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Extraction of Nanocellulose from Raw Apple Stem

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Apple stem is one of the main waste biomass resources in Aomori prefecture, Japan. Using apple stem as the raw material for the extraction of nanocellulose is attractive for treating such a waste biomass, which can lower economic cost, and add value in cultivation. In this study, the apple stem was pretreated using typical cellulose extraction method, followed by acid hydrolysis of cellulose in mild condition for production of nanocellulose. The obtained product was characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TG). The results confirmed that nanocelluloses with diameters around 10-20 nm were obtained. The nanocellulose was in whisker shape with higher crystallinity, higher thermal stability at high temperature, and no obvious composition change occurred during the acid hydrolysis.

Key Words

Nanocellulose, Raw apple stem, Hemicellulose and lignin removal, Acid hydrolysis

1. Introduction

Aomori prefecture is the highest apple production area in Japan. More than 50% of apple annual production in Japan is from Aomori. Pruning the apple tree is one of the important steps for producing the delicious apple. It can decorate the apple tree by cut some branches out for allowing the sunlight reach all parts of the tree, resulting in all apples have beautiful color. Every year, apple stem, the left branches from pruning apple trees, is one of the most waste biomass resources in Aomori prefecture.

Nanocellulose is the natural biopolymer which extracted from the cellulose, the main structure of plant cell wall. Due to its nanometer size, high crystallinity, high stiffness, and good in mechanical and optical properties, it has various applications such as nanocomposites, coating additives, food packagings, and gas barriers^{1)~3)}. Selecting the waste apple stem as the raw material for the extraction of high valued nanocellulose is very attractive for the application of this unavoidable waste in local area.

Nowadays, many researchers are studying about how to use the agriculture residues as the feedstock for

nanocellulose production. Teixeira *et al.*⁴⁾ extracted the nanowhisker-type nanocellulose from sugarcane bagasse by 6 M sulfuric acid hydrolysis at 45 °C for 30 and 75 min. Their products were in the nanometer size of 255 ± 55 nm in length and 4 ± 2 nm in diameter. Santos *et al.*⁵⁾ extracted nanocellulose from pineapple leaf by 9.17 M of sulfuric acid hydrolysis at 45 °C for 30 min. Their nanocellulose were in needle-shape. Li *et al.*⁶⁾ extracted nanocellulose from the branch-barks of mulberry with 9.17 M of sulfuric acid at 60 °C for 30 min. The obtained whisker-type nanocellulose was ranged from 20 to 40 nm in diameter. However, no study is reported on extraction of nanocellulose from apple stem.

This work focus on the extraction of nanocellulose from waste apple stem by using the typical biomass pretreatment method to get cellulose at first, followed with sulfuric acid hydrolysis in mild condition for nanocellulose production. The properties of the obtained nanocellulose were compared with those of raw apple stem, holocellulose, and cellulose based on SEM, TEM, XRD, FT-IR and TG analysis results.

2. Experimental

2.1 Materials

Apple stem was collected from local plantation in Aomori prefecture, Japan. The analytical grade of sodium chlorite, acetic acid, and sodium hydroxide were used for

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biomass pretreatment while sulfuric acid (9.17 M) was used for acid hydrolysis. All chemicals were obtained from Wako Pure Chemical Industries Ltd., Japan and were used without any further purification.

2.2 Cellulose production

Raw apple stem was pretreated for the removal of hemicellulose and lignin before acid hydrolysis. It was crushed by blender and sieved to the size between 500-250 μm . Following lignin removal process by adding a buffer solution of acetic acid, sodium chlorite and distilled water in the ratio of 0.06 ml: 0.30 g: 30 ml per 1 g of raw apple stem. The process was performed using the oil bath and reflux condenser which was controlled at a temperature of 85 °C. At every 1 h, acetic acid and sodium chlorite were added to the mixture in the same ratio for 4 times. After that, the system was left at 70 °C with continuous stirring for 15 h. The mixture was washed by distilled water and centrifuged at 8500 rpm for 10 min. This process was repeated until the neutral pH value reached. Suspension was air-dried in 70 °C oven for overnight. The product obtained from this step was called "Holocellulose".

