Studies on preparation and characterization of TEMPO-oxidized cellulose nanofibrils from non-wood resources

（非木材資源からの **TEMPO** 酸化セルロースナノフィブリルの調製と特性解析に関する研究）

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LIST OF ABBREVIATIONS

AFM	Atomic force microscopy
Ara	Arabinose
C.I.	Crystallinity index
DP	Degree of polymerization
Gal	Galactose
Glc	Glucose
HB	Hemp bast
HBH	Hemp bast holocellulose
HPLC	High-performance liquid chromatography
Man	Mannose
QSBG	Queen Sirikit Botanic Garden
Rha	Rhamnose
S.D.	Standard deviation
SEM	Scanning electron microscopy
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl radical
TOCNs	TEMPO-oxidized cellulose nanofibrils
TO-HBHs	TEMPO-oxidized hemp bast holocelluloses
Xyl	Xylose

1. INTRODUCTION

The quantitative and qualitative expansion of utilization of renewable natural resources in place of petroleum-based materials has been increasingly gaining attention in recent years for the establishment of a sustainable society. Wood is in great demand for diverse applications such as house building, furniture, paper, and also raw materials for green energy generation. Illegal harvesting of wood has caused worldwide depletion of forests, contributing to global warming. Thus, it is significant that not only wood resources but also non-wood plant resources are fully exploited. Especially in developing countries, non-wood and agricultural cellulose resources will be of significance as natural resources to revitalize regional economies.

The scientific community has paid considerable attention to non-wood and annual agricultural crops as renewable resources. Because agricultural crops are produced every year and are fast-growing, available in abundance, and are inexpensive sources of cellulose fibers, they have been used as starting materials for many industrial products (paper, films, fibers, additives, etc.). Cellulose fibers have been isolated primarily from wood resources through pulping processes on a large scale, in which wood chips are treated with chemicals under high temperature and high pressure conditions. To increase brightness or purity of the cellulose fibers, bleaching process with multi-stage sequence is often applied to the wood cellulose fibers with bleaching chemicals under various pH and temperature conditions which it is environmentally unfriendly process. Thus, low lignin content of non-wood plant resources is desirable because it mean less environmental impacts of pulping and bleaching processes of the non-wood plant resources. Furthermore, utilization of agricultural crops and residues, such as flax, hemp, jute, kenaf, sisal, bamboo, oil palm, and bagasse for industrial applications would be valuable and additional businesses for farmers, particularly in developing countries in Southeast Asia.

Cellulose is one of the structural components of plant cell walls and has become much more significant in bio-based nanotechnology. Cellulose is the most abundant and renewable polymer. Cellulose molecules are composed of β-1,4-linked D-glucosyl units and are biosynthesized as highly crystalline microfibrils, consisting of fully-extended molecular chains. Well-ordered hydrogen bonds as well as hydrophobic interactions are present between the cellulose molecules within the crystalline microfibrils.

Oxidation of native celluloses using TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical) as a catalyst is a powerful technique for dispersion of the individual cellulose microfibrils. The cellulose microfibrils originating from wood have uniform widths of ~3 nm and lengths of a few microns. The resulting TEMPO-oxidized wood cellulose nanofibers (TOCNs) can form a range of bulk materials such as films, fibers, hydrogels and aerogels, which are promising as new bio-based nanomaterials.

Regioselective oxidation of the C6-primary hydroxyl groups exposed on the wood cellulose microfibrils to carboxylate groups can be achieved by TEMPO/NaBr/NaClO system in water at pH 10 and room temperature. Aqueous TOCN dispersions are obtained by gentle mechanical disintegration of the oxidized celluloses in water. Although various pretreatment of wood cellulose for dispersing the microfibrils have been reported so far, still only TEMPO-mediated oxidation allows wood celluloses to be completely dispersed as individual cellulose microfibrils. This oxidation system is a promising process for preparation of the new bio-based nanofibers from other cellulose sources such as non-wood fibers.

Therefore, it is significant to investigate TEMPO-mediated oxidation of non-wood agricultural resources such as hemp bast, bagasse and bamboo fibers. These non-wood sources have not been investigated in detail as starting materials for TEMPO-mediated oxidation before; it has not been revealed how the TEMPO-mediated oxidation affects the morphology, chemical and physical properties, and nanofibrillation of the non-wood cellulose, including fundamental properties of the resulting TOCN films.

This thesis includes six chapters, starting with the introduction followed by the objectives. Chapter three is a literature review which describes in detail the cellulose of natural fibers in woody and non-woody plants, and the preparation of nanocellulose using TEMPO-mediated oxidation system. Chapter four is for TEMPO-mediated oxidation of hemp bast holocellulose as a representative of non-wood cellulosic material. Chapter five deals with the comparative characterization of TOCN films prepared from three different non-wood celluloses that is hemp bast, bagasse and bamboo fibers. Finally, the thesis conclusions are described as a summary in chapter six.

