

Characterization of Cellulose Nanofiber from Steam-exploded Japanese Cedar

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Cellulose nanofiber (CNF) was produced from Japanese cedar using a new environmentally friendly pulping process, *i.e.* a steam explosion treatment followed by water and acetone extractions. The effect of the steam explosion treatment on the morphological, chemical, and mechanical properties of steam-exploded CNF were clarified. The increase of steam explosion severity markedly decreased the molecular weight of α -cellulose in the steam-exploded cedar pulp. The maximum tensile strength value of 88.9 MPa and Young's modulus value of 12.9 GPa were obtained for the films made from steam-exploded CNF at a steam pressure of 35 atm for a steaming time of 5 min. The results obtained in this work were useful for developing a new production method of CNF from various steam-exploded woody biomass.

Keywords: Cellulose nanofiber; Japanese cedar; Structural properties; Mechanical properties; Steam explosion pretreatment

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INTRODUCTION

In recent years, woody fibers are one of the most widely used in various industries because of their outstanding properties. The main constituent of woody fibers, cellulose, particularly at the nano-size, has attracted considerable attention in various fields, especially to reinforce polymer composites (Yusra *et al.* 2015). Nano-size woody fibers are called cellulose nanofiber (CNF). The CNF is a material composed of nano-sized cellulose fibrils with a high aspect ratio (length to width ratio). Typical lateral dimensions are 5 nm to 20 nm and the longitudinal dimension covers a wide range, typically several micrometers.

Some of the important characteristics of cellulose that make it more applicable in the field of nanofibers are that it is hydrophilic and easily subjected to chemical modifications. These inherent features make cellulose an attractive replacement for plastics used as composite fillers or reinforcement. Different techniques have been explored for producing cellulosic nanofibers in the last few decades. These techniques would further explore the potential applications of cellulosic nanofibers in the realm of food science and identify their potential risks. The most important applications of cellulosic nanofibers in food science are: (a) immobilization of bioactive substances, such as enzymes, vitamins, and antimicrobials; (b) nutraceutical delivery systems and controlled release of materials; (c) biosensors; (d) filtration; and (e) reinforcing composites and films (Rezaei *et al.* 2015). The application of cellulose nanofiber ranges from nano to macro components. A polymer composed of cellulose nanofibers, whose functional properties are determined by the fibril structure, is called nanocellulose.

Cellulose nanofibers have some of the most desirable features, such as higher

strength and lighter weight. Despite being lighter than steel, crystalline cellulose has 8 times higher strength than steel. Apart from microorganisms as a source of cellulose production, a vast array of biomass types ranging from agricultural residues to forest resources have been employed to isolate cellulose. Different renewable resources such as wood, rice straw, and potato tubers have been utilized to prepare cellulose nanofibers, and research on their detailed characteristics has been conducted (Abe and Yano 2009). Likewise, various agricultural residues, including wheat straw and soy hull, were utilized to isolate cellulose (Alemdar and Sain 2008). Banana fibers and curaua fibers were used to isolate CNFs (Cherian *et al.* 2008; Corrêa *et al.* 2010). Sugar beet pulp was used for CNF preparation (Li *et al.* 2014). These studies imply that the popularity of cellulose utilization is increasing tremendously, while the renewable resources are being explored to isolate cellulose. Japanese cedar, which has a natural distribution between Mt. Yagura, Aomori prefecture, and Yaku Island, Kagoshima prefecture, is the most important coniferous tree in Japan. Artificial planting of Japanese cedar was brought into practice more than 500 years ago and conducted intensively after World War II. The cedar covers 40% of the total cultivated area of Japan, representing 12% of Japanese forests and they are now sufficiently mature to be utilized for research (Hirooka *et al.* 2013; Asada *et al.* 2015b). Therefore, it is desirable to make more use of these resources for not only manufacturing lumber, but also using a substrate for biofuel and green material production.

For the preparation of cellulose as a raw material of cellulose nanofiber, it is necessary to degrade and remove not only hemicellulose, but also lignin in wood biomass as a pretreatment. Among various pretreatment methods, steam explosion only uses water/steam at high temperatures, causing the formation of acetic acid from the acetylated hemicellulose component, thus catalyzing hydrolytic reactions in the wood components (Garrote *et al.* 1999; Martin-Sampedro *et al.* 2011). It follows that this pretreatment can be considered as an environmentally friendly method, because the reaction product contains only wood-derived components and water that generally do not lead to significant corrosion problems and the formation of neutralization sludges (Martin-Sampedro *et al.* 2014).

In this study, a new environmentally friendly pulping process for cellulose nanofiber production was developed using a steam explosion. Japanese cedar wood chips, a major woody biomass in Japan, were used to produce cellulose nanofiber. The effect of steam pressure on the chemical and mechanical characteristics of cellulose nanofiber obtained from steam-exploded cedar was evaluated. This will not only open numerous possibilities for future research on cellulose nanofiber production from steam-exploded woody biomass, but also fill a void in present research.

