Construction of comprehensive effective utilization method of biomass constituent using high temperature and high pressure steam treatment (バイオマス構成成分の高温高圧水蒸気処理を 用いた総合的有効利用法の構築)

2019年 3月

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Chapter 1: Characterization of Cellulose Nanofiber from Steamexploded Japanese Cedar

1.1. 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 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 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.

1.2. Materials and Methods

1.2.1. 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.

1.2.2. 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.

1.2.3. 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).

1.2.4. 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.

1.2.5. 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.

1.2.6. 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.

1.2.7. 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.

1.2.8. 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 weighted.

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.

1.2.9. 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.

1.2.10. 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 \pm 0.1 °C. According to JIS P8215 (1998), the molecular weight of the samples was measured.

1.2.11. 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).

1.2.12. 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 \times 10 mm \times 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.

1.3. Results and Discussion

1.3.1. 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.

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.

1.3.2. 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.

1.3.3. 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.

1.3.4. 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.

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.

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 (BiNFi-s WMa-10002, Sugino Machine Ltd.), similar morphological cellulose nanofibers were obtained from the steam-exploded cedar pulp.

1.3.5. 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.

The density of the CNF sheet (35 atm for 5 min) was 1.39 g/cm3; 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.

1.3.6. Figures and Tables

1.3.6.1.



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)

1.3.6.2.

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

Table 1. FTIR Adsorption Bands

1.3.6.3.



Wavenumber (cm-1)

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)

1.3.6.4.



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)





Fig. 4. FE-SEM of milled Japanese cedar





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)

1.3.6.7.



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)

1.3.6.8.



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)

1.4. Conclusion

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 steamexploded cedar pulp noticeably decreased.

3: Cellulose nanofiber was produced from steam-exploded Japanese cedar pulp after water and acetone extractions, and a NaClO2 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.

1.5. Acknowledgment

The authors are grateful for the partial support of a Grant-in-Aid for Scientific Research (A) (Grant No. 16H01790) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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Article submitted: May 8, 2017; Peer review completed: August 12, 2017; Revised version received and accepted: August 23, 2017; Published:

Chapter 2: Production of cellulose nanofibers from Aspen and Bode chopsticks using a high temperature and high pressure steam treatment combined with milling

2.1. Introduction

Recently, many plant-derived renewable materials have been reported (Cheng, Yuan, Anderson, Leitch, & Xu, 2012). Resins, such as polylactic acid and cellulose acetate obtained by the condensation polymerization of lactic acid (Zhang, Jomes, Hedrick, & Waymouth, 2016) and the acetylation reaction of cellulose (Malm, Tanghe, & Laird, 1946), respectively, have become available. However, these resins still suffer from disadvantages in terms of mechanical strength. To compensate for this limitation, the fabrication of composites has been investigated by mixing them with various fibers (Yusoff, Takagi, & Nakagaito, 2016), such as glass, carbon (Li, Cai, & Huang, 2017), and plant fibers (Takagi, Liu, Osugi, Nakagaito, & Yang, 2012). The process of mixing fibers with a resin not only aids in improving the strength of the resin but also in raising the biomass content and reducing the amount of fossil resource usage (Oliveira, Enjalbal, Frollini, & Castellan, 2008; Saba et al., 2017b). Among these reinforced fibers, cellulose nanofibers (CNF) have been attracting particular attention in recent years (Abdulkhalil, Bhat, & Yusra, 2012). CNF are nanosized fibrillated cellulose fibers that possess a high strength and low thermal expansion (Abdulkhalil et al., 2012; Yano & Nakahara, 2004). Especially with high strength, it has been reported that nanofiber from Logepole pike bark has high specific tensile strength, 66.2 MPa/(g/cm3) (Nair et al., 2015).

Both chemical and physical methods are available for fabricating CNF. In the chemical methods, cellulose is decomposed into nanosized fragments by a reagent and converted into short length CNF (Dang, Zhang, & Ragauskas, 2007; Isogai, Saito, & Fukuzumi, 2011). Therefore, the CNF produced by these methods have the advantage of mixing easily with resins. The physical methods are based on fibrillation by a grinder using a homogenizer or an electric millstone. This approach yields longer fibers than those obtained by chemical methods, so that high strength fibers can be obtained. Furthermore, the physical methods are environmentally friendly, because only water (without any additional chemicals) is used during the processing (Iwatake, Nogi, & Yano, 2008). The steaming method with high pressure and high temperature is also a chemical- free method

(no reagents) since it only uses hot steam, and represents one of the thermochemical pretreatment methods for woody and herbaceous biomass. Organic acids such as levulinic acid, formic acid, and acetic acid produced from the biomass during steaming behave like catalysts for the hydrolysis of polysaccharides and the decomposition of lignin (Sasaki et al., 2013).

The steam treatment to obtain CNF from woody and herbaceous materials is a method with a low environmental load. By using this method, pulping (delignification) is facilitated and cellulose fibers are obtained. The water-soluble component (Kurosumi et al., 2007) and the organic solvent soluble component (Asada, Basnet, Otsuka, Sasaki, & Nakamura, 2015) obtained after steam treatment can be used for other applications. Therefore, since each separated component can be effectively used, total utilization of the resources can be achieved.

In this study, we used steam pressures of 10, 15, 20, and 25 atm for steam treatment of chopsticks. Plant biomass contains not only cellulose (and hemicellulose) but also lignin, therefore, it is necessary to delignificate simultaneously with fibrillation. Mentioned above, steaming treatment is effective for delignification, we used high pressure and high temperature treatment method before fibrillation by milling method.

CNF were generated from discarded chopsticks made of Aspen (Populus tremuloides) and Bode (Styrax tonkinensis) using high pressure and high temperature steam treatment. In Japan, about 90,000 tons per year of disposable chopsticks are used and discarded (Asada, Kita, Sasaki, & Nakamura, 2011). Currently, discarded chopsticks are burned with other domestic burnable garbage. And, there is no utilization method. The materials comprising the disposable chopsticks are hardwoods such as Aspen and Bode. Hardwoods contain a lower amount of lignin than softwoods (Asada, Sasaki, Hirano, & Nakamura, 2015). To achieve efficient fiber production, a milling treatment was necessary. Therefore, a milling step was added to the treatment process after the steaming treatment. The fundamental characteristics of the produced CNF were investigated, such as the molecular weight and mechanical properties (tensile strength), and field-emission scanning electron microscopy (FE-SEM) images of the CNF surface were also obtained. Finally, we evaluated whether the optimal conditions required by the steam treatment method combined with milling to fabricate CNF could be of practical use.

2.2. Materials and Methods

2.2.1. Materials

Chopsticks were purchased in Tokushima city, Japan. Chopsticks of white poplar (Populus tremuloides) and Tonkin snowbell (Styrax tonkinensis Craib ex Hartwich) were divided into ingredients. The common names of white poplar and Tonkin snowbell are Aspen and Bode, respectively. In this study, we used a new (unutilized) chopsticks. Because, this is our first study using Aspen and Bode chopsticks, we want to confirm the only pure CNF characteristics without food contamination and other effects by organisms. The chopsticks were about 20 cm in length, 1 cm in width, and 0.5 cm in thickness. They (Aspen and Bode) were treated using various steam treatment conditions to allow delignification and were then used as raw materials for fibrillation.

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

2.2.2. Preparation of cellulose for CNF-Steam treatment

The steam treatment 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., 2015). Then, 120 g of chopsticks were introduced into the reactor and exposed to saturated steam at a pressure of 10 atm (183 °C), 15 atm (200 °C), 20 atm (213 °C), and 25 atm (224 °C) for a steaming time of 5, 10, and 15 min. The prescribed temperature was reached in a few seconds. After completion of the reaction, the valve of the reactor was gradually opened to remove the steam, and then the steamed chopsticks were removed. The steam-treated chopsticks were ground using a crusher mill (Wonder Crush Mill D3V-10, Osaka Chemical Co. Ltd) at 25,000 rpm for 10 s.

2.2.3. Extraction and separation

The steam-treated chopsticks were extracted with distilled water 60-fold of the sample dry weight, at room temperature for 24 h and separated into water soluble material and a residue using filter paper (No. 131, φ 185 mm, Advantec Co., Ltd, Tokyo, Japan). Next, the residue was extracted with acetone 30-fold of the sample dry weight at room temperature for 24 h, and after acetone extraction it was then separated using a filter paper (No. 131, φ 185 mm, Advantec Co., Ltd, Tokyo,

Japan). Fig. 1 shows the procedure.

2.2.4. Bleach treatment

The residue (25 g) obtained after the water and acetone extractions, i.e., pulp, was suspended in 1.5 L of distilled water and subjected to the following treatment (see Fig. 1). In order to remove the residual lignin in the residue, 10 g of NaClO2 was directly added to the suspended solution described above upon gentle mixing, followed by the subsequent addition of 2 mL of acetic acid. The suspended mixture was heated at 80 °C for 1 h. The addition of 10 g of NaClO2 and 2 mL acetic acid was repeated 4 times with intervals of 1 h. Finally, the mixture was filtered using a filter paper (No. 131 φ 185 mm, Advantec Co., Ltd, Tokyo, Japan), and the solid fraction was rinsed 5 times with 100 mL of distilled water. The rinsed sample, i.e., sample after bleach treatment, was maintained in a semi-wet state at 4 °C until use.