The alkali treatment was performed to purify apple stem cellulose by removing hemicellulose and the remaining lignin. The holocellulose was mixed with sodium hydroxide solution (17% wt/vol) for 1 h at room temperature with a stirring speed of 600 rpm. The suspension was washed by distilled water and centrifuged at 8500 rpm for 10 min. This process was repeated until the neutral pH value reached. Suspension was air-dried in 70 °C oven for overnight. The product obtained from this step was called "Cellulose".

2.3 Acid hydrolysis

The acid hydrolysis was performed by using 9.17 M of sulfuric acid (10 ml/g cellulose) at room temperature for 5 h. The hydrolysis was stopped by adding 20-fold cold distilled water. The suspension was centrifuged at 8500 rpm for 10 min to get the precipitates. The precipitate was suspended in distilled water, followed by centrifugation. This process was repeated until the neutral pH value reached. Subsequently, the suspension was frozen in freezer at -30 °C for overnight and then freeze-dried. The dried product was stored in vacuum for characterizations. The product obtained from this step was called "Nanocellulose".

2.4 Characterization

Surface morphologies of the samples were examined by a scanning electron microscope (SEM, SU8010, Hitachi) at an acceleration voltage of 0.5 kV. A drop of diluted product suspension was deposited on the carbon tape and

dried at 50 °C oven for 4 h. Then, sputter-coated with Pt at 15 mA for 20 s to avoid charging before measurement.

The dimensions of sample were defined by a transmission electron microscope (TEM, JEM-1400, JEOL) at an acceleration voltage of 80 kV. A drop (20 μl) of a diluted product suspension was deposited on the surface of copper grid. After 15 min waiting, the excess suspension was blotted out by filter paper. This process was repeated for 3 times, thereafter, the sample-loaded copper grid was dried at room temperature for 3 days.

XRD measurement was carried out to study the crystallinity of the sample using Rigaku Smartlab X-ray diffractometer with Cu K α radiation at 45 kV and 200 mA from 10-50° (2 θ angle range). The crystallinity of sample was calculated by peak height method. It can be calculated from the height ratio between the intensity of the crystalline peak and the total intensity after the subtraction of the background signal (non-crystalline) measured without cellulose according to equation (1)⁷⁾:

$$C (\%) = 100 \cdot \frac{I_{200} - I_{non-cr}}{I_{200}} \quad (1)$$

where C is the apparent crystallinity [%], I_{200} is the maximum intensity of the peak corresponding to the plane in the sample with the Miller indices 200 at a 2 θ angle between 22-24 degrees and I_{non-cr} is the intensity of diffraction of the non-crystalline material, which is taken at an angle of about 18° in the valley between the peaks.

The Scherrer equation was used to calculate the crystallite size, t (nm), which is determined perpendicular to the (200) planes for both cellulose I and cellulose II samples:

$$t = \frac{0.9\lambda}{\beta \cos \theta} \quad (2)$$

where λ is the radiation wavelength for Cu, θ is the diffraction angle, and β is the corrected angular width at half maximum intensity in radians⁸⁾.

Fourier transform infrared spectroscopy (FTIR) was measured using Jasco FT/IR-4200 infrared spectrophotometer at 32 scans with a resolution of 4 cm^{-1} and within the wavelength of 500-4000 cm^{-1} .

The thermal decomposition property was examined by DTA-TG apparatus (Shimadzu, DTG-60H). The amount of sample was 10 mg. The measurement was performed under a nitrogen atmosphere with a gas flow rate of 50 cm^3/min by heating the sample from room temperature to 600 °C at a heating rate of 10 °C/min. Differential thermal gravimetry (DTG) was calculated based on TGA values using a forward finite difference method as the following equation⁹⁾:

$$\text{DTG} = \frac{(w_{t+\Delta t} - w_t)}{\Delta t} \quad (3)$$

where $w_{t+\Delta t}$ and w_t are the residual weight of sample at time $t+\Delta t$ and t , respectively, and Δt is the time interval for reading residual sample weight.

