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# Cellulose Nanofibers from Cassava Bagasse: Characterization and Application on Tapioca-Film

Rumpoko Wicaksono<sup>1\*</sup>, Khaswar Syamsu<sup>2</sup>, Indah Yuliasih<sup>2</sup>, Muhamad Nasir<sup>3</sup>

1. Agricultural Technology Departement, Faculty of Agriculture, Jenderal Soedirman University, Dr. Suparno Street, Karangwangkal, Purwokerto53123, Indonesia

2. Department of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, PO BOX 220 16602, Bogor, Indonesia

3. Research Center for Chemistry, Indonesian Institute of Science, Cisitu-Sangkuriang Street, Bandung 40135,

Indonesia

\*E-mail of the corresponding author: rumpoko@unsoed.net

#### Abstract

Cassava bagasse, a solid by-product of cassava starch industry, is a source of natural fibers can be used as a reinforcement filler to overcome poor mechanical property of films. This research aims to study the character of cellulose nanofibers which produced from cassava bagasse and to study the influence of its application on the mechanical properties of tapioca-based film. Cellulose nanofibres from cassava bagasse were obtained through a series of chemical treatments (alkali treatment, bleaching, and acid hydrolysis) and mechanical treatment (high velocity mixer). Cellulose nanofibres with diameters in the range of 5-8 nm and estimated lengths of several micrometers were obtained from cassava bagasse. Zeta potential measurement indicated that cellulose nanofibers suspension has good stability. FT-IR spectroscopic of cellulose nanofibers demonstrated that the chemical treatment led to partial removal of hemicelluloses and lignin from the structure of fibers. XRD results are revealed that chemical treatment also improved crystallinity of fibers from 14.52% in cassava bagasse to 39,37% in cellulose nanofibres. It was observed that the addition of cellulose nanofibers improved tensile strength of films and decreased its elongation at break.

Keywords: Cassava bagasse, Cellulose nanofibers, Tapioca film, Filler

## 1. Introduction

Biopolymers used in films usually have poor mechanical properties when it is compared to petroleum-based polymers. Several composites have been developed by adding reinforcement (fillers) to biopolymers to enhance their performance and applicability. Natural fibers are interesting alternative as filler of films. Cellulose is an important natural fiber. Using cellulose fibers to reinforce materials have several advantages over synthetic one. They offer environmental benefits because of their availability, biocompatibility, biological degradability, sustainability, low energy consumption in production, final price reduced of material, and also reinforcement act by increasing the mechanical properties as easier process due to their non-abrasive nature (Alemdar and Sain, 2008; Chen et al., 2011; Ludueña et al., 2012).

The production of nano-scale cellulose fibers and their application in composite materials have gained increasing attention due to their high strength and stiffness. Fillers with at least one nano-sized dimension (nanofillers) have the better interfacial adhesion with the polymer matrices, when compared to the respective micro/macroscopic reinforcements. A uniform dispersion of nanofillers leads to a very large matrix/filler interfacial area, changing the molecular mobility and mechanical properties of the nanocomposite result (Azeredo et al., 2012).

Several processes are used to extract highly-purified nanofibers from the cell wall. They are generally based on successive chemical with acids or bases and mechanical treatments. The commonly acids used are  $H_2SO_4$  (Belbekhouche et al., 2011; Liu et al., 2011) or HCl (Alemdar and Sain, 2008), while bases (alkali) used are KOH (Abe and Yano, 2009) or NaOH (Elanthikkal et al., 2010). These treatments led to partial separation of the nanofibers from the cell wall, taking the advantage of its relatively low lignin and hemicellulose content. In addition, to improve the individualization of the nanofibers, several mechanical treatments can be used such as Manton–Gaulin homogenizer (Habibi et al., 2009), mechanical stirrer (Cherian et al., 2010), grinder (Siró and Plackett, 2010), and ultrasonication (Chen et al., 2011).