2.2.5. Preparation and characterization of CNF-Grinder treatment

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

2.2.6. Production of CNF sheets

After the grinder treatment of the sample, a suspension was prepared so that the dry weight became 1 g. CNF sheets were obtained by vacuum filtration using a glass fiber filter paper (GF-75, φ 110 mm, Advantec Co., Ltd, Tokyo, Japan). Sheets obtained by filtration were first sandwiched between nylon net films (11 µm NY11, Merck Millipore Ltd,) and then between two sheets of filter paper (No. 131, φ 185 mm, Advantec Co., Ltd, Tokyo, Japan). In this state press (Manual hydraulic vacuum heating press machine, 1MC-11FA, Imoto Machinery Co., LTD, Kyoto, Japan) under the conditions of 6 min, 300 kPa, and room temperature. The backing paper was replaced with a new one and press it under the conditions of 6 min, 300 kPa and 50 °C. (Nair et al., 2015)

2.2.7. Component analysis

The components of the steam treated chopsticks, i.e., the volatile material, water soluble material, acetone soluble material (low molecular weight lignin), NaClO2 soluble material (decomposed material mainly derived from high molecular weight lignin), hemicellulose, and cellulose, were separated and measured according to a modified version of the Wayman's extraction method (Chua & Wayman, 1979). Fig. 2 shows the procedure. Thus, 40 g of dry chopsticks were steam treated, then weighed, and the difference of weight before treatment was calculated giving the amount of volatile material. Then, 5 g of dry steam treated chopsticks 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., the water-soluble material, was recovered from the liquid. After water extraction, the remaining residue was extracted with 150 mL of acetone for 24 h at room temperature to afford an extract, i.e., the acetone soluble material. After concentration and drying of the extract, the acetone soluble material was weighed. The residue after acetone extraction consisted of 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 of acetic acid was subsequently added. The suspended mixture was heated at 80 °C for 1 h. Furthermore, the addition of 1 g of NaClO2 and 0.2 mL of acetic acid was repeated four times with intervals of 1 h to remove the NaClO2 soluble material. After the solid fraction was rinsed 5 times with distilled water and dried, the residue consisting of holocellulose, which is defined as steam treated chopsticks pulp in this work, was weighted.

The total sugar amount was also measured by using the following method. First, 0.2 g of chopsticks was added to 3 mL of 72% (w/w) sulfuric acid and kept at room temperature for 4 h. After 4 h, the mixture was transferred to a 100 mL conical flask and diluted with 75 mL of DW, and then autoclaved for 1 h at 121 °C. Subsequently, suction filtration was carried out using a glass fiber filter paper (GF-75, φ 110 mm, Advantec Co., Ltd, Tokyo, Japan), and the mixture was divided into a filtrate and residue. Then, 1.0 mL of 5% phenol solution was added to 1.0 mL of filtrate. Next, 5.0 mL of concentrated sulfuric acid was added quickly and dropwise upon mixing. After allowing to stand for 10 min, the reaction color turned from yellow to brown, and the absorbance was measured at 490 nm. (Hodge & Hofreiter, 1962) All the analytical determinations

were

performed in triplicate, and the means were calculated.

2.2.8. Holocellulose recovery yield (HR)

The holocellulose recovery yield (HR) was defined as follows:

Holocellulose recovery yield (HR) = Amount of NaClO2 insoluble component (g/g-dry treated sample)/Total sugar amount (g/g-dry untreated sample)

2.2.9. Severity factor

The logarithm of the severity factor (Sf) was calculated according to the following equation (Abatzoglou, Chornet, Belkacemi, & Overend, 1992):

Severity factor (Sf) = log [$t \exp\{(T - 100) / 14.75\}$]

(t: time, T: temperature)

2.2.10. Molecular weight measurement

To measure the molecular weight of the samples, 250 mg of each sample was soaked with 25 mL of 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 \pm 0.1 °C. The molecular weight of the samples was the calculated according to JIS P8215 (1998).

2.2.11. Mechanical properties

The tensile modulus and strength of the samples were 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 $60 \text{ mm} \times 10 \text{ mm}$. The gauge length was 30 mm and a testing speed of 1.0 mm/min was applied for the test.

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

2.2.12. FE-SEM

Cellulose nanofibers were observed using a FE-SEM (S-4700, Hitachi, Tokyo, Japan) operating at 1.6 kV and with a working distance of 8 mm. A small piece of the CNF mat was fixed on a carbon tape and then sputtered with Pt-Pd. (E-1020 ION SPUTTER, Hitachi, Tokyo, Japan).

2.3. Results and Discussion

2.3.1. Chemical composition of Aspen and Bode treated by steam combined with milling

Fig. 2 shows the experimental process to produce CNF from discarded Aspen and Bode chopsticks using steam treatment combined with milling as pretreatment. The produced CNF contains not only cellulose but also other materials such as hemicellulose. However, there are some researches with CNF made of the fibrillated pulp that contains cellulose and hemicellulose (Saba et al., 2017a). Therefore, we also named our product CNF. According to this process, we determined each component as follows; holocellulose, water soluble, acetone soluble, sodium chlorite soluble, and volatile components. Fig. 3 shows the component analysis of the steam treated Aspen (Fig. 3 (a)) and Bode (Fig. 3 (b)) chopsticks. The amount of holocellulose (cellulose plus hemicellulose) decreased with the increasing of the steam condition severity, i.e., pressure and steaming time. With the amount of \Box -celullose in the holocellulose (data not shown), the \Box cellulose ratios in the holocellulose of steam treated Aspen and Bode were 70~90%, the ratios increased with the increasing the treatment steam pressure and treatment time, while with untreated Aspen and Bode, the \Box -cellulose ratio was around 50%, therefore, hemicellulose part was mainly decomposed during the treatment. On the other hand, the quantity of volatile components increased with the increasing of the steam condition severity, while the water-soluble component decreased. For example, when changing the pressure from 15 to 25 atm for a steaming time of 5 min in the case of Aspen, the volatile component increased from 0.022 to 0.183, while the water-soluble component decreased from 0.125 to 0.061. Similar results were obtained for Bode. The water soluble components mainly derived from cellulose and hemicellulose, and they consisted of hydrolyzed monosaccharides such as glucose, oligosaccharides, and other decomposition materials of monosaccharides, including levulinic acid, formic acid (these organic acids serve as catalysts to promote further hydrolysis), 5-hydroxymethyl furfural, etc. (Palmqvist & Hahn-Hagerdal, 2000; Ulbricht, Northup, & Thomas, 1984). A part of these materials evaporated just after the steam treatment, thus they were named as the volatile component. The volatile component remained in the residue under milder steam conditions, and were removed by water extraction (to form the water-soluble component); however, they evaporated from the residue under harsher steam conditions.

The acetone soluble component increased with the increasing of the steam condition severity.
When the treatment pressure increased from 15 to 25 atm, the amount of acetone soluble component increased from 0.036 to 0.125, in the case of Aspen. The acetone soluble component mainly contained low molecular weight lignin derived from high molecular weight lignin. It has been reported that the acetone soluble component from steam exploded Japanese cedar could be a useful resource to synthesize epoxy resins (Asada et al., 2015). The NaClO2 soluble component mainly contained decomposed material mainly derived from high molecular weight lignin; in the NaClO2 solution, no low molecular lignin was present since it had already been extracted into the acetone soluble part. Therefore, when comparing the acetone soluble and the NaClO2 soluble components, the quantity of the former increased as the treatment pressure increased, while the latter decreased. When the treatment condition severity increased from 10 atm with a steaming time of 5 min to 20 atm for 15 min, the amount of the NaClO2 soluble component decreased from 0.162 to 0.074, in the case of Aspen. However, under a steam pressure of 25 atm the amount of the NaClO2 soluble component increased from 0.10 at 5 min to 0.14 at 15 min. This phenomenon can be attributed to the repolymerization of low molecular lignin (Asada, Kobayashi, & Nakamura, 2005). The polymerization occurs with high molecular lignin, low molecular lignin, and lignin derivatives such as phenolic compounds to form materials insoluble in acetone.

With respect to the logarithm of the severity factor (Sf), the Sf increased with the increasing of the steam condition severity, i.e. pressure and steaming time. Furthermore, it also strongly correlated with the HR value, as the Sf increased with decreases in the HR.

2.3.2. Molecular weight of cellulose of treated Aspen and Bode

Fig. 4 shows degree of polymerization of holocellulose (especially cellulose) in Aspen and Bode treated under various steam conditions. Under increasingly harsher steam conditions, the degree of polymerization decreased. For Aspen, degrees of polymerization of 910.0 and 209.9 were obtained at a steam pressure of 10 atm and steaming time of 5 min, and steam pressure of 25 atm and steaming time of 15 min, respectively. Similar results were obtained for Bode. Furthermore, we analyzed the relationship between the degree of polymerization and Sf, the relationship follow negative linear correlation.

The molecular weight of cellulose could be calculated by the degree of polymerization x 162 (Ryu, Lee, Tassinari, & Macy, 1982). Therefore, the highest molecular weight values, which were

approximately 1.5×105 (Aspen) and 1.3×105 (Bode), were obtained at a steam pressure of 10 atm and steaming time of 5 min, while the lowest molecular weight values, which were approximately 3.4×104 (Aspen) and 2.9×104 (Bode), were obtained at a steam pressure of 25 atm and steaming time of 15 min. Moreover, as the Sf increased, the decomposition of cellulose proceeded, and the molecular weight of cellulose decreased.