3. Results and Discussion

3.1 Product yield

The weight of product from each process was recorded and calculated to obtain the yield based on 100 % of raw apple stem as the substrate, as shown in Table 1. After the biomass pretreatment and lignin removal processes, the holocellulose was obtained with 74.9 % yield. Following by the alkaline treatment for the removal of hemicellulose and the remaining of lignin, the yield of cellulose was 35.8 %. Then, nanocellulose was extracted by sulfuric acid hydrolysis of cellulose and the product yield was about 5.2 %. Here, a large amount of cellulose seemed to be hydrolyzed to soluble compositions such as sugars and acids.

3.2 SEM analysis

Fig. 1 shows SEM images of the raw apple stem (A), holocellulose (B), cellulose (C), and nanocellulose (D). The diameter of raw apple stem (Fig. 1 (A)) was around 15-50 μm with an irregular shape and rough surface. After lignin removal process, holocellulose (Fig. 1 (B)) also showed the irregular shape with more flat surface compared to the raw apple stem. After the removal of hemicellulose and the remaining parts of lignin, the diameter of cellulose (Fig. 1 (C)) reduced to around 7-12 μm and the shape became to fibril structure. This is because the removal of non-cellulosic constituents in the biomass pretreatment process. For the morphology of nanocellulose (Fig. 1 (D)), the diameter was significantly decreased from micrometer to nanometer size (12-24 nm) with the fibril shape.

3.3 TEM analysis

Fig. 2 shows TEM micrograph of the obtained nanocellulose from the apple stem. It is obvious that the nanocellulose was in whisker shape with 10-20 nm in diameter. This image supported the result obtained from SEM analysis. Therefore, it can be concluded that nanocellulose can be obtained from the apple stem cellulose by hydrolysis of it in mild condition. It should be noted that

its morphology changed greatly and the size decreased to the nanometer range.

3.4 XRD analysis

The crystallinity and crystal size was examined by XRD and the results are shown in Fig. 3. XRD patterns of raw apple stem and its holocellulose showed the significant

Fig. 1 SEM images of (A) raw apple stem, (B) holocellulose, (C) cellulose, and (D) nanocellulose

Table 1 Product yields

Product	Yield (%)
Holocellulose	74.9
Cellulose	35.8
Nanocellulose	5.2

*Compared to 100% of raw apple stem

Fig. 2 TEM image of nanocellulose

Fig. 3 XRD of (A) raw apple stem, (B) holocellulose, (C) cellulose, and (D) nanocellulose

peaks at $2\theta = 16.6^\circ$, 23° , and 35° which were assigned to the crystalline planes of 110, 200, and 004 in the crystal structure of cellulose type I allomorph¹⁰. A different main diffraction peak of cellulose and nanocellulose shown around $2\theta = 12.1^\circ$ was assigned to the crystalline planes of -110 for cellulose type II¹⁰. Moreover, it is interesting that cellulose and nanocellulose showed the doublet in the intensity of the main peaks ($2\theta = 20^\circ$ and 21.9°) which were also assigned to the crystalline planes of 110 for cellulose type II. From this result, it can be concluded that alkali treatment in biomass pretreatment process led to the change of cellulose allomorph from type I; the native cellulose found in nature, to type II; the regenerated cellulose which is the most stable crystalline form¹¹. Moreover, the acid hydrolysis which removed the amorphous region out from the cellulose led to re-crystallization was the main cause to obtain the obvious peaks of doublet intensity at $2\theta = 20^\circ$ and 21.9° and the cellulose type II allomorphs in the diffraction of nanocellulose.

The crystallinity and crystal size of all samples were tabulated in Table 2. One can see that the percentages of crystallinity and crystal sizes increased from raw apple

Table 2 Crystallinity and crystal size

Samples	Crystallinity (%)	Crystal size (nm)
Raw apple stem	51.7	3.4
Holocellulose	62.3	4.7
Cellulose	66.2	8.5
Nanocellulose	69.1	9.1

stem to nanocellulose. This is because of the removal of the non-cellulosic material from the structure of biomass in biomass pretreatment and the removal of amorphous region from cellulose in the acid hydrolysis process. Moreover, the increase in crystallinity is also expected to increase the stiffness, rigidity, and strength of the product. As a result, the potential mechanical property and the reinforcing capability of nanocellulose could be increased when compared with the raw apple stem fiber¹².