Cassava (Manihot esculenta) is a root crop largely grown in tropical countries such as Indonesia. It is a starch-

rich material, also containing protein, lipid, lignocellulosic fibers and sugar. The industrial exploitation of cassava starch involves the elimination of soluble sugar and the separation of fibers resulting in a purified starch and a solid residue called cassava bagasse (Teixeira et al., 2009). In other side, this by product of tapioca industry raises problems to the environment around the industry. It contaminates the air with smelly odour due to degradation of organic compounds to volatile matters by micro-organisms. It also contaminates rivers and wells of people living near the industry (Hermiati et al., 2012).

The cassava bagasse is mainly composed of water (70–80% w/w), residual starch and cellulose fibers (Teixeira et al., 2009). Cassava bagasse contains starch and fiber in a range of 61.84-69.90 % (w/w dry basis) and 10.61-14.35 % (w/w dry basis), respectively (Srinorakutara et al., 2004). Therefore, this residue contains both a great deal of residual starch and a considerable quantity of natural fibers. These characteristics suggest the possibility of using the bagasse as a source of cellulose fibers for the extraction of new nanocellulose structures. Utilization of cassava bagasse not only reduce of environmental problem, but also improves the rural economy. This research aims to study the character of cellulose nanofibers which produced from cassava bagasse and to study the influence of its application on the mechanical properties of tapioca-based film.

## 2. Materials and methods

#### 2.1. Materials

The cassava bagasse used as raw material was obtained from tapioca industry in Purbalingga, Indonesia. Potassium hydroxide was used for alkaline treatment. Sodium chlorite and acetic acid were used as bleaching agents, while sulphuric acid was used for hydrolysis. All chemicals purchased from Merck.

2.2. Preparation of cellulose nanofibers

Cassava bagasse (CB) was purified by a series of chemical treatments and then subjected to a mechanical treatment to produce cellulose nanofibers (CN) according to the flowchart shown in Figure 1.



Figure 1. Experimental Procedure for the Preparation of Cellulose Nanofibers from Cassava Bagasse.

## 2.2.1. Alkali treatment

The alkali treatment was performed to purify the cellulose by removing lignin and hemicellulose from cassava bagasse fibres. The ground cassava bagasse (passed through100 mesh) was treated with an alkali solution (4 wt% KOH). The mixture transferred into a round bottom flask and treatment performed at 80°C for 1 h. The solid was then filtered and washed several times using distilled water. This treatment was performed twice, before and after bleaching process.

#### 2.2.2. Bleaching process

Following alkali treatment, the bleaching process was completed by adding a buffer solution of acetic acid, aqueous chlorite (5 wt%) and distilled water at  $70^{\circ}$ C for 1 h. The mixture was allowed to cool and was filtered using excess distilled water. The bleaching process is repeated two times.

## 2.2.3. Acid hydrolysis

The acid hydrolysis treatment conducted on the fibers after alkali treatment and bleaching at a temperature of  $60^{\circ}$ C using 6.5 M of sulphuric acid for 1 h under continuous stirring. The hydrolysed material is washed by centrifugation at 5,000 rpm for 10 min. This centrifugation step repeated several times by adding distilled water until constant pH of pulp in the range of 5–6 was reached.

## 2.2.4. Mechanical treatment

As about 5 g of pulp diluted in 300 ml distilled water. The slurry then homogenized with high velocity mixer at 22,000 rpm for 10 min. The suspension result was kept in refrigeration before further used.

## 2.3. Preparation of films

Tapioca starch films were prepared using a casting method. Film solution (4 wt % dry basis) was prepared by dissolving tapioca (4 wt % dry basis) in hot water (80°C), containing cellulose nanofibres suspension of 0, 1, 2, 3, and 4 wt % (dry basis) and 2.5 wt % (dry basis) sorbitol as plasticizer. Suspension was mixed directly together with hot plate magnetic stirrer for 15 min. Thus, the prepared solution was poured onto glass plate. Water was evaporated from the molds in a ventilated cabinet dryer at 50 °C for 12 h. Dried films were put in air tight container and stored at ambient temperature.

