment was demonstrated to be an effective method to reduce the hemicellulose and ash fractions of the lignocellulosic biomass, which is shown in the Appendix (Fig. S1). In this process, the liquid acetic acid flowed through the cotton stalk particles, removing ash and hemicellulose, and the ash content was decreased by 80.7%. A possible explanation for this was that there were some insoluble inorganic compounds dissolved in the acetic acid. In the kraft pulping process, 16.0% of the lignin dissolved in the black liquor was removed. The next cold alkali extraction process isolated 2.15% residual ash and 1.52% lignin from the pulps. The subsequent bleaching treatment selectively removed short-chain carbohydrates and lignin to enhance the whiteness. The final process was acid hydrolysis, which degraded the amorphous zones in the cellulose and further reduced the residual ash content at high temperatures. Overall, the increase in the $\alpha$-cellulose from 54.2% to 98.6% indicated the effectiveness of this MCC production method.

According to the United States Pharmacopoeia (2012), the ash content should not exceed 0.1%, and heavy metals (Mondal *et al.* 2015) that could cause deleterious health effects in humans should not exceed 10 ppm. In Table S2, it is shown that the elements K, Cu, P, Cl, Sr, and Fe were removed. The ash content of the S-MCC was 0.06%, which was close to the ash content in the C-MCC (0.05%) (Table 3). The reserved elements Na, Mg, Al, Si, S, and Ca were also at a lower level, and none of them are regarded as being heavy metals. Therefore, the S-MCC samples were fully consistent with the related standards.

**Table 3.** Chemical Composition of the Raw Material, S-MCC, and C-MCC

| | Cotton Stalk | S-MCC | C-MCC |
|---|---|---|---|
| Ash (%) | 5.70 | 0.06 | 0.05 |
| $\alpha$-cellulose (%) | 54.15 | 98.55 | 98.59 |
| Acid-insoluble Lignin (%) | 24.53 | 0.81 | 0.95 |
| CrI (%) | 43.01 | 80.29 | 80.60 |
| Moisture Content (%) | - | 4.64 | 4.40 |
| DP | - | 148.4 | 138.8 |

Some studies have also confirmed that the moisture content of the MCC affected the compression, tensile strength, and flowability properties (Khan *et al.* 1981; Crouter and Briens 2014). Thoorens *et al.* (2015) reported that the tablet-ability of neat MCC was statistically influenced by the moisture content, which must be measured and controlled to ensure high-quality tablets. Table 3 shows that the moisture contents of the S-MCC and C-MCC were 4.64% and 4.40%, respectively, which was consistent with other studies (Kambli *et al.* 2017).

The HCl concentration is an important factor that affects the hydrolysis of cotton stalk cellulose (Trache *et al.* 2014). Because of the unique chemical composition (cellulose, hemicellulose, and lignin) and structure of lignocellulosic biomass, various cellulose materials need different concentrations of acid. To investigate the effect of HCl concentration, a single factor experiment was done at 85 °C for 120 min, with a solid-to-liquid ratio of 1:20. Figure 1a shows that the yield and particle size decreased with an increasing HCl concentration. Over the range of 0.5 mol/L to 1.5 mol/L, the yield decreased sharply by 19.8%. During the stage of 1.5 mol/L to 2.5 mol/L, the yield slowly decreased by 7.2%, which indicated that the S-MCC entered the level-off degree of depolymerization. In these two stages, D50 decreased to 20.0 μm and 7.1 μm, while D90 decreased to 146.8 μm and 31.8 μm, respectively. In this process, D90 tended to decrease closer to the yield and thus could better represent the cellulose hydrolysis degree. Figure 1a shows that 1.0 mol/L was the most effective acid concentration for cotton stalk fiber.

The effect of the reaction temperature on the yield and particle size was performed at 2 mol/L HCl for 120 min with a solid-to-liquid ratio of 1:20. Figure 1b shows that the yield and particle size decreased as the temperature increased. Regarding the yield, hydrolysis could be divided into two phases. In phase I from 80 °C to 90 °C, the yield decreased by 3.5%. In phase II from 90 °C to 100 °C, the yield decreased by 10.5%, which was a three times higher decrease than that in phase I. During the continuous depolymerization of the amorphous regions in phase II, the crystalline regions were also destroyed. The increase in the temperature accelerated the movement of $H^+$, which led to the breakdown of β-glycosidic bonds (Zhao *et al.* 2018; El-Sakhawy *et al.* 2007). The $Cl^-$

weakened the glycosidic bonds, which facilitated the degradation of cellulose (Shi *et al.* 2018). Among the three parameters for the particle size, only D90 displayed an obvious downward trend, and decreased by 37.8 μm and 63.4 μm in phase I and II, respectively. However, the appearance of the S-MCC obtained at approximately 95 °C was gray in color. Therefore, the hydrolysis temperature should be less than 95 °C, which was consistent with the results of MCC isolation from tea waste (Zhao *et al.* 2018).