2.3.3. Tensile strength of CNF produced from treated Aspen and Bode

Fig. 5 shows the tensile strength of CNF obtained from Aspen and Bode under various steam conditions. Fig. 5(a) and 5(b) show the tensile strength of CNF treated at a steam pressure of 25 atm and steaming time of 5, 10, and 15 min, and a steaming time of 5 min and steam pressure of 10, 15, 20, and 25 atm, respectively. As the conditions increased, the strength properties decreased. In terms of time at 25 atm (Fig. 5 (a)), when comparing the results at 5 min with those at 15 min, specific tensile strengths of 59.5 and 43.6 as well as 81.6 and 34.1 [MPa/(g/cm3)] were attained for Aspen and Bode, respectively. When the pressure changed from 10 to 25 atm at a steaming time of 5 min (Fig. 5(b)), the specific tensile strength also decreased at 20, 25 atm compared with that at 10, 15 atm for Aspen and Bode. These results suggest that the high molecular weight of CNF is related to the intensity. Furthermore, the specific tensile strength of the CNF obtained at a steam pressure of 15 atm (Fig. 5 (b)), i.e., 86.9 MPa/(g/cm3), was higher than that obtained at a steam pressure of 10 atm, i.e., 84.6 MPa/(g/cm3), for Aspen. Similar results were obtained with Bode (109.9 MPa/(g/cm3) at 15 atm and 96.9 MPa/(g/cm3) at 10 atm). The molecular weights at a steam pressure of 15 atm for Aspen and Bode were around 1.2×105 (162×694 and 162×801 , respectively). Therefore, it was found that the optimal molecular weight to produce CNF was around 1.2×105 . Although treated Bode at a steam pressure of 10 atm and steaming time of 5 min also had a similar molecular weight, i.e., 1.3×105 (814 \times 162), the specific tensile strength was lower than that at a steam pressure of 15 atm. This may be thought to be related to the HR. Fig. 6 shows the holocellulose recovery rate (HR) of steam treated Aspen and Bode and the logarithm of the Sf under various steam treatment conditions. For untreated samples, i.e., milled for 1 min, and samples steamed under mild conditions, i.e., steam pressure of 10 atm and steaming time of 5 min, the HR values exceeded 1.00, as they were found to be 1.224 and 1.029 with Aspen, and 1.341 and 1.315 with Bode. The HR could be calculated from the equation described above,

i.e., amount of NaClO2 insoluble component (g/g-dry treated sample)/total sugar amount (g/gdry untreated sample). The HR values decreased with the increase of the Sf, highly negative correlation was observed (data not shown); $HR = -0.2428 \times Sf + 1.8002$ (R2= 0.9477, Aspen), $HR = -0.3508 \times Sf + 2.3033$ (R2= 0.8998, Bode). It is evident that when the HR exceeds 1.00, the undecomposed materials by bleaching remain in the NaClO2 insoluble component. On the other hand, under a steam pressure of 25 atm and steaming time of 15 min, i.e., the severest conditions used in this study, the HR was 0.545 and 0.648 for Aspen and Bode, respectively. Low HR values derived from low amounts of the NaClO2 insoluble component, i.e., low quantities of the holocellulose part (see Fig. 3). Therefore, the HR indicates not only the holocellulose recovery yield, but also the degree of fibrillation, and values around 1.00 are desirable. For example, HR was 0.899 and 0.967 at a steam pressure of 15 atm and steaming time of 5 min for Aspen and Bode, respectively, suggesting that the component separation might be sufficient under these conditions. It is necessary to analyze the HR value of steam treated other woody or herbaceous biomass and confirm the relationship between HR and tensile strength of CNF in future study. At 10 atm, The HRs of Aspen and Bode were 1.074 and 1.315, respectively, which are higher than 1.00; on the other hand, at 15 atm, the HRs of Aspen and Bode were 0.903 and 0.967, respectively, which are lower than 1.00. Therefore, it was shown that the strength is greatly affected by not only the molecular weight of cellulose, but also the HR value, and it decreases when the separation is insufficient. Overall, it was demonstrated that a steam pressure of 15 atm and steaming time of 5 min (the Sf is around 3.6) were the optimal conditions to produce CNF with a high tensile strength from Aspen and Bode using a steam treatment combined with milling method. In a previous report (Suzuki, Sasaki, Asada, & Nakamura, 2017), the maximum tensile strength value of 64.0 MPa/(g/cm3) was obtained for the CNF film produced from Japanese cedar by steam explosion at a steam pressure of 35 atm for a steaming time of 5 min.

When comparing the tensile strength results of Aspen with those of Bode, it could be seen that both Aspen and Bode showed a maximum (86.9 and 109.9 [MPa/(g/cm3)]) tensile strength at a steam pressure of 15 atm and steaming time of 5 min. Therefore, CNF with a strong tensile strength could be produced under relatively mild steam treatment conditions using Aspen and Bode as resource materials. In the case of Bode treated at a steam pressure of 25 atm and steaming time of 10 min, the tensile strength dramatically decreased (Fig. 5(a)). In terms of HR, there was no big difference for a steaming time of 5 and 10 min. It was speculated that the fibers of Bode

suffered a great damage during the treatment under these conditions. To confirm the fibrillation of CNF, we analyzed the CNF surface by field-emission scanning electron microscopy.

2.3.4. Field-emission scanning electron microscope images of CNF produced from treated Aspen and Bode

A field emission scanning electron microscope was used to investigate the changes of the surface structure in CNF obtained from steam treated Aspen and Bode. Fig. 7(a) shows the field-emission scanning electron micrographs of the CNF at a steam pressure of 25 atm and steaming time of 5, 10, and 15 min. The width of CNF decreased with the increase of the steaming time. For Aspen, a width of 170, 90, and 70 nm was observed after 5, 10, and 15 min, respectively. For Bode, a width of 120, 100, and 70 nm was observed after 5, 10, and 15 min, respectively. From these images, it was confirmed that spongy voids between the fibers were formed upon more than 10 min of steaming time. Also Fig. 7(b) shows the field-emission scanning electron micrographs of the CNF at a steaming time of 5 min and steam pressure of 10, 15, 20, and 25 atm. The width of the CNF decreased with the increase of the steam pressure, and the fibrillation proceeded faster as the treatment severity increased. From these images, it was clear that the production of CNF from Aspen and Bode by steam treatment was successfully performed.

2.3.5. Figures and Tables

2.3.5.1.



Fig. 1 Overall experimental process of production of CNF from discarded chopsticks.



Fig. 2 Component analysis procedure of untreated and treated chopsticks.

2.3.5.3.



Fig. 3 Component analysis of untreated (milling for 1 min) and steam treated Aspen (a) and Bode (b) under various steam conditions.





Fig. 4 Degree of polymerization of holocellullose in Aspen and Bode treated under various steam conditions.

2.3.5.5.



Fig. 5 Specific tensile strength of CNF obtained from Aspen and Bode treated at steam pressure of 25 atm for steaming time of 5, 10, and 15 min (a), treated at steam pressure of 10, 15, 20, and 25 atm for steaming time of 5 min (b).

2.3.5.6.





Fig. 6 Holocellulose recovery rate (HR) of untreated (milling for 1 min) and steam treated Aspen and Bode (b) and calculated severity factor under various steam conditions.





Fig. 7 FE-SEM images of CNF obtained from Aspen and Bode treated at steam pressure of 25 atm for steaming time of 5, 10, and 15 min (a), treated at steam pressure of 10, 15, 20, and 25 atm for steaming time of 5 min (b).

2.4. Conclusion

We were able to produce CNF from Aspen and Bode holocellulose with low environmental load by using a method that combines high temperature and pressure steam treatment with milling. It was demonstrated that the optimal conditions for the production of CNF from Aspen and Bode with a maximum tensile strength were a steam pressure of 15 atm and steaming time of 10 min. Furthermore, it was found that in order to produce CNF with a high tensile strength, the optimal molecular weight was around 1.2×105 . The HR value, which indicates the degree of separation and holocellulose recovery yield, may be strongly related to the tensile strength. An overall CNF amount of 22.0 g from 10 chopsticks (4.0 g per 1 chopstick) was achieved (61.2 % of holocellulose yield treated at steam pressure of 15 atm and steaming time of 5 min, and 10% of the residue decreased at grinder). The produced CNF offer potential applications due to their mechanical strength. Thus, in order to explore their practical use, further studies on the fabrication of composites with biopolymers such as polylactic acid are necessary.

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Chapter 3: Total biorefinery process of lignocellulosic waste using steam explosion followed by water and acetone extractions

3.1. Introduction

In order to move away from a fossil resource-dependent society, the development of energy and material production processes using inedible biomass by humans (lignocellulosic material) instead of edible biomass (i.e. sugar and starch material), as a raw material, has attracted increasing interest globally, since the use of edible biomass competes with food and feed supplies [1-3]. Lignocellulosic waste represents an abundant carbon-neutral renewable resource. It is used to produce biofuels and biomaterials, and its increased use would lower environmental impacts such as the emission of greenhouse gases (e.g., carbon dioxide), and fossil fuel depletion, helping to create a sustainable environment. Advances in technologies such as genetics, biotechnology, process chemistry, and engineering are leading to the concept of biorefining [4]. Biorefining is a new manufacturing concept that involves converting renewable biomass to valuable fuels and products. Recently, many researchers have attempted to convert lignocellulosic material to useful products, i.e. phenolic compound with antioxidant activity, cellulose nanofiber, epoxy resin, etc. Phenolic compound with antioxidant activity can be used in health food field [5]. The important applications of cellulose nanofiber are biosensors, reinforcing composites, and films [6, 7]. Epoxy resins are among the most important and highly valuable thermosetting resins. They are known to have good electrical characteristics, chemical resistance, mechanical strength, and low absorption of moisture. The mechanical strength and high thermal resistance properties of epoxy resins render them versatile and applicable in various fields, such as in electronics, aerospace, and automotive applications [8].

In order to develop the total biorefinery processing of lignocellulosic waste, this study focused on the efficient separation and utilization of woody structural components from white poplar chopstick waste by using a steam explosion pretreatment followed by water and acetone extractions. In East Asia, disposable wooden chopsticks are used in restaurants, school cafeterias, and homes, and are generally made of white poplar or white birch wood and bamboo [9]. The average annual amount of wooden chopsticks disposed of in Japan is about 90,000 t [9]. At present, disposable wooden chopsticks are landfilled or incinerated in Japan. Therefore, it is desirable to use them as a raw material for useful chemical production. The cellulose component was converted into cellulose nanofiber (CNF) and the lignin component was used as a raw material for the synthesis of epoxy resin. Furthermore, the mechanical and thermal properties of the CNF/Polylactic Acid (PLA) composite and cured epoxy resin were evaluated.

3.2. Materials and Methods

3.2.1. Raw Materials

White poplar (Populus Tremuloides) waste chopsticks were cut into the half, to about 10 cm in length, and steam-exploded under various operating conditions. PLA (Landy PL-2000, Miyoshi Oil & Fat Co., Ltd., Japan) was used as a filler. All chemicals were purchased from Nacalai Tesque, Inc., Japan and were of analytical grade.