2. OBJECTIVES

The purpose of this project is targeted to utilize non-wood cellulose fibers in agricultural crops and residues using TEMPO-mediated oxidation system. This investigation is significant for development of non-wood plant resources as value-added products.

This thesis addresses the following issues:

1. Preparation of cellulose nanofiber materials from hemp holocellulose by TEMPO-mediated oxidation system
2. Conversion of the hemp celluloses to optically transparent and mechanically strong films with gas-barrier function.

Expected outputs and benefits:

1. To develop hemp and other non-wood plants in the field of lignocellulose nanomaterials, and to campaign for hemp and non-wood plant-based products to be used widely in Thailand
2. To develop new forms of bio-based packaging with high functionalities in food and agricultural industry

3. LITERATURE REVIEW

From early in the 19th century, sustainability and environmental safety for the products and energy production have been considered in this world. Nowadays, the renewable feedstock has received increasing investigation as the materials in place of the current petro-chemicals and fossil fuels with the reason of depleting natural resources, regulations on utilization of synthetic materials, growing environmental awareness and economic considerations.

There are hundreds of reports concerning the use of natural and renewable feedstock such as wood, non-wood plants and agricultural crops, and their by-products. These lignocellulosic bioresources are now gaining a lot of attention from many stakeholders and being widely used in various industrial products. These bio-based materials have lower environmental impact than the petroleum-based materials.

This chapter presents general introduction on cellulose from natural plant fibers and the nanocellulose materials. The preparation of TEMPO-oxidized cellulose from natural fibers is also included.

3.1 Cellulose of natural fibers

Structure of natural plant fibers are highly complicated and differ the composition and organization depending on the origin. Most of the natural fiber is a composite in which rigid cellulose microfibrils are embedded in a soft matrix of lignin and hemicelluloses, containing small amounts of inorganics, proteins, and extractives [1-2].

In 1838, cellulose was first discovered by a French chemist, Anselme Payen. He had indicated that cellulose is long molecular chains comprised of the small repeating units [2,3]. Nowadays, it has been well known that cellulose is the most abundant polymer material, and cellulose-based materials have been used over 150 years in various applications such as food, paper, and pharmaceuticals [2,4].

The chemical formula of cellulose is $(C_6H_{10}O_5)_n$; cellulose consists of D-anhydroglucopyranose units which linked by β-1, 4-D-glucosidic bonds. The number of glucose units in cellulose molecules is referred to the degree of polymerization (DP). The native cellulose in plants has DP values ranging from several hundred to over ten thousands, and the DP of cellulose molecules depends on the origin of the cellulose and also on the method that was used in DP determination [1-2]. The chemical structure of cellulose molecule is illustrated in Figure 1. Cellulose has three hydroxyl groups in each repeating unit that are available for chemical modification. Beside, each cellulose molecular chain has different chemical structures at the both ends of chain. The one end has a D-glucopyranosyl group or reducing end. The other end has a D-glucopyranose residue or non-reducing end [1-2].

The long cellulose molecules are fully extended and directionally aligned to form crystalline microfibrils. A part of the crystalline packing of cellulose are somehow disordered. The microfibrils have lengths of over a few micron and their lengths depend on the origin [2,3,5]. Table 1 shows cellulose contents of various plant materials [5,6-11].

Table 1. Chemical composition of non-wood plant fibers and wood fibers.

Fibers	Chemical composition, %				
	Cellulose	Hemicellulose	Lignin	Ash	References
Non-wood					
Bast					
Hemp	70-74	18-22	4-6	-	[6,7]
Jute	61-72	13-20	12-13	-	[6,7]
Flax	71	19-21	2-3	-	[6,7]
Kenaf	44-57	22	15-19	-	[5,6]
Ramie	69-76	13-17	0.6-1	-	[6,7]
Cane					
Bamboo	26-43	30	21-31	-	[5,8]
Sugar	32-48	17	19-24	1-5	[5,9]
Stalk					
Rice	28-40	23-28	12-14	14-20	[7,10]
Wheat	33-38	26-32	17-19	6-8	[10]
Rye	33-50	-	14-19	2-5	[5]
Seed hull					
Cotton	85-92	6	0.7-1.6	-	[5-7]
Leaf					
Sisal	47-62	12	7-9	0.6-1	[6,7]
Palm	35	28	27	-	[11]
Wood					
Hardwood	38-49	19-26	23-30	<1	[5]
Softwood	40-45	7-14	26-34	<1	[5]