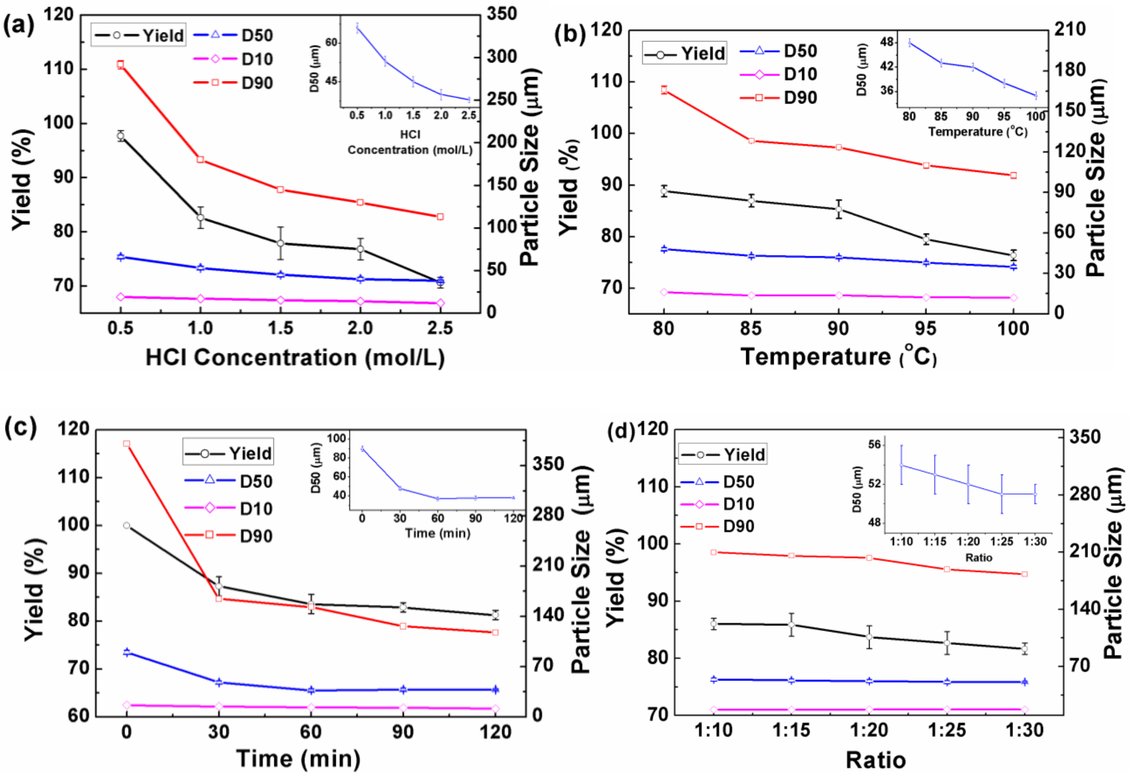


**Fig. 1.** Effect of the hydrolysis conditions on the yield and particle size (D90, D50, and D10) of the S-MCC: (a) HCl concentration; (b) reaction temperature; (c) reaction time; and (d) solid-to-liquid ratio

The effect of the reaction time on the yield and particle size was investigated at the reaction conditions of 85 °C, 2 mol/L HCl, and a solid-to-liquid ratio of 1:20. Figure 1c shows that the cotton stalk cellulose underwent rapid hydrolysis in the first 30 min. In this phase, the yield and D90 decreased by 13% and 216.4 μm, respectively. The rapid hydrolysis phase was followed by a slower phase from 30 min to 120 min, in which the yield and D90 decreased by 6.0% and 47.1 μm, respectively. A possible explanation was that it was easier for the acid solution to penetrate the amorphous region of the cellulose and hydrolyze it in the earlier stage; in the slower phase, it was harder to hydrolyze the crystallized region (Li *et al.* 2014), while cellulose depolymerization was still occurring slightly. Overall, maintaining a suitable reaction time was of great significance for the production of MCC.