3.2.2. Steam Explosion Pretreatment

Figure 1 shows a flow chart of the steam explosion process for the conversion of waste white poplar chopsticks into phenolic compound, cellulose nanofiber, and cured epoxy resin coproducts. The chopsticks were steam-exploded in a 2 L batch system reactor (steam explosion apparatus NK-2L; Japan Chemical Engineering and Machinery Co. Ltd., Osaka, Japan) [10]. The reactor was charged with 150 g of chopsticks per batch and heated to a pressure of 2.5 MPa (225 °C), 3.0 MPa (234 °C), and 3.5 MPa (243 °C) for a steaming time of 1 min and 5 min at each pressure. 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 rapidly opened to bring the reactor to atmospheric pressure in a short amount of time, thereby obtaining the product of liquid and solid materials, i.e. the steam-exploded product. The steam-exploded product was extracted with water and then acetone. It was then converted into cellulose nanofiber and cured epoxy resin co-product.

The components, viz., water extract, acetone extract (a low molecular weight lignin), bleaching extract (a high molecular weight lignin), and residue after bleaching (holocellulose), in the steam-exploded product, were separated and measured by the following procedure, with modifications according to Wayman's extraction method [11]. Five grams of dry steam-exploded product 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 (water extract) was recovered from the liquid. After water extraction, the residue was extracted with 150 mL acetone for 24 h at room temperature to dissolve the acetone extract. After concentration and drying of the extract, the acetone extract was weighed. The residue after acetone extraction consisted of holocellulose

(cellulose and hemicellulose) and residual lignin. 150 mL of 1 g NaClO2 were added to this residue (2.5 g) with gentle mixing and then 0.2 mL of acetic acid was added. The suspended mixture was allowed to react at 80 °C for 1 h. Next, the addition of 1 g NaClO2 and 0.2 mL of acetic acid was repeated four times in 1 h intervals to remove the NaClO2 extract. After the solid fraction was rinsed five times with distilled water and dried, the residue after bleaching, holocellulose, which was defined as a steam-exploded pulp in this study, was weighed. Furthermore, 1 g of the 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 while stirring for 6 min. The solid fraction was rinsed five times with distilled water and neutralized with 10% acetic acid. After drying, the residue, α -cellulose, was weighed.

3.2.3. Measurement of the Catechin Equivalent of Water Extract

Phenolic compounds in the water extract were measured according to the Folin–Ciocalteu method [12]. The extract (200 μ L) was added to the test tube containing 4 mL of distilled water, followed by the addition of 1 mL phenol reagent. The mixture was thoroughly stirred. In addition, 1 mL of 10% (w/v) sodium carbonate was added to the solution. The absorbance of the reaction was measured at 760 nm after 1 h of reaction. Estimations were carried out in triplicate and calculated from a calibration curve to obtain the amount of catechin. The amounts of phenolic compounds were expressed as a catechin equivalent (mg-catechin equiv./g-dry steam-exploded product).

3.2.4. Synthesis of Cured Epoxy Resin from Acetone Extract

Acetone extract (a low molecular weight lignin) was used as a sample for epoxidized lignin, i.e. lignin epoxy resin, and also as a curing reagent of epoxidized lignin. The cured epoxy resin was synthesized from the acetone extract according to the method reported by Asada et al. [13].

3.2.5. Preparation of Cellulose Nanofiber from Residue after Bleaching

After bleaching, 10 g of residue, holocellulose, were suspended with 1 L distilled water, and then it was passed twice through a grinder (MKCCA6-2, Masuko Sangyo Co., Ltd., Saitama, Japan) at 1500 rpm.

3.2.6. Preparation of CNF and PLA (CNF/PLA) Composite

The CNF water slurry containing 5 wt% fibers was added to the melted PLA (Landy PL-2000, Miyoshi Oil & Fat Co., Ltd., Japan), and the CNF/PLA mixture was kneaded and mixed under a vacuum. The composite was preheated in a die (100 mm \times 100 mm) at 105 °C for 1 h and then compressed at 180 °C and 1 MPa for 10 min. The resulting sheet was 1.4 mm. Specimens 80 mm long and 10 mm wide were prepared from the sheet.

3.2.7. Analyses

3.2.7.1. Fourier Transform Infrared (FTIR) Spectroscopy

Changes in the functional groups of the steam-exploded product were recorded by FTIR spectroscopy (FT/IR-670 Plus; JASCO, Tokyo, Japan) after various extraction and separation treatments. First, the samples were ground and dried at 105 °C. The sample (1.5 mg) was mixed with 200 mg of 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 to 4000 cm–1.

3.2.7.2. Molecular Weight Measurement

To measure the molecular weight of the sample (α -cellulose in the steam-exploded product), 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 ± 0.1 °C. According to JIS P8215 [14], the molecular weight of the sample was measured.

3.2.7.3. Field-Emission Scanning Electron Microscopy (FE-SEM)

After multiple extractions followed by separation treatments, the steam-exploded product and CNFs were observed using an 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.

3.2.7.4. Thermal Property of Cured Epoxy Resin and CNF/PLA Composite

The thermogravimetric (TG) curve was measured using a TG analyzer (TG/DTA SII EXSTAR 6300; Seiko Instruments Inc., Chiba, Japan) under an atmosphere of nitrogen (heating rate of 5 °C/min) using alumina as a primary standard. The thermal tests were performed in triplicate and average values are shown.

3.2.7.5. Mechanical property of Cured Epoxy Resin and CNF/PLA Composite

The tensile modulus and strength of the samples were measured using a tensile tester (Dual Column Series for Mechanical Testing 5667, INSTRON Japan Co. Ltd, Kawasaki, Japan) at room temperature. The dimensions of the samples were 80 mm \times 10 mm \times 1.4 mm. The gauge length was 30 mm and a testing speed of 2 mm/min was applied for the test. For each sample, five repetitions were performed and the average of five tests was reported.

3.3. Results and Discussion

3.3.1. Surface Structure Changes of White Poplar Chopsticks after Treatment

A field-emission scanning electron microscope (FE-SEM) was used to investigate the changes in the surface structure of the white poplar chopstick waste subjected to the steam explosion, water and acetone extractions, bleaching, and grinder treatments. Figure 2 shows FE-SEM of (A) untreated white poplar chopstick waste, (B) steam-exploded product at 2.5 MPa for 5 min, (C) residue after water extraction, (D) residue after acetone extraction, (E) residue after bleaching, and (F) cellulose nanofiber. Though the rough and linty surface of untreated white poplar chopstick waste was observed, the woody fibers were defibrillated by the steam explosion and the fiber size became about 100 nm wide as shown in Fig. 2(B). However, there are variations in the degree of disintegration, and it has not been fibrillated to a uniform thickness. The residue after water extraction had a rough and spherical surface as shown in Fig. 2(C), but the residue after acetone extraction had a clean and smooth surface as shown in Fig. 2(D). This means that acetone extraction removed a low molecular weight lignin from the residue after water extraction. When compared before and after bleaching, little change of surface was observed by FE-SEM as shown in Figs. 2(D) and (E); the sample was decolorized from brown to white due to the removal of high molecular weight lignin. The CNF, which was produced from the residue after bleaching, holocellulose, by a grinder treatment had comparatively smaller nanofibers (about 20 nm wide) as shown in Fig. 2(F).

3.3.2. Chemical Composition of Steam-Exploded White Poplar Chopsticks

Figure 3 shows the ratios of the components, i.e. water extract, acetone extract, bleaching extract, and residue after bleaching, to the dry weight of the steam-exploded white poplar chopsticks. Three treatment conditions, viz., steam pressures of 2.5, 3.0, and 3.5 MPa for a steaming time of 5 min, were evaluated. The highest ratio of component of the residue after bleaching, 56.6% holocellulose (hemicellulose plus cellulose), was obtained at a steam pressure of 2.5 MPa for a steaming time of 5 min. This component could be used as a raw material for the CNF. Similar values of the residue after bleaching, 53.1% and 51.1%, were observed with a steam pressure of 3.0 and 3.5 MPa for a steaming time of 5 min, respectively. At severe treatment conditions, i.e. extended time, high temperature, and high concentration of an acidic catalyst, the amount of

holocellulose decrease due to its decompose first to sugars and then to further decomposed materials, such as furan derivatives and organic acids [15]. Kurosumi et al. [16] reported that water extract from steam-exploded bamboo plant (Sasa palmate (Bean) Nakai; a steaming pressure of 3.9 MPa for a steaming time of 1 min) contained phenolic compounds derived from high molecular weight lignin and indicated antioxidant activity. In this study, the highest ratio of component of the water extract, 16.7%, was observed at a steam pressure of 2.5 MPa, and the amount of phenolic compounds in the water extract corresponded to 76 mg-catechin equiv./g-dry steam-exploded product. However, since the phenolic components were not separated, purified, and identified in this study, this point is a future subject. Furthermore, the acetone extract mainly contained a low molecular weight lignin derived from a high molecular weight lignin. Asada et al. [13] mentioned that a low molecular weight lignin extracted from steam-exploded plant biomass could be a useful resource for synthesizing epoxy resin. The highest ratio of component of acetone soluble material, 36.7%, was observed at a steam pressure of 3.5 MPa, which means that the depolymerization reaction was promoted by increasing treatment pressure (severity). As a result, it seems that each component of steam-exploded product, residue after bleaching, water extract, and acetone extract, could be a promising resource for various useful chemicals.

3.3.3. FTIR Analysis of the Steam-Exploded Product after Various Extraction and Separation Treatments

Figure 4 shows changes in the functional groups of the steam-exploded product at 2.5 MPa for 5 min by water extraction, acetone extraction, bleaching, and NaOH treatment using FTIR analysis. The assignments of FTIR absorption bands are indicated in the caption of Figure 3 [17-21]. Although the peaks at (4) and (12) ascribed to the lignin structure were observed in the steam exploded product, the residue after water extraction, and the residue after acetone extraction, they became weak in the residue after bleaching (holocellulose) and disappeared in the residue after NaOH treatment (α -cellulose). This means that the acetone extraction cannot remove high molecular weight lignin, but the NaClO2 and NaOH treatments can degrade and remove high molecular weight lignin. Since the peaks of (4) and (12) are completely eliminated, it seems that lignin was completely removed by alkali treatment. Also, trace amounts of lignin or lignin-derived compounds were present in holocellulose. The intensity of the peaks at (6), (9), (10), and (11),

which relate to COO' stretching, stretching of C-O in a ring, or bending of C-OH, C-O-C stretching, and O-H bending, respectively, in the holocellulose (cellulose and hemicellulose), increased due to the removal of lignin that strongly covered the cellulose and hemicellulose.