EXPERIMENTAL

Materials

Japanese cedar wood, *i.e.* *Cryptomeria japonica* D. Don, was chopped into small fragments with approximately 2 cm to 4 cm in length and 1 cm to 3 cm in width. Raw cedar was ground by a crusher mill (Wonder Crush Mill D3V-10, Osaka Chemical Co., Ltd.) at 15,000 rpm for 1 min and observed by field-emission scanning electron microscopy (FE-SEM).

The cedar chips were treated using various steam explosion conditions to allow delignification and used as a raw material for fibrillation.

All chemicals were purchased from Nacalai Tesque Co., Ltd. (Kyoto, Japan) and were of analytical grade.

Preparation of cellulose for CNF- Steam explosion

Steam explosion was performed in a batch system with a 2-L reactor (steam explosion apparatus NK-2L; Japan Chemical Engineering and Machinery Co. Ltd., Osaka, Japan) (Asada *et al.* 2012). Then, 200 g of cedar chips were introduced into the reactor and exposed to saturated steam at a pressure of 30 atm (234 °C), 35 atm (243 °C), and 40 atm (250 °C) for a steaming time of 5 min and 15 min. The prescribed temperature was reached in a few seconds. After exposure to the saturated steam, a ball valve at the bottom of the reactor was opened suddenly to bring the reactor rapidly to atmospheric pressure, thereby obtaining the product of liquid and solid materials, *i.e.*, the steam-exploded cedar.

Extraction and separation

The steam-exploded cedar was extracted with distilled water 60 times at room temperature for 24 h and separated into water-soluble material and residue using filter paper (No. 131, 185 mm, Advantec Co., Ltd., Tokyo, Japan). Next, the residue was extracted with acetone 30 times at room temperature for 24 h and then the residue after acetone extraction was separated using filter paper (No. 131, 185 mm, Advantec Co., Ltd., Tokyo, Japan).

Bleach treatment

The 25 g of residue after water and acetone extractions, *i.e.* Japanese cedar pulp, was suspended in 1.5 L distilled water, and the following treatment was applied. To remove residual lignin in the residue, 10 g NaClO₂ was directly added to the suspended solution described above with gentle mixing, and then 2 mL acetic acid was subsequently added. The suspended mixture was reacted at 80 °C for 1 h. Furthermore, the addition of 10 g NaClO₂ and 2 mL acetic acid was repeated 4 times in 1 h intervals. Finally, the mixture was filtered using filter paper (No. 131, 185 mm, Advantec Co., Ltd., Tokyo, Japan), and the solid fraction was rinsed 5 times with distilled water. The rinsed sample, *i.e.* sample after bleach treatment, was maintained in a semi-wet state at 4 °C until its use.

Alkaline treatment

To remove hemicellulose completely from the sample after bleach treatment, 1 g of the sample was soaked with 25 mL of 17.5 wt.% NaOH (aq) at room temperature for 30 min. Next, 25 mL distilled water was added and stirred for 6 min. Finally, the mixture was filtered using glass fiber filter paper (GFD2-854-08, 5.5 cm, ASONE Co., Ltd., Osaka, Japan), and the solid fraction was rinsed 5 times with distilled water and neutralized with 10% acetic acid. The rinsed sample, *i.e.* α-cellulose, was maintained in a semi-wet state at 4 °C until its use.

Preparation and characterization of CNF- Grinder treatment

A 10 g sample after the bleach treatment was suspended with 1 L distilled water and passed twice through a grinder (MKCCA6-2, Masuko Sangyo Co., Ltd., Saitama, Japan) at 1500 rpm.

Production of CNF sheet

After the grinder treatment of the sample described above, a CNF sheet was obtained by vacuum filtration using a glass fiber filter paper (GF-75, 110 mm, Advantec Co., Ltd., Tokyo, Japan). Then, each CNF sheet in a semi-wet state was vacuum-dried at 50 °C overnight.

Methods

Component analysis

The components, *i.e.* water soluble material, acetone soluble material (a low molecular weight lignin), NaClO₂ soluble material (a high molecular weight lignin), hemicelluloses, and cellulose, in the steam-exploded cedar were separated and measured by the following procedure, with modifications according to Wayman's extraction method (Chua and Wayman 1979). Then, 5 g of dry steam-exploded cedar were added to 100 mL of distilled water and extracted for 24 h at room temperature. The solid and liquid materials were separated by filtration, and the filtrate, *i.e.* water soluble material, was recovered from the liquid. The residue after water extraction was extracted with 150 mL acetone for 24 h at room temperature to dissolve an extract, *i.e.* acetone soluble material. After concentration and drying of the extract, the acetone soluble material was weighed. Residue after acetone extraction consisted holocellulose (cellulose and hemicellulose) and residual lignin. Approximately 150 mL of 1 g NaClO₂ was added to this residue (2.5 g) with gentle mixing and then 0.2 mL acetic acid was subsequently added. The suspended mixture was reacted at 80 °C for 1 h. Furthermore, the addition of 1 g NaClO₂ and 0.2 mL acetic acid was repeated 4 times in 1 h intervals for removing the NaClO₂ soluble material. After the solid fraction was rinsed 5 times with distilled water and dried, the residue, *i.e.* holocellulose, which was defined as a steam-exploded cedar pulp in this work, was weighed.