The effect of the ratio on the yield and particle size was analyzed at 85 °C with 2 mol/L HCl for 120 min. Figure 1d shows that the solid-to-liquid ratio had a slight effect on the yield and particle size of the S-MCC. The yield decreased from 86.1% to 81.6% and the D90 value decreased slightly from 205.7 μm to 182.9 μm. A possible explanation was that an excessive solid-to-liquid ratio would not have a further influence on the degradation of cellulose into MCC when the amount of HCl employed was enough to hydrolyze part of the amorphous cellulose (Trache *et al.* 2016). Thus, maintaining a suitable ratio was of great significance for MCC production.

The orthogonal experiment was an experimental design method utilized for MCC isolation optimization. Based on the results that are shown in Table 4, the temperature had a significant effect on the yield ($P < 0.05$) and cellulose particle size (D90). The order of the factors with the highest effect was as follows: hydrolysis temperature > HCl concentration > hydrolysis time > solid-to-liquid ratio. Considering that the hydrolysis temperature should not be higher than 95 °C to protect the MCC samples, A1B1C2D3 (1 mol/L HCl, 85 °C, 90 min, and 1:10) was selected as the optimum operation condition. This could provide a scientific basis for acid hydrolysis of cotton stalk. After acid hydrolysis, the D90, D50, and yield values were 150.4 μm, 40 μm, and 93.5%, respectively. Finally, the S-MCC was dried with a spray dryer. The DP of the S-MCC was 146, which was comparable to that of the C-MCC (Table 3).

**Table 4.** SPSS Analysis of the Yield and D90 of the S-MCC from the Orthogonal Experiment

| Factor | A | B | C | D | Yield (%) | D90 |
|---|---|---|---|---|---|---|
| 1 | 2 | 2 | 1 | 2 | 92.2 | 191.0 |
| 2 | 1 | 2 | 2 | 1 | 98.2 | 343.0 |
| 3 | 1 | 3 | 3 | 2 | 90.1 | 133.4 |
| 4 | 2 | 3 | 2 | 3 | 93.0 | 91.0 |
| 5 | 3 | 1 | 2 | 2 | 99.2 | 135.4 |
| 6 | 2 | 1 | 3 | 1 | 94.9 | 147.6 |
| 7 | 3 | 3 | 1 | 1 | 87.1 | 105.9 |
| 8 | 3 | 2 | 3 | 3 | 92.8 | 105.2 |
| 9 | 1 | 1 | 1 | 3 | 99.3 | 262.1 |
| K1 | 287.6 | 293.4 | 278.6 | 280.2 | | |
| K2 | 280.1 | 283.2 | 290.4 | 281.5 | | |
| K3 | 279.1 | 270.2 | 277.8 | 285.1 | | |
| R | 7.5 | 23.2 | 12.6 | 4.9 | | |
| Order | B > A > C > D | | | | | |
| Optimum Condition: A1B1C2D3 | | | | | | |
| * Data is the analysis results of the yield | | | | | | |

The FTIR spectra of the raw material, S-MCC, and C-MCC are shown in Fig. 2. There were two main typical absorbance regions, from 3500 $cm^{-1}$ to 3000 $cm^{-1}$ and from 800 $cm^{-1}$ to 1800 $cm^{-1}$. The first broad band ranging from 3500 $cm^{-1}$ to 3000 $cm^{-1}$ was from the O-H stretching vibration of hydrogen-bonded hydroxyl groups belonging to cellulose. The peaks at 800 $cm^{-1}$ to 1800 $cm^{-1}$ were attributed to C=O, C-O-C, C-H, and C=C bands. Earlier studies (Mirmohamadsadeghi *et al.* 2016) indicated that the bands at 1750 $cm^{-1}$ to 1680 $cm^{-1}$ can be attributed to components of hemicellulose. The bands of 1515 $cm^{-1}$ to 1505 $cm^{-1}$ were assigned functional groups of lignin. Generally, the spectrum of MCC was relatively simple, and these bands represented the removal of lignin and hemicellulose by the chemical treatment. In this study, the S-MCC sample displayed similar characteristic peaks compared with C-MCC, which indicated that they had similar chemical compositions. The absorption bands at 2901 cm$^{-1}$ to 2902 cm$^{-1}$ were from C-H stretching and the crystalline order of cellulose, which was comparable to that of MCC reported by Kambli *et al.* (2017). The water absorption peak was observed from 1640 cm$^{-1}$ to 1642 cm$^{-1}$ because of the strong interaction between the cellulose and water. The bands located at 1430 cm$^{-1}$ to 1431 cm$^{-1}$ corresponded to CH$_2$ bending vibrations. The peak around 1372 cm$^{-1}$ was related to C-H and C-O groups. The peaks around 1059 cm$^{-1}$ were associated with C-O-C pyranose ring skeletal deformation, and the peaks at 897 cm$^{-1}$ were related to C-H rocking vibrations of cellulose.