3.3.4. Synthesis of Cured Epoxy Resin from Acetone Extract

The synthesis of epoxy resin from acetone extract was attempted. Table 1 shows the characteristics of acetone extracted from steam-exploded product at 2.5 MPa after 5 min. The purity of lignin contained in the extract was 99%, which implies that high-purity lignin was obtained in this work. The number-average molecular weight, the weight-average molecular weight, and the hydroxyl equivalent of the extract were 1200, 5100, and 130, respectively. Asada et al. [13] reported that the weight-average molecular weight and the hydroxyl equivalent of methanol extract from various steam-exploded plant biomasses were in the range of 1330–1600 and 115–118, respectively. The reason why the weight-average molecular weight and the hydroxyl equivalent of acetone extract were higher than those of methanol extract seems to be that acetone can extract more lignin than methanol. This means that not only a small molecular weight lignin but also a comparatively large molecular weight lignin was extracted by acetone extraction.

The resinification of the acetone extract was carried out with epichlorohydrin. Figure 5 shows the 1H NMR spectrum of the acetone extract and the epoxidized lignin synthesized from acetone extract. Both varied significantly. Hydroxyl signals were observed at 8–9 ppm in the acetone extract but they were not observed in the epoxidized lignin. Furthermore, the epoxide signals appeared at 2.7–2.9 ppm in the epoxidized lignin. These results suggest the incorporation of an epoxy group into the acetone extract, i.e. a low molecular weight lignin.

Epoxidized lignin, i.e. epoxy resin synthesized from the acetone extract, was cross-linked with the acetone extract as a curing agent. The thermal properties (thermal stability and thermal decomposition) of cured epoxy resin were investigated by using TG/DTA analysis. Figure 6 shows the TG/DTA profiles of epoxy resin cured in a nitrogen atmosphere. The thermal decomposition temperature at 5% weight loss (Td5), 10% weight loss (Td10), and 30% weight loss (Td30) were 260, 294, and 358 °C, respectively. Benyaha et al. [22] reported that the thermal decomposition temperature at 30% weight loss of cured bio-based epoxy resin using a green tea extract, i.e. catechin with isophorone diamine, was 299 °C. Because the value was much lower

than that of the cured epoxy resin obtained in this study, the low molecular weight lignin is a more suitable biopolymer than catechin for the synthesis of heat-resistant bio-based epoxy resin. Since Td5 exceeded the temperature of heat-stability property for solder-dip resistance, i.e. beyond 250 °C [23], it can be used in the electronic board material field. Furthermore, the tensile strength of cured epoxy resin obtained in this study was about 30 MPa and this value was within the values of conventional fossil-derived epoxy resins, i.e. 27-80 MPa.

3.3.5. Degree of Polymerization of α-Cellulose in Residue after Bleaching

The degree of polymerization of α -cellulose in the residue after bleaching, i.e. holocellulose, obtained from the steam-exploded cedar white poplar chopstick waste, was compared to that of BiNFi-s WMa-10002 (a commercial cellulose nanofiber, Sugino Machine Ltd., Japan) as shown in Fig. 7. When the treatment condition severity was increased, the degree of polymerization decreased. The molecular weight of cellulose can be calculated by the formula (degree of polymerization×162) [24], therefore, the lowest molecular weight in this study, approximately 17,000, was obtained at a steam pressure of 3.5 MPa for a steaming time of 5 min. The degree of polymerization at 2.5 MPa and 5 min was a little lower than that of BiNFi-s WMa-10002. However, since a comparatively high degree of polymerization, approximately 500, was obtained from the residue at 2.5 MPa and 5 min, it seems to be the most adequate for the production of CNF as a reinforcement material. Suzuki et al. [7] reported that CNF obtained from steam-exploded Japanese cedar at 3.0 MPa and 5 min could be used for a practical applications and its degree of polymerization of α -cellulose was about 400. This value was lower than that obtained in this study.

3.3.6. Effect of CNF on Mechanical and Thermal Properties of CNF/PLA Composite

The reinforcement effects of CNFs produced from holocellulose and α -cellulose on the mechanical and thermal properties of CNF/PLA composites were evaluated using CNFs obtained from steam-exploded white poplar chopstick waste at 2.5 MPa and 5 min. Figure 8 shows the tensile strength and Young's modulus of various composites. As can be seen, the tensile strength and Young's modulus of PLA with 5 wt% CNF obtained from holocellulose increased to 3.7 and

27.8 times in comparison to neat PLA, respectively. The tensile strength of the CNF/PLA composite from holocellulose was a little stronger than that from α -cellulose. This means that hemicellulose contained in the holocellulose fibers binds not only cellulose fibers but also PLA resin, resulting in the high strength of CNF/PLA. Though the tensile strength and Young's modulus of CNF/PLA composite from holocellulose were a little lower than those of CNF/PLA composite with a commercial CNF, due to a lower degree of polymerization of α -cellulose as shown in Fig. 6, the significant reinforcement effect of CNF obtained from steam-exploded product on PLA resin was confirmed.

The TG/DTA profiles of the CNF/PLA composite show their thermal stability and degradation characteristics. Figure 9 shows TG/DTA profiles of PLA, CNF/PLA composite from holocellulose, and CNF/PLA composite with a commercial CNF. Since the thermal decomposition temperatures at 5% weight loss (Td5) were almost the same and the similar TG/DTA profiles were observed regardless of samples, it was found that the addition of 5 wt% CNF to PLA did not affect the thermal property of neat PLA.

3.3.7. Figures and Tables

3.3.7.1



Fig. 1 Flow chart of steam explosion followed by water and acetone extractions from white poplar chopsticks for phenolic compound, cellulose nanofiber and cured epoxy resin co-product





Fig. 2 FE-SEM of (A) untreated white poplar chopsticks waste, (B) steam-exploded product at 2.5 MPa for 5 min, (C) residue after water extraction, (D) residue after acetone extraction, (E) residue after bleaching, and (F) cellulose nanofiber





Fig. 3 Ratios of components to dry weight of steam-exploded white poplar chopsticks waste. (A) 2.5 MPa and 5 min, (B) 3.0 MPa and 5 min, (C) 3.5 MPa and 5 min





Wavenumber [cm⁻¹]

Fig. 4 FTIR of (A) steam-exploded product, (B) residue after water extraction, (C) residue after acetone extraction, (D) residue after bleaching, and (E) α -cellulose (E) obtained from white poplar waste treated at 2.5 MPa for 5 min. Assignments of FTIR absorption bands: (1) O-H

stretching, (2) C-H stretching, (3) CO2, (4) C=O band from ester groups, (5) O-H stretching, (6) COO' stretching, (7) CH2 bending, (8) O-H in plane bending or C-H bending, (9) stretching of C-O in ring or bending of C-OH, (10) C-O-C stretching, (11) O-H bending, (12) C-H bending or CH2 stretching amorphous, (13) O-H out-of-plane bending



Fig. 5 1H NMR of (A) acetone extract and (B) epoxidized lignin synthesized from acetone extract

3.3.7.6.



Fig. 6 TG/DTA profiles of cured epoxy resin made from acetone extract, i.e. low molecular weight lignin



Fig. 7 Degree of polymerization of α -cellulose in residue after bleaching obtained from steamexploded white poplar chopsticks waste and commercial cellulose nanofiber (BiNFi-s WMa-10002, Sugino Machine Ltd.). (A) 2.5 MPa and 5 min, (B) 3.0 MPa and 5 min, (C) 3.5 MPa and 5 min, (D) BiNFi-s WMa-10002



70

3.3.7.7.

3.3.7.8.



Fig. 8 Tensile strength and Young's modulus of various composites. (A) PLA, (B) PLA with
5% CNF obtained from holocellulose, (C) PLA with 5% wtCNF obtained from α-cellulose, and
(D) PLA with 5% wtCNF (a commercial CNF, BiNFi-s WMa-10002)


Fig. 9 TG/DTA profiles of PLA and CNF/PLA composite. Solid line: PLA, dashed line: PLA with 5 wt% CNF obtained from holocellulose, and dotted line: PLA with 5 wt% CNF (a commercial CNF, BiNFi-s WMa-10002)

3.4. Conclusions

This work proposed a new effective and environmentally friendly biorefinery process of lignocellulosic waste using a steam explosion followed by water and acetone extractions. The water extract, acetone extract, and the residue obtained after bleaching holocellulose derived from steam-exploded white poplar chopstick waste, were converted into useful eco-materials. The water extract corresponded to a 76 mg-catechin equiv./g-dry steam-exploded product and can be used as an antioxidant. The acetone extract was converted into a cured lignin epoxy resin with high heat-resisting properties. The residue after bleaching was used as the raw material for the CNF, and its reinforcement effect on the PLA resin was demonstrated.

3.5. Acknowledgement

The authors are grateful for the partial support of a Grant-in-Aid for Scientific Research (A) (Grant No. 16H01790) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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Chapter 4: Synthesis of bamboo –derived lignin epoxy resin

4.1. Introduction

Extracted fractions obtained by steam explosion treatment and steam treatment include a water extracting component and an organic solvent extracting component in addition to holocellulose and are easily dissolved because they are dissolved in a solvent. In particular, a large amount of low molecular weight lignin is extracted in the organic solvent extraction component. Lignin is a type of polyphenol and has many highly reactive phenolic hydroxyl groups. In addition, there is no effective use method of lignin itself, and lignin produced in the papermaking process, the saccharification process, etc. is currently not discarded and discarded. Further, in these processes, since the acid-base reagent is used and the delignification treatment is carried out, the produced lignin is difficult to use and has low reactivity. In addition, since the solution in which lignin is dissolved has a large environmental burden, at present, it is only a method of utilizing it as a heat source by burning these.

However, the structure of lignin has many possibilities for use in various applications. Therefore, I focused on phenylpropane structure which is the basic skeleton of lignin. This structure currently has many common structures with bisphenol A which is a raw material of epoxy resin, and I thought that lignin could be used as an alternative to bisphenol A derived from petroleum. In addition to being a resource derived from petroleum, bisphenol A is at risk of having environmental hormone action, and substitution for biomass resources without toxicity like lignin is required.