Next, 1 g of this residue was soaked with 25 mL of 17.5 wt.% NaOH (aq) at room temperature for 30 min and then added to 25 mL distilled water with stirring for 6 min. The solid fraction was rinsed 5 times with distilled water and neutralized with 10% acetic acid. After drying, the residue, *i.e.* α-cellulose, was weighed.

Fourier transform infrared (FTIR) spectroscopy

Changes in the functional groups of the steam-exploded cedar after various extraction and separation treatments were recorded by FTIR spectrometry (FT/IR-670 Plus; JASCO, Tokyo, Japan). First, the samples were ground and dried at 105 °C. The sample (1.5 mg) was mixed with 200 mg potassium bromide (KBr). The role of KBr was to hold the fiber flour during the test. Transparent pellets were prepared from the blend and analyzed from 400 cm⁻¹ to 4000 cm⁻¹.

Molecular weight measurement

To measure the molecular weight of the samples, 250 mg of the sample was soaked with 25 mL distilled water and stirred at room temperature for 1 h. Next, 25 mL of copper ethylene diamine solution (CEDs) was added and stirred for 30 min. The temperature of the water bath was maintained at 25 °C ± 0.1 °C. According to JIS P8215 (1998), the molecular weight of the samples was measured.

FE-SEM Analysis

Cellulose nanofiber was observed using a FE-SEM (6400F, Hitachi, Tokyo, Japan), operating at 1.6 kV and a working distance of 8 mm. A small piece of the CNF mat was fixed on carbon tape and then sputtered with Pt (E-1020 Ion Sputter, Hitachi, Tokyo, Japan).

Mechanical properties

The tensile modulus and strength of the samples was measured using a tensile tester (Dual Column Series for Mechanical Testing 5667, INSTRON Japan Co. Ltd., Kamasaki, Japan) at room temperature. The dimensions of the samples were 60 mm × 10 mm × 1 mm. The gauge length was 30 mm, and a testing speed of 1.0 mm/min was applied for the test.

For each sample, five repetitions were performed, and the average of five tests was reported.

RESULTS AND DISCUSSION

Chemical Compositions of Steam-exploded Japanese Cedar

To prepare the cellulose material from Japanese cedar, a pretreatment of steam explosion was performed. Four steam explosion conditions, steam pressures of 30 atm, 35 atm, and 40 atm for a steaming time of 5 min, and a steam pressure of 35 atm and for a steaming time of 15 min were evaluated. Figure 1 shows the chemical composition analysis of the steam-exploded Japanese cedar.

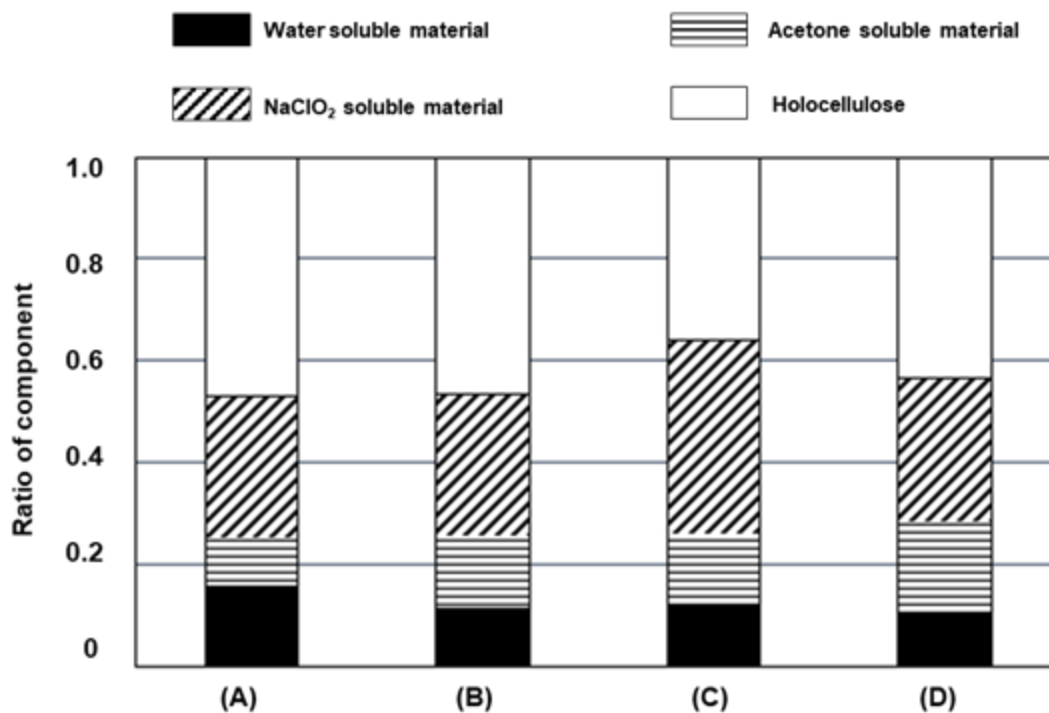


Fig. 1. Ratio of component to dry weight of steam-exploded Japanese cedar treated at 30 atm for 5 min (A), 35 atm for 5 min (B), 35 atm for 15 min (C), and 40 atm for 5 min (D)