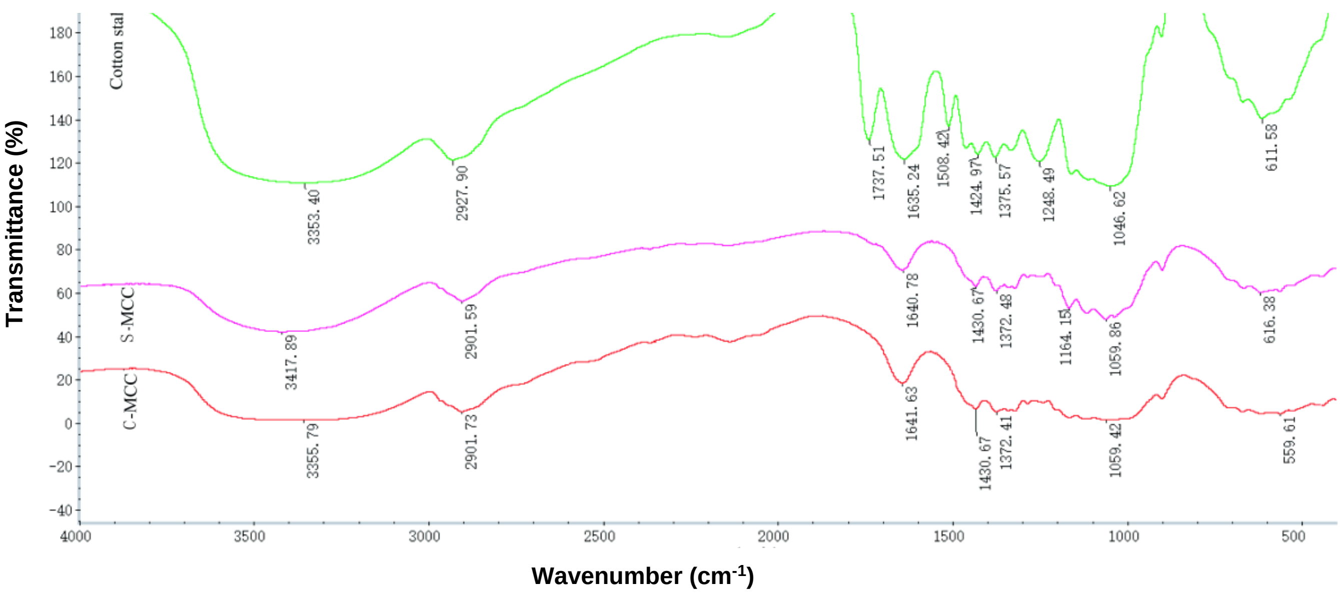


**Fig. 2.** FTIR spectra of the cotton stalk, S-MCC, and C-MCC

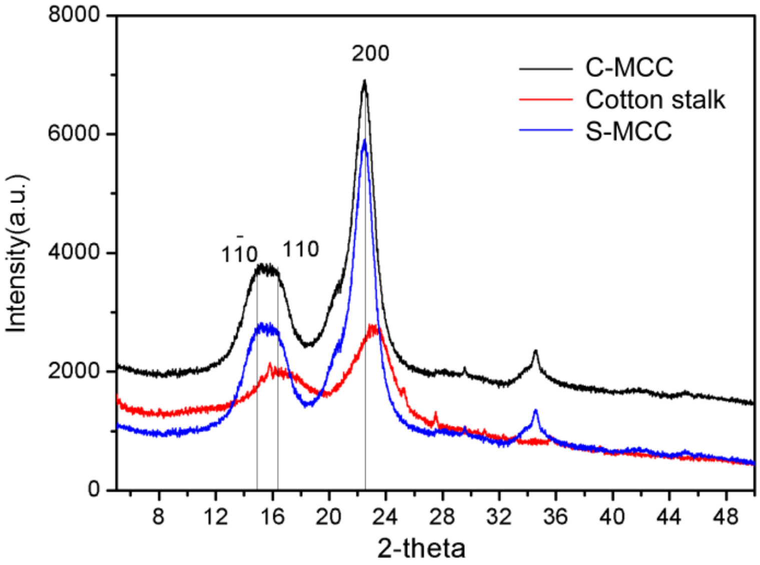
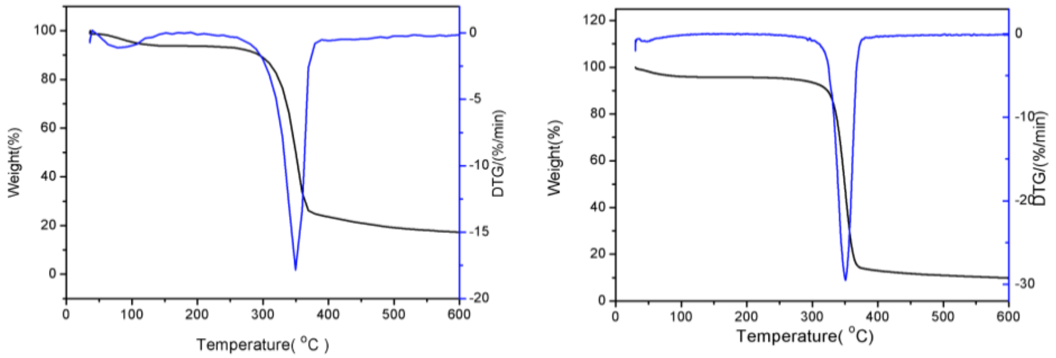


**Fig. 3.** X-ray diffraction patterns of the cotton stalk, S-MCC, and C-MCC

X-ray diffraction is a versatile technique that provides in-depth crystallographic structure information for materials. The CrI value represents the degree of cellulose crystallization and is one of main factors that affects MCC characteristics. According to the literature, the crystallization of MCC particles is important because of its effect on a variety of properties, including the stabilizing capacity for suspension and emulsion, thermal properties, and hydration capacity (Thoorens *et al.* 2015). Additionally, Xiang *et al.* (2016) suggested that a higher CrI is more suitable for use as a reinforcing material in biocomposites. The XRD pattern of the raw material, S-MCC, and C-MCC samples is shown in Fig. 3. The diffractograms showed a peak at $2\theta$ =22.6° and a shoulder in the region $2\theta$ = 14° to 17°, which originated from cellulose I ( Elanthikkal *et al.* 2010; Zhao *et al.* 2018; Wang *et al.* 2010). Table 3 shows that the CrI values of the of the raw material, S-MCC, and C-MCC were 43.0%, 80.3%, and 80.6%, respectively, which indicated a significant improvement through the isolation process. Furthermore, the structural parameters also depended on the origin of the raw material. The CrI of the S-MCC isolated from the cotton stalk in this study was higher than the MCC samples obtained from oil palm, alfa fibers, and roselle fibers (Trache *et al.* 2014; Hussin *et al.* 2016; Kian *et al.* 2017).