## 2.4. Characterization of cassava bagasse and cellulose nanofibres

## 2.4.1. Microscopies

Scanning electron microscopy (SEM) (Zeiss EVO<sup>®</sup> 50) was used to observe the surface morphology of cassava bagasse. The accelerating voltage was 1 kV. Transmission electron microscopy (TEM) (JEOL JEM-1400) was used to determine the dimensions of the cellulose nanofibres obtained from cassava bagasse. A drop of suspension was deposited on the surface of a clean copper grid and dried at ambient temperature before TEM analysis was carried out with an accelerating voltage of 100 kV. As for contrast in TEM, the cellulose nanofibres were stained in a 1 wt% solution of ammonium molybdate.

## 2.4.2. Zeta potential measurement

The zeta potential of the cellulose suspensions was measured using a Delsa Nano C Beckman Coulter. The mobility of the particles undergoing electrophoresis was measured using dynamic light scattering studies. This measured electrophoretic mobility was then converted to the zeta potential.

## 2.4.3. Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectra were recorded using a Bruker Tensor 37 FTIR spectrophotometer. Samples were mixed with potassium bromide, KBr. The mixture was then compressed into pellet form. FTIR spectral analysis was performed within the wave number range of  $400-4000 \text{ cm}^{-1}$ .

## 2.4.4. X-ray diffractometer

X-ray Diffraction (XRD) patterns were obtained using a XRD-7000 Shimadzu X-ray Diffractometer with Cu radiation and operated at 40 kV and 30 mA. The sample was placed in a sample holder, and analysis is carried out in a static position. Scattered radiation was detected in the range  $2\theta = 5-40^{\circ}$ , at speed  $2^{\circ}/\text{min}$ . Cristallinity index was ratio of crystalline peak area to total area in percent.

## 2.4.5. Tensile properties

The tensile strength and percent elongation at break of films were determined using Zwick instrument. The dumbbell form samples were clamped between grips with initial distance between grips of 50 mm. Force were recorded during extension at 10 mm min<sup>-1</sup>.

## 3. Results and discussion

## 3.1. Microscopical characterization

Chemical and mechanical treatment is applied to cassava bagasse to form nano-sized fibers. Microscopical observation of CB and CN can be seen in Figure 2. The diameter of CB fibers as about 50  $\mu$ m. The granules from cassava tubers also appear in SEM with a diameter in a range 270-290  $\mu$ m and length of about 0.5 mm. CN had an average diameter of around 5-8 nm and several micrometer in length.



Figure 2. SEM of CB and TEM of CN.

Chemical and mechanical treatments are applied to CB in this study succeed to produce nano-sized fibers (CN). The individualization of CN from cassava bagasse was based on protocols combining chemical and mechanical treatments. During chemical treatments, constituents like pectins and hemicelluloses were hydrolyzed by the action of alkaline solutions, whereas lignin was removed during additional steps using sodium chlorite. Alkali treatment and high temperatures cause hydrolyzed hemicellulose and soluble in water.

Bleaching treatments help remove most of the lignin. Lignin is believed to be linked with the carbohydrate moiety through two types of linkages. One is alkali sensitive and another alkali resistant. The alkali sensitive linkage forms an ester type combination between lignin hydroxyls and carboxyls of hemicellulose uranic acid. The ether type linkage occurs through the lignin hydroxyls combining with the hydroxyls of the cellulose. The degradation of lignin leads to the formation of hydroxyl, carbonyl and carboxylic groups. These groups help in solubilizing the lignin content in the alkali medium and there by facilitates the purification of cellulose (Zuluaga et al., 2009; Cherian et al., 2010). Acid treatments can also weaken the structure of the aggregate fibers, thus causing more easily disintegrated fibers, while mechanical treatment is used to separate the fiber aggregates into individualized nanofibers (Cao et al., 2012).