Through the steam explosion, glycosidic- and hemicellulose-lignin bonds in the wood are hydrolyzed, and the depolymerization reaction of lignin occurs, resulting in the production of a water soluble material, *i.e.* sugars and organic acids, and an organosolv material, *i.e.* a low molecular weight lignin (Li and Gellerstedt 2007; Asada *et al.* 2012). Higher composition ratios of holocellulose (hemicellulose and cellulose), *i.e.* 0.46 and 0.47, respectively, were obtained at 30 atm and 35 atm for 5 min. A similar value of holocellulose, *i.e.* 0.43, was obtained at 40 atm for 5 min. In contrast, a minimum value of holocellulose, *i.e.* 0.38, was observed at 35 atm for 15 min due to its longer steaming time. With cellulose degradation, the structure of cedar fiber was destroyed, which caused a decrease in the mechanical properties, which is not desired for the textile industry. In general, hemicellulose and cellulose decompose into sugars and more decomposed materials such as furan derivatives and organic acids through severe treatment conditions, *i.e.*, long time, high temperature, and high concentration of acid (Palmqvist and Hahn-Hagerdal 2000). Moreover, the decomposed materials polymerized with high molecular lignin at the severe treatment conditions, *i.e.* (C) and (D), which caused an increase in the amount of NaClO₂ soluble material.

Because acetone-soluble material mainly contains a low molecular weight lignin derived from a high molecular weight lignin, Asada *et al.* (2015a) mentioned that an acetone-soluble material, *i.e.* a low molecular weight lignin, from steam-exploded wood could be a useful resource to synthesize epoxy resin. The use of epoxy resin as coatings, electrical or electronic laminates, adhesives, flooring, and paving shows the application versatility of epoxy resin. The highest composition ratio of acetone soluble material, *i.e.* 0.18, was observed at 40 atm for 5 min. A similar value, *i.e.* 0.14, was obtained at 35 atm for 5 and 15 min. However, only 0.08 of acetone-soluble material was observed at 30 atm for 5 min. Therefore, considering the effective utilization of not only cellulose but also lignin, it suggested that 35 atm for 5 min was the most optimal condition because it contained high amounts of holocellulose, a raw material for CNF, and an acetone-soluble material, a raw material for epoxy resin.

FTIR Analysis

Changes in functional groups of steam-exploded Japanese cedar by water extraction, acetone extraction, and NaClO₂ treatment were revealed by FTIR analysis. Figure 2 shows the FTIR spectra of residue after water extraction (a), residue after acetone extraction (b), residue after NaClO₂ treatment (c) of steam-exploded Japanese cedar at 35 atm for 15 min, and (d) microcrystalline cellulose powder (Alfa Aesar). Table 1 shows the assignments of FTIR absorption bands. The peaks at 2, 3, 5, and 7 ascribed to lignin structure were observed in the residues after water and acetone extractions, but they disappeared in the residue after NaClO₂ treatment. This suggests that the acetone extraction cannot remove high molecular weight lignin, but the NaClO₂ treatment can degrade and remove lignin completely (Sasaki *et al.* 2016).

The intensity of the peaks at 1, 4, and 6, which relate to O-H bending, and the stretching bands of C-O and C-H in the cellulose and hemicellulose, increased due to the removal of lignin that covered the cellulose and hemicellulose strongly. Furthermore, the peaks of residue after NaClO₂ treatment were similar to those of microcrystalline cellulose powder, a standard cellulose compound. As a result, it was found that the bleach treatment removed impurities from the cellulose.

Table 1. FTIR Adsorption Bands

| Number | Peak Location (cm ⁻¹) | Assignments of IR Absorption Bands |
|--------|-----------------------------------|---|
| ① | 1635 | O-H bending of absorbed water |
| ② | 1604 to 1609 | Aromatic skeleton vibration, and C=O stretching |
| ③ | 1512 to 1513 | Aromatic skeleton vibration |
| ④ | 1282 | C-H stretching |
| ⑤ | 1271 | C-O stretching |
| ⑥ | 1235 | C-O stretching |
| ⑦ | 857 | C-H out of plane |