The thermodynamic properties of MCC were analyzed by TGA and the derivative thermogram (DTG). In the S-MCC TGA curve (Fig. 4a), there were four stages based on the weight. In the first stage, the weight decreased from 100% to 94% because of the evaporation of water and other volatile substances from 36 °C to 131 °C. The weight ranged from 93% to 90% from 142 °C to 290 °C, which indicated its stability at 290 °C. In the third stage, there was a sharp weight reduction from 299 °C to 370 °C, and the weight decreased from 92% to 26% because of the breaking of chemical bonds, such as C-C, C-O, and C-H, and thermal cracking of S-MCC. In the fourth stage with the ash formation process, the solid residue of the S-MCC was approximately 24% to 17% from 380 °C to 600 °C. Overall, the onset decomposition temperature was 289.7 °C and the peak temperature was 369.5 °C with an 82.7% weight loss. In the DTG curve of the S-MCC, the onset and peak temperatures were 299.7 °C and 349.5 °C, respectively. Additionally, in the DTG curve of the C-MCC (Fig. 4b), the peak temperature was 349.5 °C, which was the same as for the S-MCC. The onset and peak temperatures of the degradation process were 319.1 °C and 350.7 °C, respectively. These differences were because of the different raw materials. Based on the above results, the MCC from cotton stalk presented an excellent thermal stability, and therefore it can be used in pharmaceuticals, food, and other biocomposites on a large scale (Nsor-Atindana *et al.* 2017).