## 3.2. Zeta potential measurement

Zeta potential is an important parameter for examining dispersion stability of colloidal, indicating the degree of repulsion between adjacent, similarly charged particles in dispersion. Particles that are small enough, a high zeta potential will confer stability, i.e., the solution or dispersion will resist to aggregation. When the potential is low, attraction exceeds repulsion and the dispersion will break and flocculate. Colloids with high zeta potential (negative or positive) are electrically stabilized while colloids with low zeta potentials tend to coagulate or flocculate. Briefly, zeta potentials from 0 to  $\pm 30$  mV indicate instability, while zeta potentials higher than  $\pm 30$  mV indicate stability (Silva *et al.*, 2012). The higher values of zeta-potentials indicate the higher capacity of dispersion, while low values indicate low dispersion stability (Tonoli et al. 2012).

The ZP measurement is showed that CN suspension was 52.45 mV, indicates that cellulose nanofibers suspension have good stability. Cellulose nanofibers suspension obtained is not easy to agglomerate. This situation is much different with CB that easy to agglomerate if suspended in the water. These results indicate that the hydrolysis process which applied to the CB is not only sufficient to reduce the fibers size into nano-sized fibers, but also has good stability in the form of suspension. Insufficient hydrolysis of cellulose may result in larger particles (less surface area per unit mass) with a lower mean surface charge, favouring particle–particle interaction and making it easy to agglomerate (Elanthikkal et al. 2010).

## 3.3. FTIR spectroscopic analysis

FTIR spectroscopy is a nondestructive method for studying the physico-chemical properties of lignocellulosic materials.FT-IR analysis which conducted to determine the presence of certain functional groups in the material. The FTIR spectra of CB and CN are shown in Figure 3.



Figure 3. FTIR Spectra of CB and CN.

The FTIR spectra of CB and CN have exhibited a broad band in the region 3500-3200 cm<sup>-1</sup> that indicates the free O-H stretching vibration of OH groups in cellulose molecules (Mandal and Chakrabarty, 2011). Moreover, the spectra of all samples showed the characteristic C-H stretching vibration around 2900 cm<sup>-1</sup> (Rosa et al., 2012). The peak present at 1730–1740 cm<sup>-1</sup> in the spectrum corresponding to the raw fibres could be due to the presence of C=O linkage, which is a characteristic group of lignin and hemicelluloses (Abraham et al., 2011). The peak at 1647.59 cm<sup>-1</sup> in CB and 1645.14 cm<sup>-1</sup> in CN are associated with –OH bending of adsorbed water (Alemdar and Sain, 2008; Abraham et al., 2011). All the FTIR spectra were developed after the same carefully drying process, however the water adsorbed in the cellulose molecules is very difficult to extract due to the cellulose–water interaction (Abraham et al., 2011).

Lignin presented characteristic peaks in the range  $1200-1300 \text{ cm}^{-1}$  corresponding to the aromatic skeletal vibration. The signal is thought from aryl group (Abraham et al., 2011; Mandal and Chakrabarty, 2011). The FTIR spectra of CB and CN have exhibited the peak at  $1200-1300 \text{ cm}^{-1}$ . These peaks are are associated with C-O stretching of hemicellulose and lignin (Abraham et al., 2011; Rosa et al., 2010). The intensity of these peaks decreased in CN, which was attributed to the partial removal of hemicellulose and lignin.

The presence of cellulose can also be expected from the appearance of the signal at 896.69 cm<sup>-1</sup>, which is typical structure of cellulose (Alemdar and Sain, 2008). The peak at wave numbers around 900 cm<sup>-1</sup> indicate the presence of  $\beta$  glycosidic linkages between glucose units in cellulose (Adel et al., 2010; Mandal and Chakrabarty, 2011; Rosa et al., 2012). The presence of this peak showed the increase in the percentage of cellulosic components after removal of non-cellulosic materials by chemical treatments (Neto et al., 2013).

## 3.4. X-ray diffraction

X-ray diffraction patterns of CB before and after the chemical process to produce cellulose nanofibers (CN) are presented in Fig. 4. Diffractogram of CB shows two main peaks at  $2\theta = 17^{\circ}$  and  $2\theta = 22.85^{\circ}$ , while CN shows main peak at  $2\theta = 15.5^{\circ}$  and  $2\theta = 22.13^{\circ}$ . As shown in Figure 4, two samples have a typical crystal lattice for cellulose I, which arises from the fact that there is no doublet in the intensity of the main peak (Adel et al., 2011).