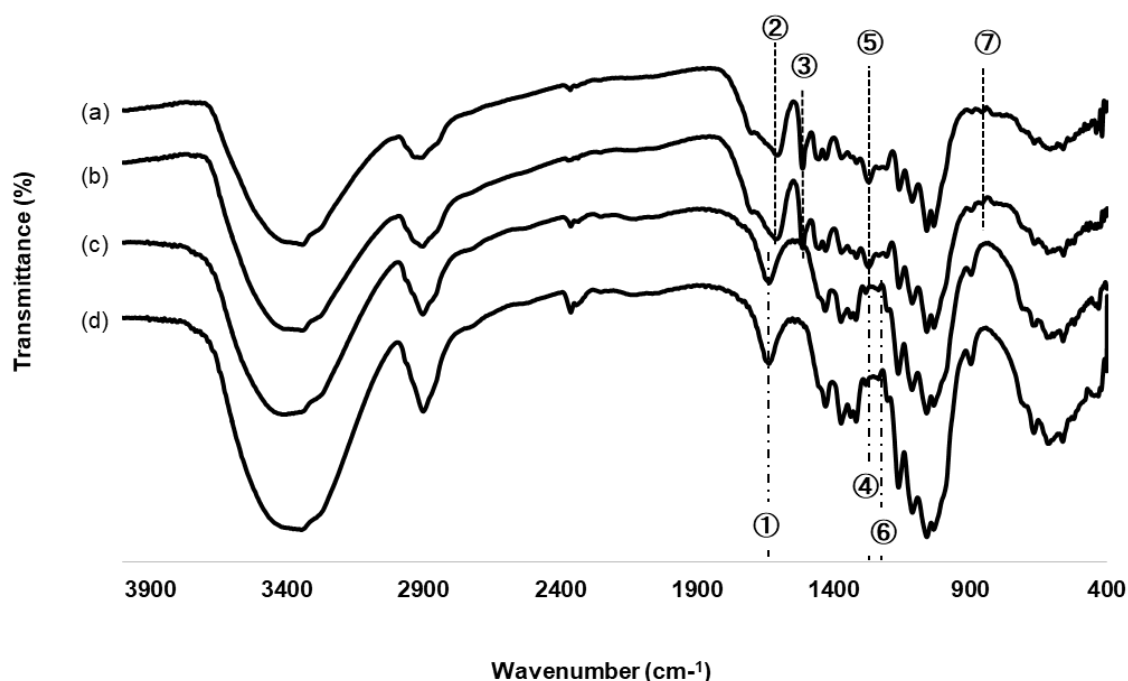


Fig. 2. FTIR spectra of residue after water extraction (a), residue after acetone extraction (b), residue after NaClO₂ treatment (c) of steam-exploded Japanese cedar at 35 atm for 15 min, and microcrystalline cellulose powder (Alfa Aesar) (d)

Molecular Weight Measurement

Figure 3 shows the degree of polymerization of α -cellulose in the steam-exploded cedar pulp. With the increase of steam explosion severity, the degree of polymerization decreased substantially from 387 at a steam pressure of 30 atm for 5 min to 119 at a steam pressure of 40 atm for 5 min.

The molecular weight of cellulose was calculated by the degree of polymerization \times 162 (Ryu *et al.* 1982); therefore, the lowest molecular weight, approximately 20,000, in this study was obtained at a steam pressure of 40 atm for 5 min.

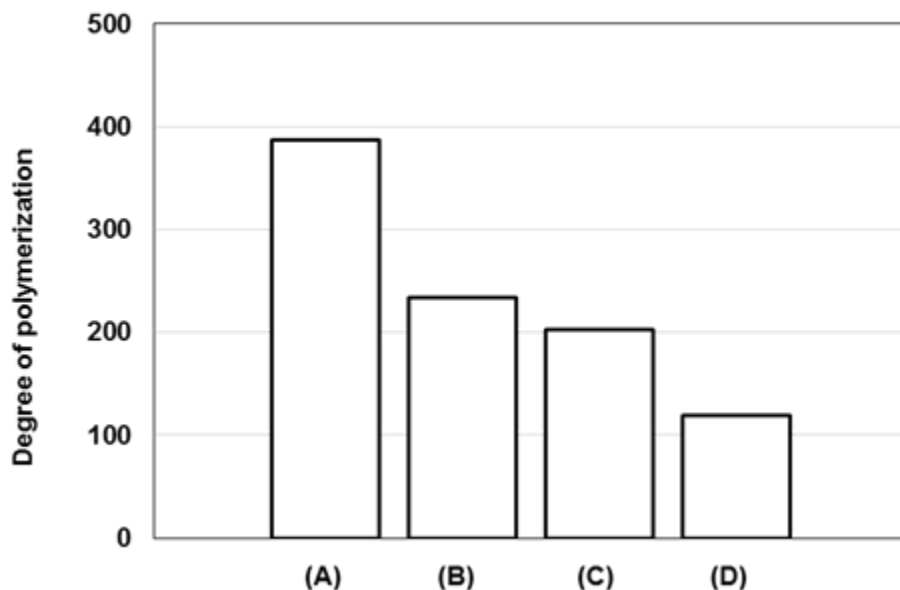


Fig. 3. Degree of polymerization of α -cellulose in steam-exploded Japanese cedar pulp treated at 30 atm for 5 min (A), 35 atm for 5 min (B), 35 atm for 15 min (C), and 40 atm for 5 min (D)

FE-SEM Analysis

The surface structure of the steam-exploded fiber after bleach treatment provided fiber behavior in subsequent applications, including the interactions between the fiber and polymer matrix in composite materials (Ramesh *et al.* 2015; Zhang *et al.* 2016). A field-emission scanning electron microscope was used to investigate the change of surface structure in the raw cedar (Fig. 4) and steam-exploded cedar after water and acetone extractions followed by bleach treatment (Fig. 5). Figure 4 shows the rough and linty surface of raw cedar that was ground by a crusher mill at 15,000 rpm for 1 min.