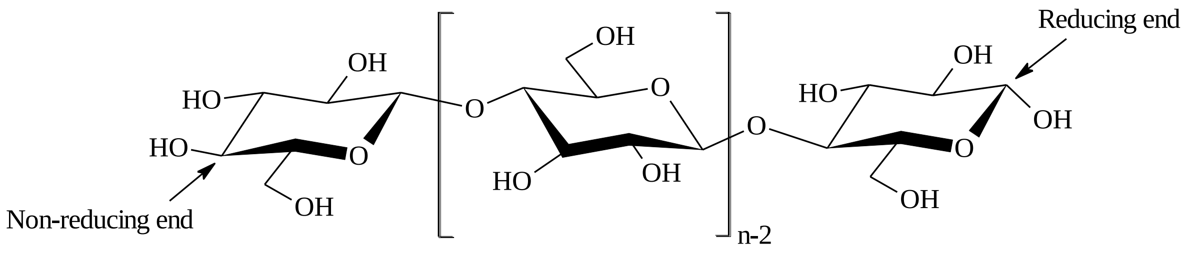


Figure 1. Chemical structure of cellulose

3.2 Nanocellulose materials

Cellulose is biodegradable, low cost, and renewable. Wood is the major source in pulp and paper manufacturing. There are hundreds of reports on wood cellulose fibers, and wood cellulose has gained a lot of attention especially in the field of the nanocellolose materials. The nanocellulose has been getting tremendous attention since material properties of cellulose drastically changes as the dimensions of cellulose materials are decreased to the nano scale [12-13].

During the biosynthesis of the plants, the cellulose chain bristles with polar -OH groups. These groups form many hydrogen bonds with OH groups on adjacent chains, bundling the chains together. The chains also pack regularly in places to form hard, stable crystalline regions that give the bundled chains with higher stability and strength [12-14]. Figure 2 shows the schematic structure of cellulose microfibrils pattern.

Cellulose fibers are classified to their origins; not only wood such as poplar trees [6] and radiata pine [16], but also agricultural crops including bamboo [17], cotton [18], wheat straw, soy hulls [19], hemp [20], sisal [21], pineapple leaf [22], coconut husk fibers [23], banana rachis [24], sugar beet [25-26] and palm leaves [27] are the origins of cellulose fibers. In addition, some bacteria [28] and sea animals [29-30] produce cellulose. In this review, I would focus on only plant cellulose. Many studies have been done on producing nanocelluloses from various natural fibers. The typical diameters of these nanocelluloses are between 2 to 40 nm. The degrees of nanofibrillation of the natural cellulose fibers depend on the degree of polymerization and morphology of the original cellulose. The degrees of nanofibrillation are also related to the methods used for nanofibrillation [5,31].