Figure 4. X-ray Diffraction Patterns of CB and CN.

Changes in the intensity of diffraction peaks indicate a change in the structure or crystallinity cellulose molecular chain regularity (Elanthikkal et al., 2010; Chen et al., 2011). High crystallinity indicates higher perfection of the crystal lattice (Lu and Hsieh, 2010). Crystallinity index of CB and CN are 14.52% and 39.37%, respectively. It can be seen that CN is more crystalline than CB. The increase in the overall order of the hydrolysed fibres can be attributed to the removal of the hemicelluloses and lignin which exist in amorphous regions during the chemical treatment (Elanthikkal et al., 2010; Chen et al., 2011).

The results above demonstrate that hydrolysis took place preferentially in the amorphous region. (Cherian et al., 2011). The series of chemical treatments using alkali (KOH) can eliminate residual starch, hemicellulose, and pectin, whereas bleaching treatment (with  $NaClO_2$ ) can reduce lignin, thus more soluble in alkaline solution (Abe and Yano, 2009). The use of sulfuric acid can promote hydrolysis process of hemicellulose or lignin during treatment (Adel et al., 2010).

## 3.5. Mechanical properties of films

The measurement results of the mechanical properties of films, including tensile strength and elongation at break are presented in Figure 5.



Figure 5. Tensile strength (a) and elongation at break (b) of tapioca film with adding CN.

Figure 5 (a) shows the reinforcing ability of CN on tensile strength of films. The tensile strength of films tends to increase with the increase in CN content. The tapioca film with 1% CN loading is showed an improvement of tensile strength up to 69% over pure tapioca film. Strengthening mechanisms associated with the ability of the fiber to form a network structure of the film matrix and attributed to the high contact surface area of the smaller fibers. The high contact surface area of fibers promoting the formation of hydrogen bonds between matrix films and CN. Enhancement of hydrogen bonds formation might have lead to a higher efficiency of the stress transfer from the matrix to the fibers (Bilbao-Sainz et al., 2011).

The removal of surface impurities on plant fibers is advantageous in fibre-matrix adhesion, as it facilitates both mecanical interlocking and the bonding reaction due to the exposure of the hydroxyl groups to film matrix (Abraham et al., 2011). Lignin is a relatively hydrophobic material, while starch is a hydrophilic polymer. Lignin removal can increase the interaction between the fiber and the matrix of starch-based films (Azeredo et al., 2012). Good interaction between cellulose fibers and starch film allowing good distribution of fibers in the film matrix (Guimaraes et al., 2010). Poor distribution of fiber can reduce the effectiveness of films reinforcement (Kengkhetkit and Amornsakchai, 2012; Savadekar and Mhaske, 2012).

In general, addition of fibers can improve the tensile strength, but lower elongation capacity (Müller et al., 2009). Elongation at break of films tend to decrease with the increase of CN content, as shown in Figure 5 (b). Reduce in elongation at break is caused by a restriction of the molecular mobility of the polymer chains due to the addition of fibers. The ability of fiber reduce water absorption of films may influence the decrease of elongation at break. Water can act as a plasticizer. Interaction between starch chains and cellulose fibers can prevent the interaction between starch chains and water, thus reducing the hygroscopic properties of starch-based films. This makes the film becomes more rigid and reduces its flexibility.

## 4. Conclusion

Cellulose nanofibres with diameters in the range of 5-8 nm and estimated lengths of several micrometers were extracted from cassava bagasse. Zeta potential measurement indicated that cellulose nanofibers suspension has good stability. FT-IR spectroscopic of cellulose nanofibers demonstrated that the chemical treatment led to partial removal of hemicelluloses and lignin from the structure of fibers. XRD results revealed that chemical treatment also improved crystallinity of fibers from 14.52% in cassava bagasse to 39,37% in cellulose nanofibers. It was observed that the addition of cellulose nanofibers improved tensile strength of films and decreased its elongation at break.

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