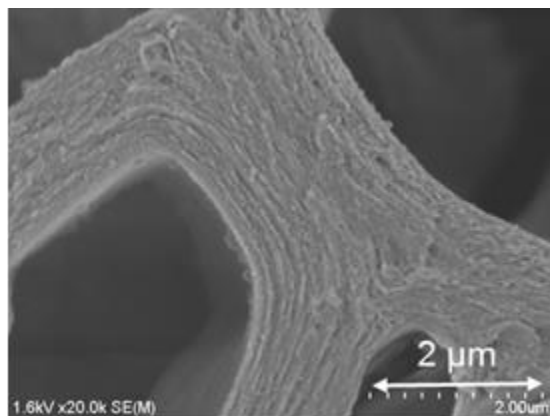


Fig. 4. FE-SEM of milled Japanese cedar

As illustrated in Fig. 5, the bleached cedar pulps had a relatively smooth and lint-free surface, due to the removal of impurity compounds by steam explosion. The increase of steam pressure and steaming time remarkably promoted the fibrillization of pulp, which resulted in the production of a large amount of small fibers. From FE-SEM imaging, it was clear that as fibrillation continued to increase in steam pressure and steaming time, there

was an increase in Japanese cedar pulp disintegrated to smaller fibers. Furthermore, it was found that the pulp at 40 atm was finely fibrillated even by the steam explosion treatment alone and some cellulose nanofibers were observed without the grinder treatment.

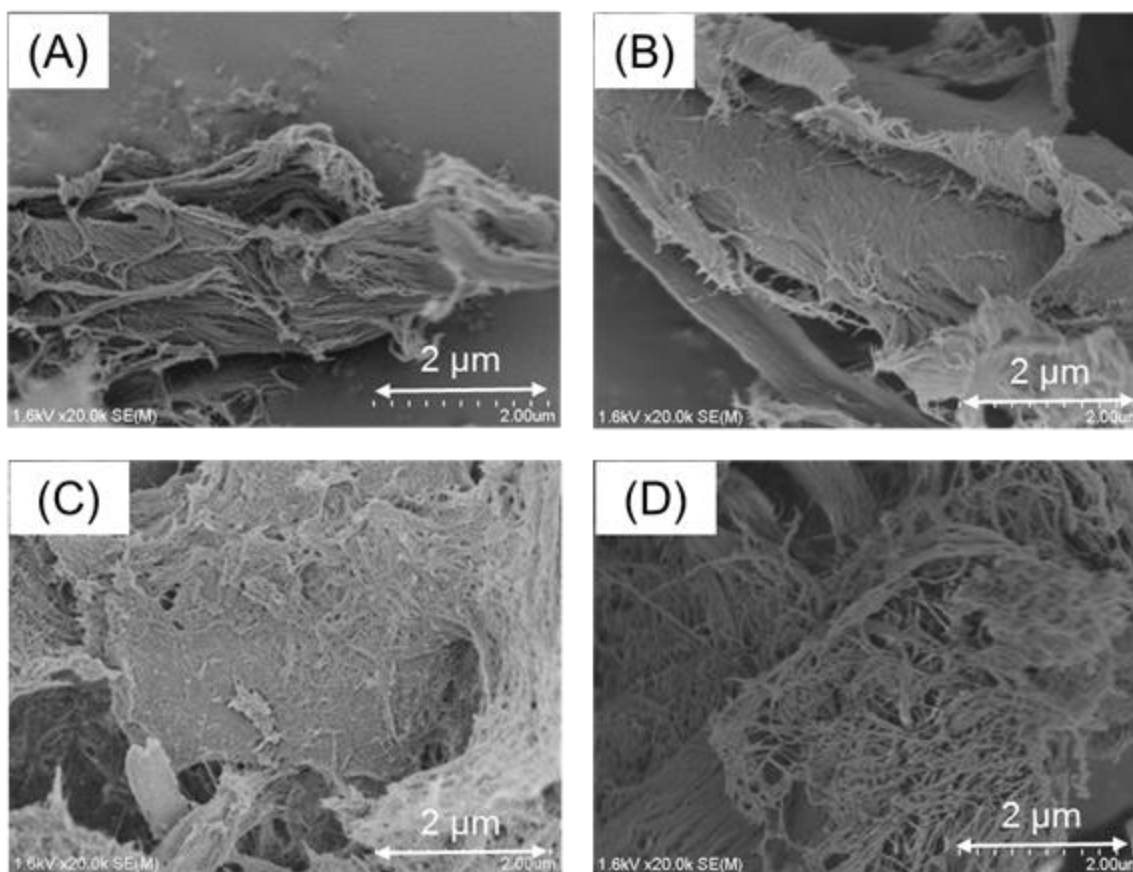


Fig. 5. FE-SEM of Japanese cedar pulp treated at 30 atm for 5 min (A), 35 atm for 5 min (B), 35 atm for 15 min (C), and 40 atm for 5 min (D)

Figure 6 shows the field-emission scanning electron micrographs of the cellulose nanofiber from bleached cedar pulp with the grinder treatment. Regardless of the steam explosion condition, cellulose nanofiber was obtained with the grinder treatment. The width of cellulose nanofiber decreased with the increase of steam pressure and steaming time. Though the cellulose nanofiber at 30 atm for 5 min had some large nanofibers, those beyond 30 atm had comparatively smaller nanofibers. Compared with the commercial cellulose nanofiber (BiNF-i-s WMa-10002, Sugino Machine Ltd.), similar morphological cellulose nanofibers were obtained from the steam-exploded cedar pulp.