The change of cellulose allomorph and the increase in crystallinity as well as crystal size of nanocellulose was also observed by other researchers. Mandal and Chakrabarty¹³ extracted nanocellulose from waste sugarcane bagasse. Their XRD patterns were also changed from the characteristic of cellulose type I of raw sugarcane bagasse to cellulose type II in nanocellulose after the acid hydrolysis. Their crystallinity of nanocellulose was also increased.

3.5 FTIR analysis

FTIR spectroscopy presents the information of chemical changes that occur during various treatments. FTIR spectra of raw apple stem, holocellulose, cellulose, nanocellulose are respectively shown in Fig. 4. The broad peak around $3500\text{--}3200\text{ cm}^{-1}$ of all samples was the free O-H stretching vibration of the OH groups in the cellulose

Fig. 4 FTIR Spectra of (A) raw apple stem, (B) holocellulose, (C) cellulose, and (D) nanocellulose

molecules¹³⁾. The peak around 2895 cm^{-1} of all samples showed the characteristic of C-H stretching vibration¹⁴⁾. The spectra at 1730 cm^{-1} for the raw apple stem and holocellulose was attributed to the C=O stretching vibration of acetyl and uronic ester groups from hemicelluloses or the ester linkage of carboxylic group of lignin or hemicelluloses¹³⁾. This peak was only found in the raw apple stem which contains lignin, hemicellulose, and cellulose, and holocellulose which consists of hemicellulose and cellulose. The peak around 1640 cm^{-1} in all spectra correlated to the absorption of water¹²⁾¹³⁾. The spectra at 1507 cm^{-1} was associated to the aromatic C=C in lignin¹⁴⁾¹⁵⁾. Thus, this peak was only found in raw apple stem structure. The peak at 1254 cm^{-1} corresponded to the axial asymmetric strain of =C-O-C, which was commonly observed in ether, ester, and phenol groups⁵⁾.

The peak at 1061 cm^{-1} which increased in cellulose and nanocellulose, was assigned to the C-O stretching and the C-H rock vibrations of the cellulose⁵⁾. The increase of this peak in nanocellulose compared to other fibers was due to the higher cellulose content. In addition, the peak at 900 cm^{-1} which associated with the β -glycosidic linkages between glucose units in cellulose¹⁴⁾, was gradually increased in cellulose and nanocellulose, respectively. This peak also stood for the cellulose type II allomorphs and corresponded to the result from XRD and is also agreement with the result in other study¹⁴⁾.

From the results of FTIR, one can see that biomass pretreatment removed lignin and hemicellulose from the raw apple stem. Acid hydrolysis removed the amorphous region but the chemical composition had no significant change when compared to the cellulose. In other words, the cellulose molecular structure remained unchanged after the acid hydrolysis. This results are similar to other previous studies. For example, Haafiz et al. hydrolyzed microcrystalline cellulose (MCC) with 9.17 M of H_2SO_4 . Their FTIR spectra showed that the chemical groups of the resulting material were stable and no strong chemical structure change occurred¹⁶⁾. Reddy and Rhim hydrolyzed mulberry pulp with 6.73 M of H_2SO_4 . The FTIR spectra of nanocellulose also showed no distinctive change in absorption peak position when compared to mulberry fiber¹⁷⁾.

3.6 TG analysis

The thermal stability of product was analyzed by using TG and the results are shown in Fig. 5. TG curve, which shows the weight loss due to the thermal degradation in each temperature, is shown in Fig. 5(a), while the derivative weight of fiber in each temperature, DTG curve, is revealed in Fig. 5(b). The thermal