Epoxy resins are widely used as adhesives and electronic substrate materials due to strength, high insulating properties, and the like. Epoxy resins synthesized from lignin are difficult to use as adhesives due to color and reactivity, and will be studied for use as an electronic substrate material. It is indispensable for our life by the development of home appliances. Therefore, demand is expected to increase more and more in the future. Therefore, making electronic substrate materials from biomass seems to play an important role in the future society.

In this study, bamboo is treated by steam explosion treatment. Bamboo is a fast-growing plant, a plant that exists abundantly in Japan. The bamboo is delignified by steam explosion treatment, and an epoxy resin is synthesized from low molecular weight lignin extracted with acetone. Further, the extracted lignin is also used as an epoxy resin curing agent to obtain a cured epoxy resin. Based on the strength and thermal stability of the obtained cured product, we investigate whether the biomass-derived epoxy resin can be used as an electronic substrate material.

4.2. Materials and methods

4.2.1. Sample

The bamboos were about 5 cm in length, 3 cm in width, and 1 cm in thickness. The bamboos were treated using steam explosion treatment conditions to allow delignification.

4.2.2. Preparation of acetone soluble lignin

4.2.2.1. 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). Then, 200 g of bamboo chips were introduced into the reactor and exposed to saturated steam at a pressure of 35 atm (243 °C) for a steaming time of 5 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 bamboo.

4.2.2.2. Extraction and separation

The steam-exploded bamboo was extracted with distilled water 60-fold of the sample dry weight, at room temperature for 24 h and separated into water soluble material and a residue using filter paper (No. 131, φ 185 mm, Advantec Co., Ltd, Tokyo, Japan). Next, the residue was extracted with acetone 30-fold of the sample dry weight at room temperature for 24 h, and after acetone extraction it was then separated using a filter paper (No. 131, φ 185 mm, Advantec Co., Ltd, Tokyo, Japan). Advantec Co., Ltd, Tokyo, Japan). Acetone was removed from the filtrate by an evaporator. The acetone soluble lignin (low molecular weight lignin) was obtained.

4.2.3. Synthesis of epoxy resin from acetone-soluble lignin

Synthesis was carried out according to the method of Asada et al. (2015). A 1000 mL fournecked flask equipped with a condenser, septum cap and a stirrer was charged with 0.1 mol of acetone soluble lignin dissolved in 50 mol of epichlorohydrin (For e.g. 10 g lignin in about 400 mL of epichlorohydrin). The gram equivalent weight, which is defined as the mass of a given substance which contains one part by weight of replaceable hydroxyl groups, was found 116.9 g/eq, belonging to bamboo, eucalyptus and bamboo lignin, respectively (determined by the acetic anhydride and pyridine method; Hitachi Chemical Techno Service Co., Ltd.). Hence, the obtained hydroxyl equivalent weight of the lignin refers to the mass of hydroxyl groups that could be replaced during reaction with epichlorohydrin. The solution of TMAC (0.25 g in 9.25 m)mL DW) was added dropwise after 10 min of the reaction. The suspension was heated at 70 °C under nitrogen (N_2) flow with continuous stirring at 250 rpm for 8 h until the completion of the reaction. The water-soluble TMAC catalyzes the reaction resulting the opening of the epoxy ring and detaching the hydrogen chloride. Next, the epoxy ring was reconstructed with addition of sodium hydroxide. The resulting mixture was then cooled to 45 °C, followed by the addition of concentrated sodium hydroxide (50 wt%, 4.8 g in 4.8 mL DW) and maintained under N2 flow until completion, with stirring for another 3 h. The organic layer was then separated and washed three times with DW (100 mL each time) to remove NaCl (by product of reaction) and the remaining catalyst. Finally, the excess epichlorohydrin and organic solvent was distilled off under reduced pressure at 70 °C to obtain the epoxidized resins from different biomasses i.e., bamboo.

The obtained lignin epoxy resins were further dissolved in methyl ethyl ketone (MEK) as a solvent. MEK was used as a solvent during ring closure reaction because it can dissolve lignin epoxy resin whilst insoluble in water. This property of MEK is favorable to wash out NaCl produced as a byproduct during ring closure reaction leaving final epoxidized product in the organic portion. The volume of MEK was fixed at 20 times the amount of epoxy resin, e.g., 11.6 g of epoxy resin in 348 g MEK. The ring closure reaction of the synthesized resins was conducted in a four-necked 1000 mL flask, which was charged with epoxidzed lignin obtained from different biomasses followed by the addition of aqueous NaOH (30 wt%, 0.39 g in 0.93 mL DW) at 70 °C under N2 flow with continuous stirring at 250 rpm for 1 h. After that the temperature was cooled down and the reaction mixture was washed with DW (100 mL, three times) and the organic solvents (MEK and remaining epichlorohydrin) were distilled off under reduced pressure using an oil bath at 120 °C. The final product was then dried in a vacuum oven at 40 °C overnight and final epoxidized resin from bamboo with epoxy equivalent weights of 345.5 g/eq, respectively. The epoxy equivalent was determined by titration of the product

dissolved in HCl/dioxane solution with a solution of NaOH in methanol. All the chemicals used in this experiment were supplied by Wako Pure Chemical Industries Ltd., Japan.

4.2.4. Synthesis of cured epoxy resin

The cured networks of lignin epoxy resins and a commercial epoxy resin EP828 (diglycidyl ether bisphenol A; Japan Epoxy Resins Co. Ltd.) were prepared with lignin as a bio-based curing agent, in 1:1 molar ratio of epoxy group to hydroxyl equivalent groups, to obtain the optimal cross-linking of cured epoxy samples. Furthermore, 1-(2-cyanoethyl)-2-ethyl-4- methylimidazole (2E4MZ-CN), a hardening accelerator, was also added in the amount of per hundred ratio (phr). The mixture was dissolved in tetrahydrofuran (THF, about 30 mL) then stirred for 15 min at room temperature. The solution mixture was then transferred onto a sheet of PTFE to remove THF in a vacuum oven at 50 °C for 1 h. The curing process of epoxy resin was performed at 120 °C for 0.1 h, 150 °C for 2 h, and then 165 °C for 3 h, as determined from the DSC curve.

4.2.5. Analyses

A nuclear magnetic resonance (NMR) spectrometer (ECX-400; JEOL, USA) was used to obtain ¹H NMR spectra. Each 5 mg sample was dissolved in 500 μ L of DMSO-d6 and was measured by NMR spectroscopy. The FTIR spectral analysis of bamboo lignin, and their epoxy resins, was performed on an FTIR spectrophotometer (FTIR 420; JASCO Co.,) using KBr pellets (KBr : sample = 200 mg : 1.5 mg). Each spectrum was recorded using 32 scans ranging from 4000 to 500 cm⁻¹, with a resolution of 4 cm⁻¹ in the transmission mode. The thermogravimetric (TG) curve was measured using a TG analyzer (TG/DTA SII EXSTAR 6300; Seiko Instruments Inc., Chiba, Japan) under an atmosphere of nitrogen (heating rate of 5 °C/min) using alumina as a primary standard. The thermal tests were performed in triplicate and average values are shown.

4.3. Results and Disccusions

4.3.1. Synthesis and curing resin

As shown in Table 1, 34 g of acetone-soluble lignin could be extracted per 100 g of exploded sample. In addition, a large amount of holocellulose was recovered, and bamboo was a good sample in this experiment.

NMR and FTIR were measured to confirm the addition reaction of epoxy group to lignin. Comparing Fig. 1 and 2, the peak of CO stretching vibration of the epoxy group was confirmed in the vicinity of 800 cm-1 in FT-IR in Fig. Further, in NMR, a peak peculiar to the epoxy group was detected at 2.5-3.0 ppm, and it was confirmed that the epoxidation of lignin was carried out smoothly.

NMR was also used to confirm the curing reaction. Fig. 3 and 4 compared, Fig. 3 shows a mixture of epoxidized lignin and lignin before curing, and Fig. 4 shows a mixture after curing reaction. After the reaction, since the peak of the epoxy group disappeared, it was confirmed that the curing reaction was carried out. The epoxy group of epichlorohydrin shows an addition reaction to the hydroxyl group of lignin and an epoxy group is introduced. Of the hydroxyl groups of lignin, the phenolic hydroxyl group is the most reactive. For this reason, the epoxy group is first added to the phenolic hydroxyl group, and then the hydroxyl group bonded to the hydrocarbon reacts to form lignin having two epoxy groups. Since the epoxy resin is a compound having a structure having two or more epoxy groups, the epoxidized lignin undergoes a reaction as an epoxy resin.

In the curing reaction, the epoxy group reacts with the hydroxyl group, and the resin and the curing agent polymerize to cure. The epoxy group of the epoxidized lignin is cleaved and reacts with the hydroxyl group of lignin to cause polymerization. Therefore, after the curing reaction, the number of hydroxyl groups and epoxy groups decreases. By seeing these reactive groups by FT-IR and NMR, it can be confirmed whether the curing reaction is progressing steadily.

4.3.2. Characteristic resin

Fig. 5 shows the results of the tensile strength test. Both resin and hardener are derived from lignin and both epoxy resins derived from petroleum and cured products of curing agent are

compared. There was no significant difference in strength, suggesting that lignin-derived epoxy resin can be substituted for strength.

In Fig. 6, in order to examine the heat resistance, the thermogravimetric reduction temperature was measured. The lignin epoxy resin has a somewhat lower heat resistance than that derived from petroleum. However, it exceeded the soldering heat resistant temperature which is the heat resistance sound required for ordinary industrial products, $250 \degree C$. Therefore, it was suggested that lignin epoxy resin can also be used as a substitute for heat resistance.

From the results shown in Fig. 5 and Fig. 6, it was confirmed that the lignin epoxy resin is comparable to the general epoxy resin currently used. This result showed that lignin can be used as an electronic substrate material, which is the main use of epoxy resin.