Strength Properties of CNF

The tensile strength and young's modulus of CNF sheets obtained from Japanese cedar pulp ranged from 55.5 MPa to 88.9 MPa and 7.9 GPa to 13.6 GPa, respectively (Fig. 7). The maximum tensile strength and young's modulus, *i.e.* 88.9 MPa and 12.9 GPa, were obtained at 35 atm for 5 min, similar result 80.3 MPa (tensile strength) and 13.6 GPa (Young's modulus) were obtained at 35 atm for 15 min.

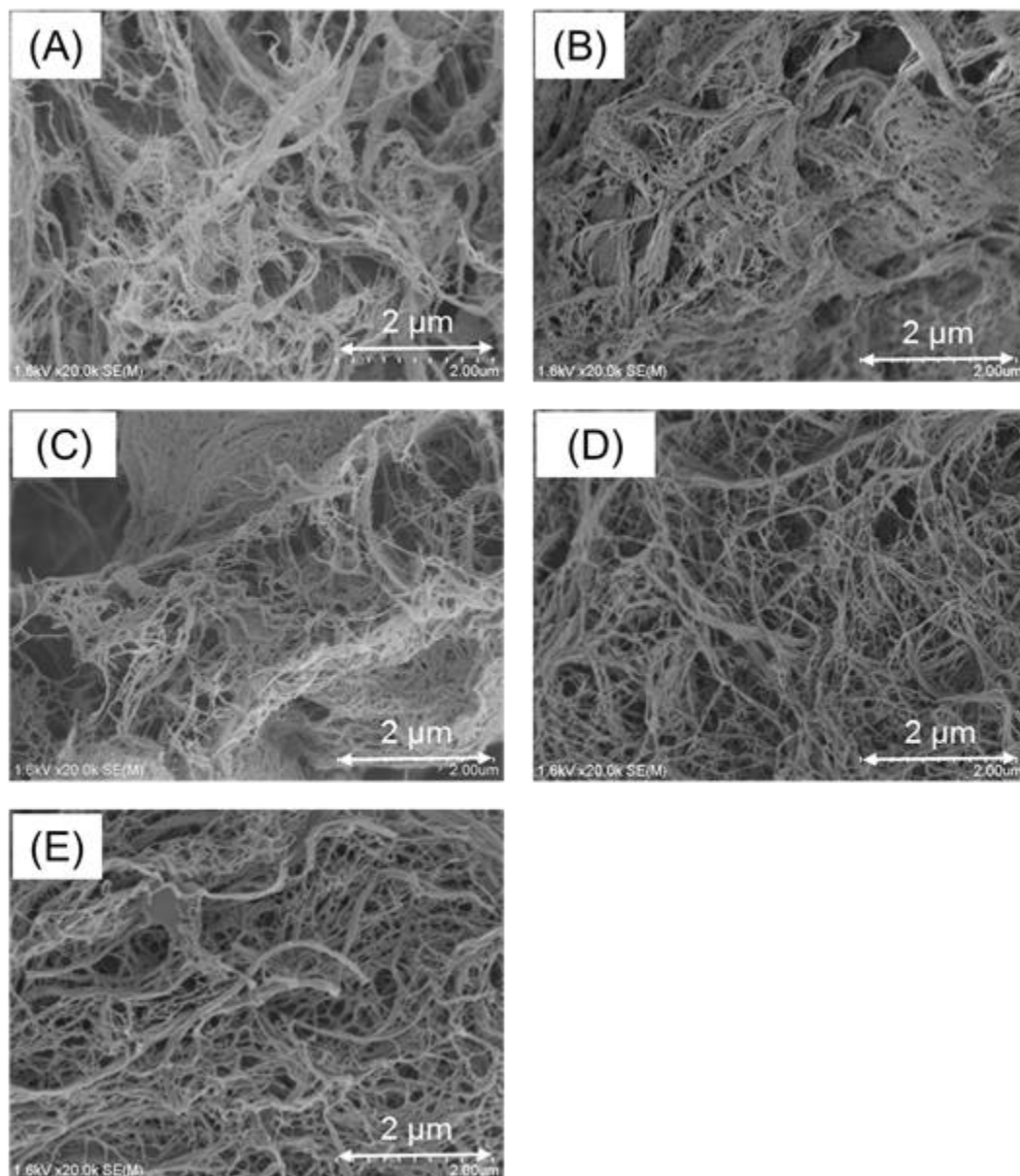


Fig. 6. FE-SEM of cellulose nanofiber obtained from Japanese cedar pulp treated at 30 atm for 5 min (A), 35 atm for 5 min (B), 35 atm for 15 min (C), 40 atm for 5 min (D), and commercial cellulose nanofiber (BiNFi-s WMa-10002, Sugino Machine Ltd.) (E)