(a) (b)

**Fig. 4.** Typical TGA and DTG curves for the (a) S-MCC and (b) C-MCC

The surface morphology of the S-MCC and C-MCC were analyzed by SEM. Figure 5 (a-i, a-ii, b-i, and b-ii) shows that the surface of both MCC samples were smooth and rod-shaped because of the removal of lignin, hemicellulose, and some amorphous cellulose. Compared with the C-MCC, the most of S-MCC showed individualized fibers with long, thread-like structure, and few were rod-liked structures. As was previously reported, these differences can be attributed to their origins from different cellulose sources (Kalita *et al.* 2013).

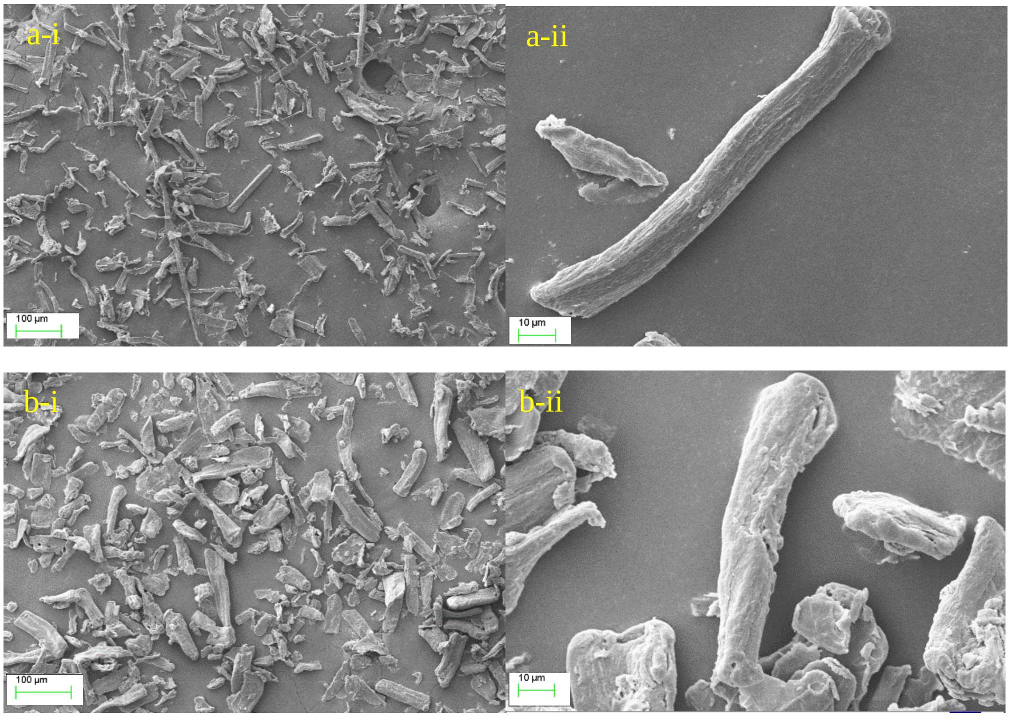


**Fig. 5.** Typical SEM micrographs of S-MCC (a), C-MCC(b)

## CONCLUSIONS

1. The optimum hydrolysis conditions were determined (1 mol/L HCl, 85 °C, 90 min, and 1:10). A great amount of ash content (80.7%) was removed by acetic acid pretreatment. Upon completion of hydrolysis, the ash, cellulose, and moisture contents of the microcrystalline cellulose (MCC) prepared from cotton stalk waste satisfied the industrial requirements for pharmaceutical applications.
2. The overall results demonstrated that the MCC from cotton stalk (S-MCC) was similar to commercial MCC (C-MCC). A typical cellulose I structure and good thermal stability were seen in the S-MCC.
3. The S-MCC has great application potential in food, pharmaceuticals, and biocomposites.