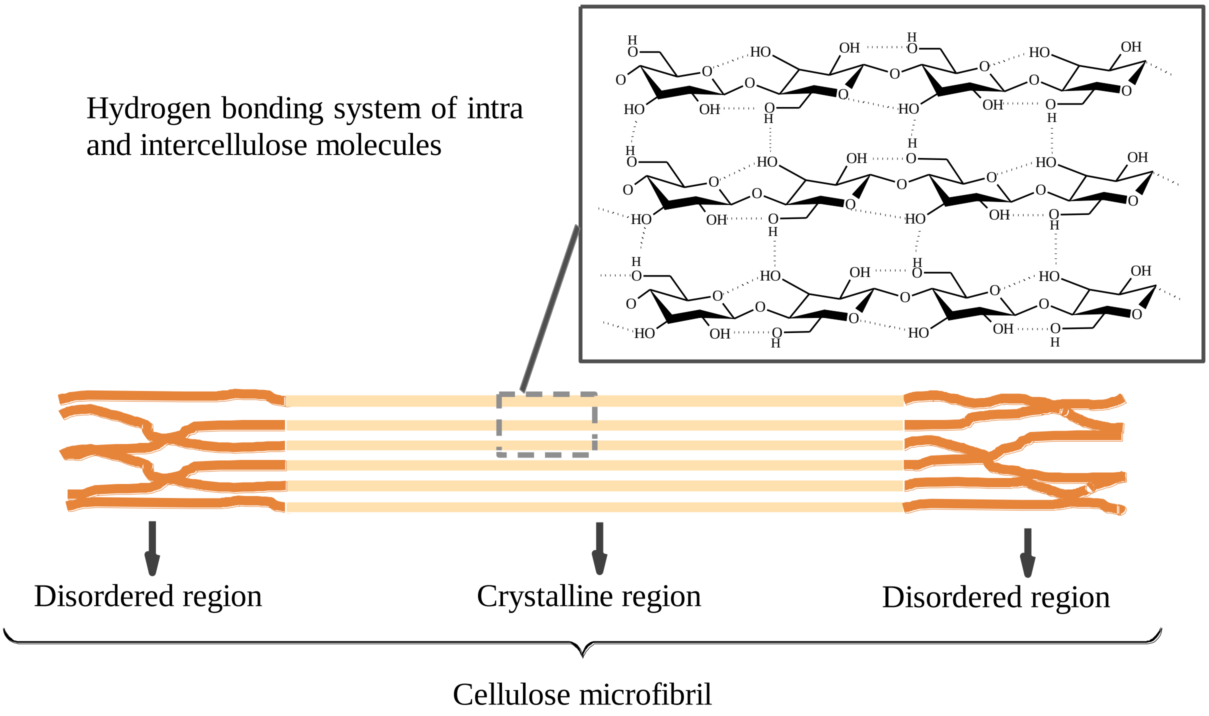
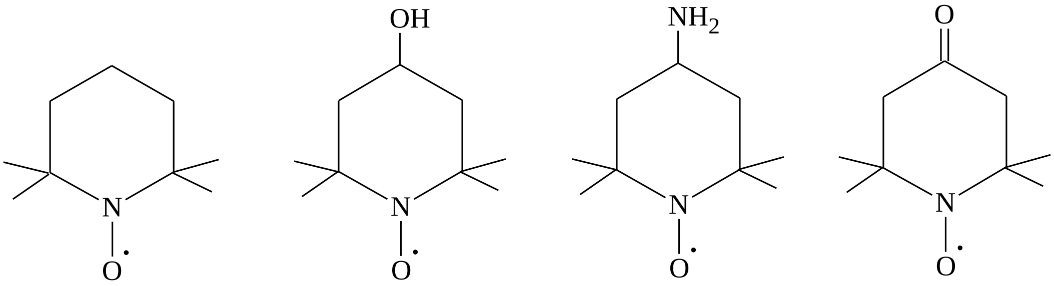


Figure 2. Structure of cellulose microfibrils

3.3 TEMPO-oxidized cellulose

TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) is a stable nitroxide radical and is soluble in water. Figure 3 shows a variety of TEMPO derivatives. These radical species are utilizable in catalytic carbohydrate oxidation [32]. TEMPO and its derivatives can regioselectively oxidize the hydroxymethyl groups of water-soluble polysaccharides such as starch, amylodextrin and pullulan, and the secondary hydroxyls remained unoxidized.



(1) TEMPO

(2) 4-hydroxyl-TEMPO

(3) 4-amino-TEMPO

(4) 4-oxo-TEMPO

Figure 3. Structures of TEMPO derivatives

1) 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)
2) 4-hydroxyl-2,2,6,6-tetramethylpiperidine-1-oxyl (4-hydroxyl-TEMPO)
3) 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (4-amino-TEMPO)
4) 4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl (4-oxo-TEMPO)

Recently, the use of the catalytic oxidation system with TEMPO followed by very mild mechanical treatment was found to be the most efficient method to convert native cellulose materials such as cotton and bleached wood pulp into nanofibrilar celluloses [33–35]. The most common procedure for this oxidation is to add a primary oxidant, co-catalyst, NaBr, to cellulose/water suspensions in the presence of TEMPO at pH 10 and room temperature. This method can selectively oxidize the C6 primary hydroxyl groups exposed on the surface of the cellulose microfibrils and converted to carboxylate groups via aldehydes formed as intermediates by consuming only NaClO and NaOH [34]. The reaction scheme of the TEMPO-mediated oxidation is shown in Figure 4. The TEMPO-oxidized cellulose can be mechanically disintegrated into the individual microfibrils with ~3 nm width and a few microns in length.