Table 1 steam-exploded (35 atm and 5 min) bamboo had componenets.

This is a table showing the ratio of water-soluble component, acetone-soluble component and holocellulose from bamboo exploded sample.

component	(%)		
Water soluble component	13.1		
Water insoluble component	86.9		
Acetone soluble component	34.0		
Acetone insoluble component	52.9		
Sodium Chlorite Soluble Component	6.1		
Holocellulose	46.8		



Fig. 1 FT-IR was measured lignin and epoxylated lignin

FT-IR spectrum of acetone extracted lignin and epoxylated lignin that acetone extrated lignin epoxy group added.



Fig. 2

This is the result of ¹H-NMR measurement of acetone extracted lignin (exploded sample at 35 atm 5 min).



Fig. 3

This is the result of ¹H-NMR measurement of acetone extracted lignin was added epoxy group (exploded sample at 35 atm 5 min).



Fig. 4

This is the result of ¹H-NMR measurement of before curing mixture lignin and epoxy lignin.



Fig. 5

This is the result of ¹H-NMR measurement of after curing mixture lignin and epoxy lignin.





Comparison of tensile strength test results of cured products of acetone extracted lignin and epoxy lignin obtained from explosive bamboo and cured products of petroleum derived epoxy resin and curing agent.



Fig. 7

Comparison of thermal weight work test results of cured products of acetone extracted lignin and epoxy lignin obtained from explosive bamboo and cured products of petroleum derived epoxy resin and curing agent. Td 5 is the temperature when the thermal weight loss (-%) is 5%, and Td 10 is the temperature when it is 10%.

4.4. Conclusion

Steam explosion treatment (high temperature and high pressure steam treatment) was used as a pretreatment, and a lignin epoxy resin was synthesized from extracted and separated lignin. Almost all of lignin was extracted from bamboo and used. The synthesized lignin epoxy resin was produced as a cured epoxy resin using lignin as a curing agent. The cured product produced exhibited the same degree of tensile strength and heat resistance as the commercially available epoxy resin cured product derived from petroleum. These values were available as electronic substrate materials. Therefore, it was suggested that lignin is used as an alternative to petroleum resources in industrial products such as electronic substrate materials.

4.5. Reference

Epoxy resin synthesis using low molecular weight lignin separated from various lignocellulosic materials

Chikako Asada, Sunita Basnet, Masaya Otsuka, Chizuru Sasaki, Yoshitoshi Nakamura International Journal of Biological Macromolecules (2015) 74, 413-419

Chapter 5: Analysis of steam-treated biomass extract components

5.1. Introduction

We have explained in the previous chapter that holocellulose and acetone extraction components separated by water vapor treatment (steam explosion treatment and steam cooking treatment) can be used. Separated fractions that may be available are water extractables. Water extraction components include low molecular weight sugars and polyphenols. Organic acids such as hemicellulose-derived acetic acid are produced by hydrolysis of high-temperature and high-pressure steam. These organic acids act as catalysts to break the bonds between cellulose, lignin and hemicellulose and show the utility as delignification treatment. Furthermore, it decomposes the intramolecular bonds of the respective polymers to produce monosaccharides such as glucose and xylose, phenol compounds, and the like.

In this study, we focused on phenolic compounds. There are many kinds of phenol compounds forming lignin, and they are hydrolyzed by water vapor treatment. Phenolic compounds have antioxidative activity, have attracted attention as functional foods, and also attract attention as starting materials for pharmaceutical products. As mentioned in the previous chapter, it is also expected to be used in the field of industrial products such as resin. There are various phenol compounds, and their properties and characteristics are also various. Among them, we focused on antioxidation and conducted research.

The word lifestyle disease has now become a general term. One of these causes is said to be active oxygen. It is one of the biological control mechanisms of the human body, but it is toxic to the human body as soon as it exists excessively. Antioxidant substances have the action of protecting the human body from active oxygen. It is expected that people's health will be maintained by reacting with active oxygen and reducing active oxygen.

In this study, we will study the antioxidant power of components extracted from water from various plant biomass treated by steam treatment.

5.2. Materials and Methods

5.2.1. Materials

Cedar (Cryptomeria japonica), eucalyptus (Eucalyptus globulus), and bamboo (Phyllostachys pubescens) were chopped into wood chips (2–4 cm in length and 1–3 cm in width) and used as plant biomass samples. They (cedar, eucalyptus and bamboo) were treated using various steam treatment conditions to allow delignification and were then used as raw materials for fibrillation.

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

5.2.2. Preparation of water soluble components

The steam treatment 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., 2015). Then, 120 g of biomass samples were introduced into the reactor and exposed to saturated steam at a pressure of 10 atm (183 °C), 15 atm (200 °C), 20 atm (213 °C), 25 atm (224 °C), 30 atm (234 °C), 35 atm (243 °C) and 40 atm (250 °C) for a steaming time of 5 min. The prescribed temperature was reached in a few seconds. After completion of the reaction, the valve of the reactor was gradually opened to remove the steam, and then the steamed chopsticks were removed. The steam-treated biomass samples were ground using a crusher mill (Wonder Crush Mill D3V-10, Osaka Chemical Co. Ltd) at 25,000 rpm for 10 s.

5.2.3. Extraction and separation

The steam-treated biomass samples were extracted with hot distilled water (70 °C) 60-fold of the sample dry weight, at room temperature for 10 seconds and separated into water soluble material and a residue using filter paper (No. 131, φ 185 mm, Advantec Co., Ltd, Tokyo, Japan). Next, the extract was dried in freeze drying.

5.2.4. Analysis

5.2.4.1. DPPH radical scavenging activity and total phenolic contents

Determination of free radical scavenging activity and total phenolic contents was Aksoy et al. (2013) method.

Free radical scavenging activities of solutions of the steam treated-biomass sample extracts in the study prepared in diatilled water at concentrations of 1.0, 0.4, 0.2, 0.1 and 0.05 g/L were determined in accordance with the Shimada (1992) method, which is based on the principle of scavenging the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. DPPH was added to the solutions prepared with plant extracts and stirred. Each mixture was kept in the dark for 30 min and the absorbance was measured at 517 nm against a blank (Shimada et al., 1992).

The total phenolic content of the steam treated-biomass sample extracts was determined according to the Folin–Ciocalteu method (Gamez-Meza et al., 1999). Folin–Ciocalteu reagent was added to the extract. After 5 min, Na2CO3 was added and the mixture was stored at room temperature for 2 h. The absorbance of the mixture was measured at 760 nm against water on a UV spectrophotometer. The results were calculated using the standard calibration curve of (+)-catechin and expressed as (+)-catechin equivalents (g/g-steam treated biomass sample).

5.2.4.2. The amount of sugars

Measurement of the amount of glucose and the amount of xylose was determined using a kit (Glucose kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and Xylose kit (BIOCON(JAPAN)LTD., Nagoya, Japan)). Total sugar content was analyzed using the phenolsulfuric acid method (Sasaki et al., 2011). The amount of carotenoid was determined by the Wellburn (1994) method. The amount of flavonoid was determined by the Nakamura (2016) method.

5.2.4.3. Analysis of extracted components (purification and structural analysis)

The steam treated water soluble samples were analyzed by high-performance liquid chromatography (HPLC) (GE Healthcare Japan, Tokyo, Japan). ODS (Octa Decyl Silyl) column (COSMOSIL 5C18-AR-II Packed Column, 4.6 mmI.D.×250 mm and 20 mmI.D.×250 mm, Nacalai Tesque Co., Ltd., Kyoto, Japan) was used for analysis. The mobile phase was 100% pre-filtered HPLC-grade acetonitrile and Milli-Q water.

Molecular weight of samples were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). LC-MS/MS was measured (LC-MS/MS (API 2000, AB Sciex, Framingham, MA., USA) and column (Scherzo SM-C18, 2.0 mmI.D.×75 mm, Imtakt, Kyoto, Japan)) in a negative ion mode. A nuclear magnetic resonance (NMR) spectrometer (ECX-400; JEOL, USA) was used to obtain 1H NMR spectra. Each 5 mg sample was dissolved in 500 µL of DMSO-d6 and was measured by NMR spectroscopy.

The FTIR spectral analysis of bamboo lignin, and their epoxy resins, was performed on an FTIR spectrophotometer (FTIR 420; JASCO Co.,) using KBr pellets (KBr : sample = 200 mg : 1.5 mg). Each spectrum was recorded using 32 scans ranging from 4000 to 500 cm-1, with a resolution of 4 cm-1 in the transmission mode.

5.3. Results and Discussions

5.3.2. Total polyphenol

Fig. 1 is a graph showing the change in the total amount of polyphenols at a pressure change of 10 to 40 atm for each sample.

The amount of polyphenols is increasing compared to those that were not subjected to all bamboo steam treatments. It is speculated that lignin was hydrolyzed by steam treatment and low molecular weight polyphenol dissolved in water. Especially at 25 atm, sugar and extraction amount are large. At this pressure, decomposition proceeded and reaggregation did not occur and many polyphenols were extracted.

It was shown that 25 atm is the optimum condition for pretreatment in bamboo component separation.

However, with respect to cedar, the untreated one has the greatest amount of polyphenols. As the processing pressure increases, the amount of polyphenol also increases, but this is presumed to be due to decomposition of lignin as well as bamboo.

The filtrate extracted from the untreated cedar has a strong aroma. This was no longer 10 atm. Reduced aromatic phenols and reduced total polyphenol content. In steam treatment, the pressure after treatment is reduced to atmospheric pressure. At this time the steam is discharged from the released valve. At that time, it is considered that the scent component was released together with steam, did not remain in the water extract component, and the amount of polyphenol decreased. (table 1) Cedar is a coniferous tree, coniferous trees are hard in woody structure, and it was shown that high conditions are necessary up to decomposition. Eucalyptus showed high value when untreated, 30 to 40 atm. Eucalyptus is similar to cedar, but eucalyptus is hardwood, the woody structure is softer than coniferous trees, decomposition occurred relatively quickly.