The density of the CNF sheet (35 atm for 5 min) was 1.39 g/cm^3 ; similar densities were obtained for all the conditions. The minimum tensile strength and Young's modulus, *i.e.* 55.5 MPa and 7.9 GPa, were obtained at 40 atm for 5 min. Nair and Yan (2015) reported that the maximum tensile strength and Young's modulus, *i.e.* 76 MPa and 15 GPa, were obtained for the films made from fibrillated CNFs obtained by 5 passes of the grinder treatment of milled and NaOH-treated lodgepole pine bark. The grinder setup, number of samples, and solid consistency used for their work was similar to that used in this work. However, in the current experiment almost the same maximum mechanical properties were obtained within 2 passes through the grinder treatment. In this work, the reason why the tensile strength and Young's modulus of CNF at 30 atm for 5 min were low seemed to be that the fibrillation was not carried out completely by the grinder treatment with 2 passes,

as shown in Fig. 6. Although it is necessary for obtaining a strong CNF to increase the number of passes in the grinder treatment, it requires a high-energy consumption (Spence *et al.* 2011). Therefore, it was found that the steam explosion at 35 atm for 5 min to 15 min was adequate to obtain strong CNF from Japanese cedar and conserve energy. In contrast, the reason why the tensile strength and Young's modulus of CNF at 40 atm for 5 min were lower than those at 35 atm for 5 min to 15 min was that the degree of polymerization of cellulose at 40 atm for 5 min was too small, *i.e.* 119, which resulted in the weak strength (Fig. 3). Compared to commercial CNFs, *i.e.* a comparatively high tensile strength with a comparatively low Young's modulus CNF (BiNFi-s WMa-10002) and a comparatively low tensile strength with a comparatively high Young's modulus CNF (BiNFi-s FMa-10002), the mechanical properties of CNF obtained from steam-exploded Japanese cedar pulp at 35 atm for 5 min to 15 min almost corresponded with those of BiNFi-s FMa-10002. This meant that steam-exploded CNF can be used for a practical applications.

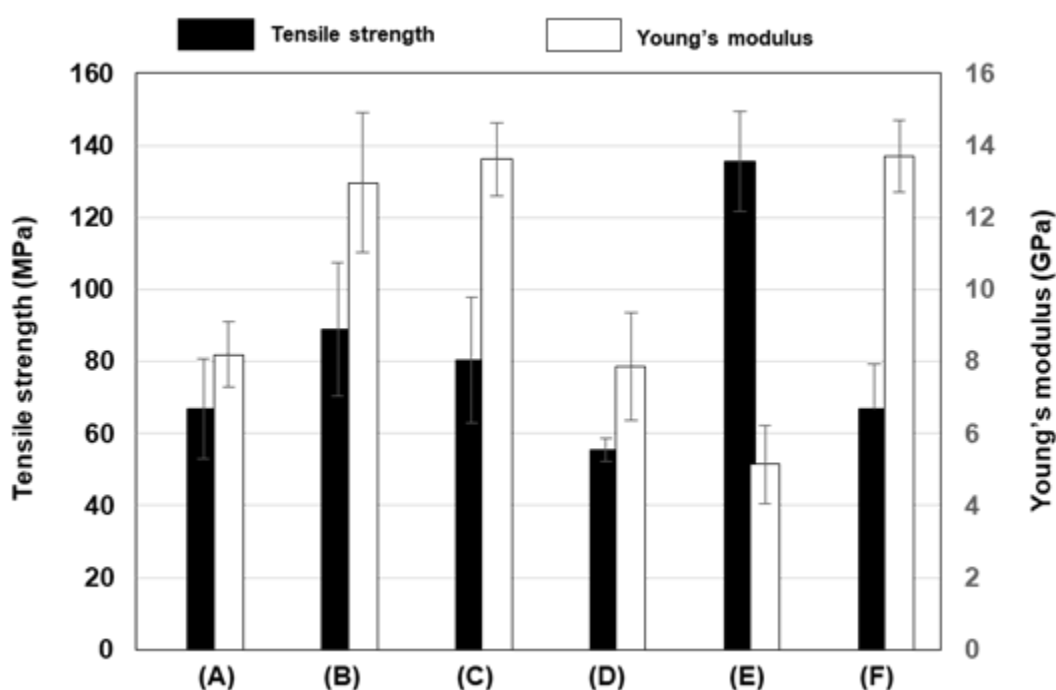


Fig. 7. Tensile strength and Young's modulus of CNF sheet obtained from Japanese cedar pulp treated at 30 atm for 5 min (A), 35 atm for 5 min (B), 35 atm for 15 min (C), 40 atm for 5 min (D), BiNFi-s WMa-10002 (E), and BiNFi-s FMa-10002 (F)

CONCLUSIONS

1. Japanese cedar was treated by steam explosion with different pressures and times. The effect of steam explosion on the morphological and chemical characteristics of steam-exploded products was evaluated.
2. With the increase of steam explosion severity, the molecular weight of α -cellulose in the steam-exploded cedar pulp noticeably decreased.
3. Cellulose nanofiber was produced from steam-exploded Japanese cedar pulp after water and acetone extractions, and a NaClO_2 treatment.

4. The maximum tensile strength and young's modulus values, *i.e.* 88.9 MPa and 12.9 GPa, were obtained for the CNF made from steam-exploded cedar at 35 atm for 5 min.
5. The results obtained in this work provide a better understanding of the production of CNF from steam-exploded softwood biomass. In case of herbaceous plants and hardwoods, the results are also valuable with further consideration due to their difference of lignin structure and component ratio.

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