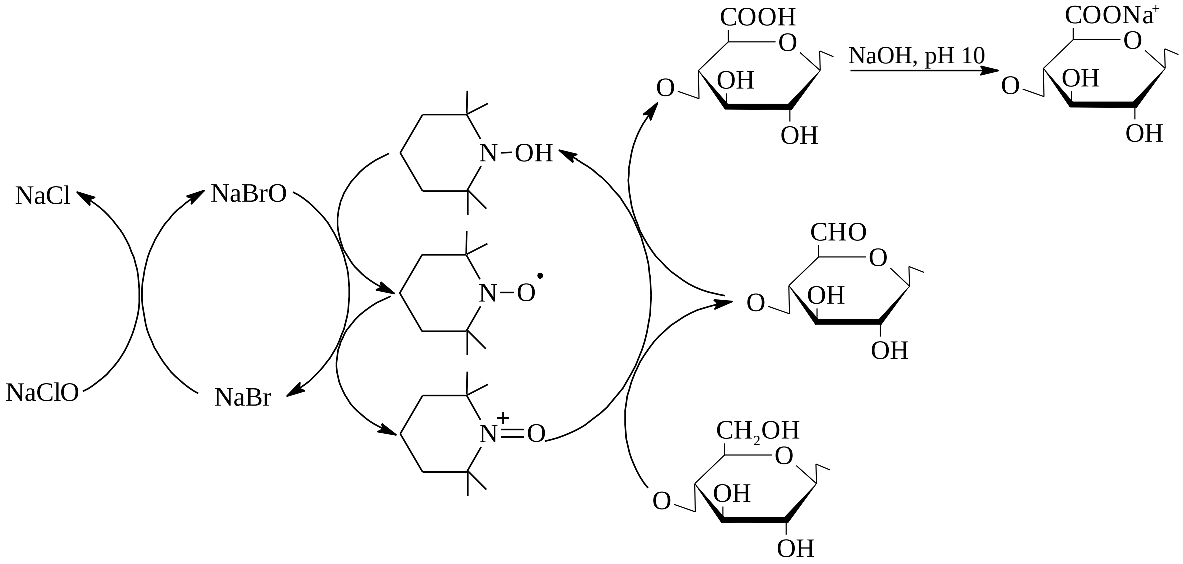


Figure 4. TEMPO-mediated oxidation of cellulose under alkaline condition.

In 2004, Prof. Isogai’s group in the University of Tokyo has first reported on the effect of TEMPO-mediated oxidation on the chemical and crystal structures of native cellulose. The use of NaClO varied from 0–9.67 mmol g^{-1} of cellulose together with TEMPO and NaBr at pH 10.5 and room temperature. The results showed that the more NaClO is added, the more carboxylate groups are formed in the solid native celluloses with preserving the crystalline structure. However, this oxidation system significantly influences the degree of polymerization of the cellulose when an excess amount of NaClO is added; accordingly, the DPv is dramatically decreased [36].

Saito et al. (2007) applied the TEMPO-oxidation system to never-dried cellulose at pH 9.5, 10 and 10.5, respectively. It was shown that pH 10 is the optimal condition for shortening the reaction time; at pH 10, cellulose was consumed NaClO of 5 mmol g^{-1} per gram of cellulose for 130 minutes while at pH 9.5 and 10.5, the reaction times were 285 and 195 minutes, respectively [34].

Saito et al. (2006) also reported that the efficiency of TEMPO oxidation depends on the structures of raw cellulose materials. They applied the TEMPO-oxidation to wood pulp, cotton linters, tunicate, bacterial cellulose, ramie and spruce holocellulose, and calculated the oxidation efficiencies to be 70, 95, 62, 85 and 96%, respectively [33].

3.4 Summary

On the basis of research in TEMPO-mediated oxidation system, it is indicated that there is potential for producing individualized nanofibrils from natural fibers with specific properties at the commercial levels. Significantly, TEMPO-oxidized cellulose could be dispersed in water more efficiently under suitable conditions than other celluloses reported so far. Moreover, natural fibers used as starting materials in this system strongly influence the properties of nanofibrils. In view of better utilization of natural fibers, we expect to use other fiber sources from agricultural crops, which may be utilized in producing nanofibrils for high functional properties as packaging films.

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4. TEMPO-MEDIATED OXIDATION OF HEMP BAST HOLOCELLULOSE TO PREPARE CELLULOSE NANOFIBRILS DISPERSED IN WATER

ABSTRACT

Hemp bast holocelluloses fiber (*Cannabis sativa L.* Subsp. Sativa) was oxidized by TEMPO-mediated oxidation at various NaClO addition levels in water at pH 10. When carboxylate contents of the oxidized products were 1.5-1.7 mmol/g, TEMPO-oxidized cellulose nanofibrils almost completely dispersed at the individual nanofibril level were obtained by mechanical disintegration of the TEMPO-oxidized hemp bast holocelluloses in water, where the nanofibrillation yields were 98-100%. The sugar composition analysis revealed that most of hemicelluloses originally present in the hemp bast holocellulose were degraded and removed from the solid oxidized products, providing almost pure TEMPO-oxidized celluloses. X-ray diffraction patterns of all TEMPO-oxidized hemp bast holocelluloses had the same cellulose I crystal structure and similar crystallinity indices and crystal widths, indicating that carboxylate groups formed by the oxidation were selectively present on the crystalline cellulose microfibril surfaces in the holocellulose. However, the weight recovery ratios and viscosity-average degrees of polymerization of the TEMPO-oxidized hemp bast holocelluloses decreased to 69-59% and 470-380, respectively, when their carboxylate contents increased to 1.5-1.7 mmol/g by the TEMPO-mediated oxidation. Atomic force microscopy height images showed that the nanofibril widths were 2.7-2.9 nm, and the average nanofibril lengths decreased from 590 to 400 nm as the NaClO addition level was increased from 7.5 to 12.5 mmol/g in the TEMPO-mediated oxidation.