Bamboo that is classified as herbaceous type has decomposed from cedar and eucalyptus, decomposition proceeds from low pressure, and eucalyptus and cedar which are woody species are decomposed unless it is high pressure. There are differences in easiness of decomposition depending on whether it is a conifer tree or a hardwood tree among woody plants, and coniferous trees require treatment under more stringent conditions.

5.3.3. DPPH radical scavenging activity

The EC50 is a value showing antioxidation, and the smaller the value, the stronger the antioxidant power. Since this is a value indicating a concentration showing 50% activity, it is preferable that the value is small. Significant differences appeared between herbs and woody plants. The antioxidant activity of bamboo, which is an herbaceous species, improved by steam treatment. However, cedar and eucalyptus trees, which are woody species, were best untreated and could not exceed steam treated ones. (Fig. 2) This result showed that in woody plants, polyphenols obtained by steam treatment did not produce strong activity, and the components released with steam showed strong activity. However, since the antioxidant capacity increases as the treatment pressure increases, it is suggested that if it can be treated at even higher pressure, a large amount of compound exhibiting activity can be produced and a high antioxidant activity can be exhibited It was.

5.3.4. About sugars

Subsequently, the results of glucose, xylose, total sugar, carotenoid and flavonoid amount are shown in Table 2. First, sugar (glucose, xylose and whole sugar), eucalyptus showed remarkable effect of steam treatment. Xylose has a fast rate of decomposition and is decomposed from hemicellulose at low pressure (low temperature) to produce xylose. However, as the pressure increases, xylose is decomposed to produce an overdecomposition product such as furfural and the amount of xylose decreases.

On the other hand, glucose decomposes on the higher temperature side than xylose. The peak of xylose is 20 atm and the glucose has a peak of maximum yield on the considerably high

temperature side of 35 atm. When the temperature further increased, over-decomposition occurred, an over-decomposition product such as 5'-HMF was formed, and the amount of glucose decreased.

Since the extract was yellowish, carotenoids were measured. As the condition got tighter, it increased and the appearance color became darker. Flavonoids are present in a representative group of compounds with antioxidation. The higher the activity, the more the amount of flavonoid was increased. Furthermore, it increases as the treatment pressure increases, and it is inferred that the flavonoid detected here is one produced by decomposition of lignin.

5.3.5. Analysis extracted bamboo

The bamboo where steam treatment dominated the antioxidant power was analyzed in detail. Extracts of bamboo treated at 25 atm by HPLC were collected. Measured at a wavelength (280 nm) at which a benzene ring or the like is detected and at a wavelength (370 nm) at which a flavonoid or the like is detected and separated. (Fig. 3) For each classified fraction, DPPH radical scavenging activity activity was measured. NMR and FT-IR, LC-MS, and GC-MS were performed to identify compounds contained in the fractions which showed the strongest activity.

The LC-MS result showed that the target compound had a molecular weight of 192. This peak was fragmented by LC-MS / MS. The result is shown in Fig. 4. Fig. 5 shows the results of NMR, and Fig. 6 shows the result of FT-IR.

From Fig. 4, peaks can be confirmed at m / z values of 91.02 and 92.94, which are fragments of toluene and phenol, and it is presumed that 107.99 is cresol from toluene and phenol. First, from these results, it is shown that the target compound is a phenol compound. From the NMR results, peaks are observed at 10.42 and 8.78 ppm, 8.78 ppm is the peak of phenolic hydroxyl group, and 10.42 ppm is aldehyde or carboxylic acid. The peaks at 7.48 and 7.21 ppm are the peaks of the benzene ring, and from the shape and integral values it was shown that it is a trisubstituted benzene ring. Since the peaks of 3.36 and 2.51 ppm are the peaks of the solvent (DMSO) and the peak of 1 to 4 ppm is the peak of the hydrocarbon, it has a benzene ring from NMR, the aldehyde or the carboxylic acid, the hydroxyl group and the hydrocarbon group are substituted on the benzene ring It is inferred that this compound is a compound. (Fig. 5) From the results of FT-IR, stretching vibration of -OH is shown around 3400 cm-1, C = O stretching vibration in the vicinity of 1600 cm-1, and bending vibration of -OH near 1200 cm-1 are shown.

Furthermore, in the vicinity of 1100 cm-1, C = C deformation vibration was shown, and from the peak near 800 cm-1, it was shown that the substitution of the benzene ring was 1,2,3trisubstituted benzene. (Fig. 6) From the facts found by FT-IR and NMR, it is applied to the fragment indicated by LC-MS and it is shown as follows: (1) $C_6H_3(OH)(COOH)(C_4H_7)$ (2) $C_6H_3(OH)(CHO)(C_5H_{11})$ (3) $C_6H_3(OH)(CHO)(C_4H_7O)$. It is conceivable that C = C is present from the length of the intense wavelength or the measurement result, and the possibility of being the structure of (1) is the highest. Therefore, the structure of the compound is considered to be $C_6H_3(OH)(COOH)(C_2H_4CH = CH_2)$.

It is highly probable that the structure that we have deduced this time is a compound generated from lignin. It also suggests that it possesses antioxidant ability because it has a carboxylic acid hydroxyl group and a phenolic hydroxyl group.

Table 1

Volatile component and water extraction component amount (75 $^{\circ}$ C, 10 seconds) and its total value in steaming treatment per gram sample of bamboo, cedar and eucalyptus.

g/g-sample	atm	untreated	10	15	20	25	30	35	40
bamboo	Volatile components	0.000	0.033	0.060	0.120	0.181	0.217	0.250	0.289
	Water extract components	0.058	0.065	0.059	0.129	0.145	0.114	0.079	0.079
	Total	0.058	0.098	0.119	0.249	0.326	0.331	0.329	0.368
cedar	Volatile components	0.000	0.000	0.002	0.022	0.044	0.071	0.148	0.169
	Water extract components	0.005	0.013	0.041	0.052	0.095	0.035	0.036	0.027
	Total	0.005	0.013	0.043	0.074	0.139	0.106	0.184	0.195
eucalyptus	Volatile components	0.000	0.036	0.063	0.101	0.161	0.196	0.207	0.237
	Water extract components	0.032	0.026	0.090	0.103	0.074	0.055	0.041	0.065
	Total	0.032	0.062	0.153	0.204	0.236	0.251	0.248	0.302

Table 2

The amounts of glucose, xylose, total sugar, carotenoid and flavonoid in water extract component solution (1 g / L) of bamboo, cedar and eucalyptus

	atm	untreated	10	15	20	25	30	35	40
glucose (g/L)	bamboo	0.2255	0.0913	0.0618	0.0403	0.0389	0.0443	0.0456	0.0550
	cedar	0.0712	0.0443	0.0443	0.0644	0.0483	0.0806	0.0644	0.0193
	eucalyptus	0.0430	0.0309	0.0309	0.0376	0.0215	0.0524	0.0752	0.0389
xylose (g/L)	bamboo	0.0031	0.0019	0.0054	0.0307	0.0474	0.0024	0.0094	0.0057
	cedar	0.0043	0.0234	0.0024	0.0170	0.0373	0.0071	0.0035	0.0024
	eucalyptus	0.0002	0.0241	0.0889	0.1637	0.0677	0.0092	0.0035	0.0031
total sugar (g/L)	bamboo	0.3952	0.3161	0.4753	0.7482	0.4095	0.1643	0.2438	0.2291
	cedar	0.2878	0.5272	0.6278	0.7338	0.9002	0.4365	0.3158	0.3158
	eucalyptus	0.1402	0.6735	0.8603	0.7239	0.3785	0.3857	0.3959	0.3669
carotenoid (mg/L)	bamboo	0.1518	0.1625	0.1099	0.0940	0.2275	0.2032	0.2218	0.2014
	cedar	0.7878	0.1199	0.0818	0.1063	0.0765	0.1442	0.1512	0.1667
	eucalyptus	0.7721	0.0339	0.0350	0.0520	0.0814	0.1526	0.1418	0.1669
flavonoid (g/L)	bamboo	0.0112	0.0094	0.0075	0.0116	0.0204	0.0270	0.0234	0.0235
	cedar	0.0272	0.0103	0.0061	0.0436	0.0046	0.0151	0.0166	0.0197
	eucalyptus	0.0769	0.0046	0.0061	0.0086	0.0134	0.0248	0.0243	0.0267





EC50 value of DPPH radical scavenging activity activity of water extract solution (1 g / L) under each pressure condition of bamboo, cedar and eucalyptus.





Amount of total polyphenol in water extraction solution (1 g / L) under each pressure condition of bamboo, cedar and eucalyptus.





HPLC results by reversed phase column. UV absorption at 370 nm of a water extract solution (1 g / L) of steamed sample (25 atm, 5 min and 10 sec) and untreated (60 sec) water extraction solution. The conditions of solvent (A: water, B: acetonitrile) and% B were measured at the flow rate of 1 ml / min under the following conditions. 0 - 9 min: 5%, 9 - 11 min: 5-30%, 11 - 36 min: 30 - 60%, 36 - 40 min: 100%.



Fig. 4

LC-MS/MS spectrum peak of the most active fraction among the fractions obtained by purifying bamboo water extract (25 atm 5 min) by HPLC.



Fig. 5

¹H-NMR spectrum peak of the most active fraction among the fractions obtained by purifying bamboo water extract (25 atm 5 min) by HPLC.



Fig. 6

FT-IR spectrum peak of the most active fraction among the fractions obtained by purifying bamboo water extract (25 atm 5 min) by HPLC.

5.4. Conclusion

As for bamboo, by treating with steam, the antioxidant capacity and polyphenol content of the extract increased, indicating the usefulness of the treatment. However, it did not show much effect in woody species such as eucalyptus and cedar. However, if the treatment conditions were further strengthened, the possibility of increasing in polyphenol amount was shown. Separation of xylose and glucose monosaccharides showed that separation occurred on the low temperature side of xylose, glucose was found to occur on the high temperature side, and at higher temperatures it decomposed due to excessive decomposition.

The compound contained in the bamboo extract was found to be a phenol compound and was found to be a compound having a molecular weight of 192 and having a trisubstituted structure. Since the extract contained substances showing sugar and antioxidant, it could be expected to be used in the food field.

5.5. Reference

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