Fig. 5 TG (a) and DTG curves (b) of (A) raw apple stem, (B) holocellulose, (C) cellulose, and (D) nanocellulose

properties of all samples are tabulated in Table 3. The initial weight loss of all samples occurred from room temperature to $100\text{ }^{\circ}\text{C}$, which exhibited the evaporation of moisture because of the hydrophilic character of the lignocellulosic fibers¹²⁾. For raw apple stem, the thermal decomposition showed several steps because the differences in chemical structures between lignin, hemicellulose, and cellulose which decomposed at different temperatures. Two main degradation points were revealed: the first one started from $160\text{--}310\text{ }^{\circ}\text{C}$ with the maximum decomposition temperature at $280\text{ }^{\circ}\text{C}$ and the weight loss of 24% and the other one started from $310\text{--}400\text{ }^{\circ}\text{C}$ with the maximum decomposition temperature at $340\text{ }^{\circ}\text{C}$. These two different thermal degradations of raw apple stem implied the decompositions of lignin and cellulose, respectively. This result is similar to that of Abraham *et al.*¹⁸⁾ who reported the thermal degradation of coir fiber consisted of degradation of lignin at $270\text{ }^{\circ}\text{C}$ and α -cellulose at $335\text{ }^{\circ}\text{C}$.

For holocellulose, the remaining fibers after lignin

Table 3 Thermal Properties of all Samples

Samples	Main Thermal Degradation Range (°C)	Maximum decomposition temperature in each stage (°C)	Weight loss in each stage (%)	Residual weight (%) at 600 °C
Raw apple stem	160-310	280	24	23.1
	310-400	340	37	
Holocellulose	160-370	304	53	6.5
Cellulose	190-400	337	71	15.2
Nanocellulose	100-270	165	34	30.8
	270-450	360	22	

removal process, there was only one main decomposition temperature started from 160-370 °C with the main degradation temperature at 304 °C and the weight loss in this step was 53 %. This revealed the decomposition of cellulosic fibers; hemicellulose and cellulose, which were the main compositions in holocellulose.

For cellulose, only one significant decomposition started from 190-400 °C with the main degradation temperature at 337 °C was found with a 71 % of weight loss. This implied the decomposition of cellulose material.

Nanocellulose showed obviously different thermal degradation characteristics when compared to others. Two main gradual degradation peaks were observed: the first one started from 100-270 °C with 165 °C of the maximum decomposition temperature and a 34 % of weight loss followed by the other main degradation from 270-450 °C with 360 °C of the main degradation temperature and a 22 % of weight loss. This two-step of gradual degradation process should be related to the breakdown of the different surface structures of nanocellulose particle due to the introduction of sulfate group during acid hydrolysis process^{19) 20)}. The lower thermal degradation stage revealed the decomposition of the more accessible and highly sulfated amorphous regions while the higher thermal degradation range corresponded to the breakdown of the interior of unsulfured crystals^{6) 16)}. This result is similar to that from Haafiz *et al.*¹⁶⁾ who used sulfuric acid hydrolysis and found that nanocellulose showed the two-step thermal degradation processes at 201 °C and 490 °C. Li *et al.*⁶⁾ also used the sulfuric acid to hydrolyze the mulberry branch and the obtained nanocellulose also showed significant two different degradations at 220 °C and 335 °C.

Furthermore, it should be noted that the nanocellulose had the highest residue at 600 °C when compared to other samples. It is possible that the sulfate groups acting as the flame retardants which serving as the barrier from the burning surface to the attached polymeric chains in nanocellulose^{6) 16)}. Moreover, the higher crystallinity in nanocellulose could also improve the inherent flame resistant¹⁵⁾. From the analysis of TG, one can see that

although the thermal stability of nanocellulose decreased at the initial decomposition process, it became more stable at higher temperatures when compared to other fibers.

4. Conclusions

Raw apple stem, one of the main waste biomass resources in Aomori prefecture, can be the source of cellulose for nanocellulose extraction. With a typical biomass pretreatment way and a mild sulfuric acid hydrolysis process, the whisker shape nanocellulose with the diameter of 10-20 nm was obtained. The obtained nanocellulose had the significant morphology change, higher crystallinity, larger crystal size, and higher thermal stability at high temperatures. Moreover, from FT-IR results, there was no significant difference in chemical composition after the acid hydrolysis reaction when compared to the cellulose. It is expected that the present way could be applied for the nanocellulose production from waste biomass.

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