4 TEMPO-mediated oxidation of hemp bast holocellulose to prepare cellulose nanofibrils dispersed in water

4.1 INTRODUCTION

Cellulose is a naturally occurring and highly crystalline polysaccharide with fibrous morphologies mostly present as one of the major plant components, and is expected to be used as the most abundant and renewable carbon resource on earth for establishment of sustainable society [1,2]. Plant cellulose microfibrils have lengths and widths of greater than 1 μm and approximately 3 nm, respectively, and thus have high aspect ratios. Moreover, cellulose microfibrils have high elastic moduli (~140 GPa) due to high crystallinities [3]. These unique properties provide potential applications of cellulose microfibrils as bio-based nanomaterials in various high-tech and commodity fields.

In this context, nanofibrillated celluloses and nanocrystalline cellulose whiskers have recently been attracted much attention because of their promising properties as finely molecular-assembled, highly crystalline and bio-based nanomaterials [4-7]. Nanofibrillated celluloses are prepared by mechanical disintegration of native cellulose fibers and pre-treated native celluloses in water, and generally have fibril lengths >200 nm [6,7]. On the other hands, nanocrystalline cellulose whiskers are prepared by acid hydrolysis of native celluloses with, for instance, 64% sulfuric acid and the successive mechanical disintegration of the water-insoluble hydrolyzed products in water, and have spindle-like morphologies and lengths < 100 nm [4,6].

Many cellulose resources have been studied to prepare and characterize nanofibrillated celluloses, which are prepared by repeated mechanical disintegration treatment in water. Mild cellulase treatment or partial carboxymethylation has been applied to wood chemical pulps as a pre-treatment to improve fibrillation efficiency to decrease energy consumption [6]. When never-dried wood holocelluloses are used as the starting materials, nanofibrillation proceeds more smoothly compared with once-dried wood pulps [8,9]. As non-wood cellulose recourses, bamboo [10,11], rice and wheat straws [9,12], cotton [13], hemp bast [14], sisal [15], pineapple leaf [16], coconut husk fibers [17], banana rachis [18], bagasse [19], and sugar beet [20,21] have been used for preparation of nanofibrillated celluloses.

However, since the above nanofibrillated celluloses generally consist of some bundles of cellulose nanofibrils, it has been difficult to prepare completely individualized cellulose nanofibrils with high aspect ratios without serious damages in molecular weight or crystal structures. On the other hand, it has been found that mostly individualized cellulose nanofibrils with high aspect ratios can be prepared from wood celluloses in high yields by TEMPO-mediated oxidation under suitable conditions and successive gentle mechanical treatment of the oxidized celluloses in water [7,22-24]. The obtained TEMPO-oxidized cellulose nanofibrils (TOCNs) have uniform widths of 3-4 nm, high aspect ratios, and high elastic moduli of 145 GPa [7,25]. Therefore, TOCNs would have potential application as new bio-based and functional nanomaterials.

Non-wood cellulose resources have been increasing great interest as alternatives of wood celluloses particularly in developing countries, because non-wood celluloses can be used also for productions of paper, board, textile and other functionalized and commodity goods. Hemp is an annual herbaceous plant in *Cannabis sativa* species, flourishes in tropical areas, and grows first to 2-4 m in height in approximately three months. The hemp stem can be converted to cellulose fibers as commercial products by pulping and bleaching processes at the industrial scale [19]. Thus, hemp bast is one of the promising non-wood cellulose resources [19,26-28]. TEMPO-mediated oxidation has been applied to palm leaf and hemp bast fibers as chemical modification of the cellulose fibers [29,30]. Particularly, Milanovic et al. [30] studied TEMPO-mediated oxidation of lignin-containing hemp bast fibers in detail under various conditions to add new functionalities to hemp fibers for textile use. However, there have been no reports concerning TEMPO-mediated oxidation of hemp bast holocellulose fibers for conversion of the oxidized products to individualized cellulose nanofibrils dispersed in water.