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# APPENDIX

## Supplementary Material

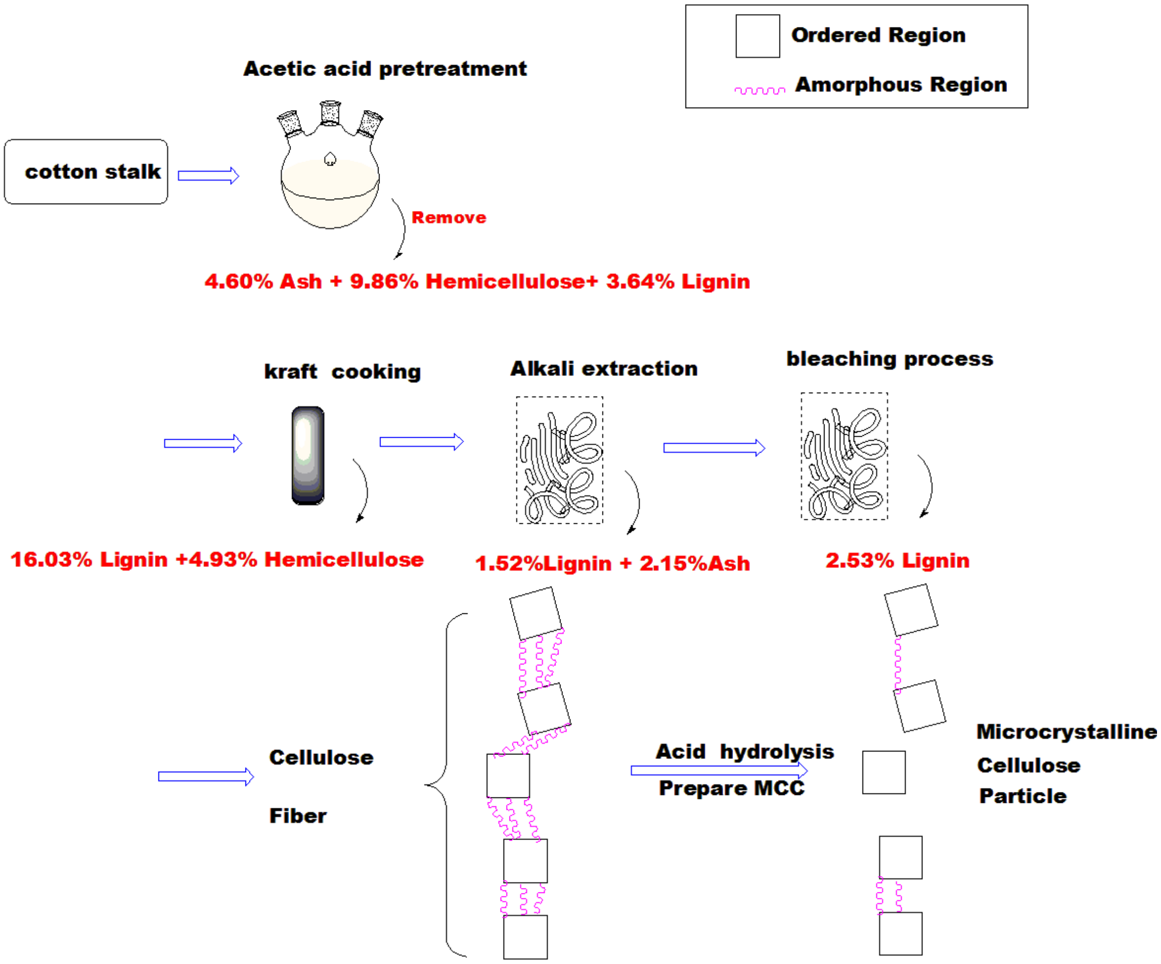

**Fig. S1.** Schematic diagram of S-MCC preparation

**Table S1.** Chemical Composition of Ash in Raw Material and S-MCC

| Element | Chemical compound | | Chemical formula |
|---|---|---|---|
| | Cotton stalk ash (‰) | C-MCC ash(‰) | |
| Na | 1.51 | 0.06 | $Na_2O$ |
| Mg | 3.54 | 0.15 | MgO |
| Al | 0.80 | 0.02 | $Al_2O_3$ |
| Si | 8.25 | 0.05 | $SiO_2$ |
| S | 2.68 | 0.04 | $SO_3$ |
| Ca | 25.63 | 0.28 | CaO |
| K | 9.83 | | $K_2O$ |
| Cu | 0.12 | | CuO |
| P | 3.59 | | $P_2O_5$ |
| Cl | 0.06 | | |
| Sr | 0.19 | | SrO |
| Fe | 0.80 | | $Fe_2O_3$ |
| Total | 57.00 | 0.60 | |