In this work, therefore, a hemp bast holocellulose was prepared from hemp bast, and TEMPO-mediated oxidation was applied to the hemp bast holocellulose under various conditions. Carboxylate and aldehyde contents, degrees of polymerization, weight recovery ratios, X-ray diffraction patterns and sugar compositions of the TEMPO-oxidized hemp bast holocelluloses prepared under various conditions were studied. Moreover, the TEMPO-oxidized hemp bast holocelluloses were mechanically disintegrated in water, and degrees of individualization of celluloses nanofibrils prepared from the TEMPO-oxidized hemp bast holocelluloses were evaluated from light transmittances of the aqueous dispersions. Average widths and lengths of these cellulose nanofibrils were measured from their atomic force microscopy (AFM) images.

4.2 EXPERIMENTAL

4.2.1 Materials

The hemp (*Cannabis sativa* L. Subsp. Sativa) bast sample used was kindly provided by Queen Sirikit Botanic Garden (QSBG) Project in Chiang Mai Province, Northern Thailand. The hemp bast separated from the stem was air-dried, cut into small pieces 5-10 mm in length by scissors, and de-waxed by stirring in an acetone/water mixture (9:1 by vol.) at room temperature for 1 day, thus obtained were washed thoroughtly with distilled water by filtration using a glass filter 14-40 µm in pore size. The moisture content of the specimen was determined by drying a corresponding specimen to constant weight in oven at 105±3°C, placing in a desiccator for a half hour and weighing. The percent of moisture in the sample was calculated as below:

$$\text{Moisture content, \%} = \left(\frac{A - B}{A}\right) \times 100$$

When

A = weight of specimen at 23°C and 50% R.H.

B = oven-dry weight of specimen

TEMPO, sodium bromide, sodium chlorite, a 13% sodium hypochlorite solution, sodium hydroxide, acetone and other chemicals and solvents were of laboratory grade (Wako Pure Chemicals, Co. Ltd., Japan) and used without further purification.

4.2.2 Preparation of holocellulose (Chlorite holocellulose)

According to the Wise method [31], to 5 g of de-waxed hemp bast, added 160 mL of distilled water, 1.0 mL acetic acid and 2 g of sodium chlorite in a 250 mL Erlenmeyer flask. An optional 25 mL Erlenmeyer flask was used as a cap of the reaction flask. The mixture was heated on an oil bath at 75°C for 1 h with shaking. This delignification treatment was repeated four times with fresh chemicals to prepare hemp bast holocellulose. At the end of reaction, the flask was cooled and the hemp holocellulose was thoroughly washed with water by filtration on a glass filter 14-40 μm in pore size until the yellow color and the odor of chlorine dioxide were removed. The hemp bast holocellulose was diluted with deionized water to 2000 mL (1.2% consistency) and disintegrated in water using a domestic blander at 3000 rpm until all fiber bundles were dispersed in order to prepare individualized hemp bast holocellulose fibers, which was kept in the wet state (~10% consistency) at 4°C in refrigerator before use. Samples were oven dried for 3 h at 105±3°C, placed in a desiccator for a half hour, and weighed. The yield of holocelullose was calculated as below:

$$\text{Holocellulose content, \%} = \left(\frac{\text{C}}{\text{D}}\right) \times 100$$

When

C = oven-dry weight of holocellulose residue

D = oven-dry weight of the original specimen

4.2.3 TEMPO-mediated oxidation of hemp bast holocelluloses

The wet hemp bast holocellulose (HBH) fiber corresponding to 1 g dry weight was suspended in water (100 mL), containing TEMPO (0.016 g) and sodium bromide (0.1g). TEMPO-mediated oxidation was started by adding a designed amount of NaClO (3, 5, 7.5, 10 and 12.5 mmol g^{-1} of HBH) to the slurry. The slurry was stirred at room temperature and pH 10 by addition of 0.5M NaOH using a pH stat until no NaOH consumption was observed [23,24]. The TEMPO-oxidized hemp bast holocelluloses (TO-HBHs) thus obtained were washed thoroughly with distilled water by filtration using a glass filter 14-40 μm in pore size and stored in the wet state at 4°C before further treatment or analysis [7,23,24]. Weight recovery ratios of the TO-HBHs were calculated from their dry weights, which were measured after freeze-drying and then heating at 105±3°C for 3 h. The weight recovery ratios of TO-HBHs were calculated as below:

$$\text{Weight recovery ratios, \%} = \left(\frac{\text{E}}{\text{F}}\right) \times 100$$

When

E = oven-dry weight of TEMPO-oxidized cellulose

F = oven-dry weight of the original holocellulose

4.2.4 Nanofibrillation of TEMPO-oxidized hemp bast holocelluloses

The never-dried TO-HBH was suspended in water (20 mL) at a 0.1% (w/v) solid content, and the slurry was homogenized at 7500 rpm for 1 min at room temperature using a double-cylinder-type homogenizer (Physcotron, Microtec Nition Co. Ltd., Japan) [32]. The gel thus obtained was then sonicated for 4 min using an ultrasonic homogenizer with a 7-mm-diameter probe tip at 19.5 kHz and 300 W output power (US-300T, Nissei, Japan) to prepare an aqueous dispersion of TO-cellulose nanofibrils. Nanofibrillation yields were calculated as weight percentages of the TO-cellulose nanofibrils present in the supernatant fractions after centrifugation of the dispersions at 12,000g for 20 min [32].

4.2.5 Analyses

The neutral sugar composition analysis was carried out to the HBH and TO-HBHs by two-step acid hydrolysis with 72 and 3% sulfuric acid treatments [33]. First of 0.1 g of each sample, 72% H_2SO_4 (1 ml) was added and then the slurry was kept at 30°C for 1 h in water bath. The slurry was diluted to 3% H_2SO_4 with distilled water, and heated at 121°C for 1 h. An adequate amount of 2% myoinositol used as an internal standard was added to the solution containing the hydrolyzates. Sulfuric acid in the solution was removed as $CaSO_4$ precipitates by an excess addition of $CaCO_3$ and the successive centrifugation. After concentration of the supernatant by evaporation, acetonitrile was added to the solution to adjust the volume ratio of acetonitrile:water to 1:1. The solution (~1 mL) was filtered through a 0.45μm poly(tetrafluoroethylene) membrane filter (Millex-LH, Millipore, USA), and the filtrate (20 μL) was injected to a high-performance liquid chromatography (HPLC) system. The HPLC system had one column of Shodex Asahipak NH2P-50 4E (φ 4.6 mm × 250 mm) and a refractive index detector (Optilab T-rEx, Shodex, Wyatt Technology, USA). Acetonitrile/water (3:1 by vol.) was used as a mobile phase at a flow rate of 1.0 mL/min at 30°C [34]. The amounts of monosaccharides in the samples were determined using calibration curves obtained beforehand.

The lignin and cellulose contents in HBH were measured by the micro Kappa number [35] and α-cellulose methods [36], respectively. The residual lignin content of hemp bast holocellulose fiber was determined using the micro kappa number technique according to the method reported by Okita et al., [35]. The micro kappa number evaluation is applicable to very small lignin content samples with low permanganate consumption. The test sample was disintegrated in water until homogenous dispersion, then the total volume was adjusted with distilled water to 80 ml, and a mixture of 10 ml of 4 N sulphuric acid and 10 ml of 0.1 N potassium permanganate were added to the slurry at 25±2°C. After stirring for 10 minutes, 2 ml of 1.0 N potassium iodide was added. The liberated iodine was titrated with 0.1 N sodium thiosulfate using a starch indicator.

Alpha cellulose content of hemp bast holocellulose fiber was determined using 1 g of oven dried hemp bast holocellulose fiber, which was treated with 25 ml of 17.5 % NaOH solution at 20°C in a water bath for 30 min. The mixture was mixed with a glass rod until the sample saturated with the NaOH solution. Then, 25 ml of distilled water was added and the mixture was allowed to stand at 20°C for 5 min before filtering the sample using a crucible with medium porosity and washing it with water. Finally, the sample was dried in an oven at 105±3°C. The α-cellulose content of in the sample was calculated as below:

$$\alpha\text{-cellulose}, \% = \left(\frac{G}{H}\right) \times 100$$

When

G = oven-dry weight of α-cellulose

H = oven-dry weight of the original holocellulose

A part of TO-HBH was post-oxidized with $NaClO_2$ in water at pH 4-5 for 2 days to convert C6-aldehyde groups partly formed and present as intermediates in the TO-HBH to C6-carboxylate groups. The carboxylate contents of freeze-dried TO-HBHs and post-oxidized TO-HBHs were determined by electric conductivity titration method to obtain carboxylate and aldehyde contents of the TO-HBHs according to a previously reported method [7,23,24,32,37].

The freeze-dried HBH and post-oxidized TO-HBH samples (0.04 g each) were dissolved in 0.5M copper ethylenediamine. Inherent viscosities of the sample were measured using a capillary viscometer at 20°C, and the values were converted to viscosity average degrees of polymerization (DP_v) according to a reported method [32]. Freeze-dried HBH and TO-HBHs (0.1 g each) were pressed into pellets using a hydraulic press at 600 MPa for 1 min. X-ray diffraction patterns were collected for the pellets using a Rigaku RINT 2000 X-ray diffractometer by monochromator-treated CuKα radiation (λ 0.15418 nm) at 40 kV and 40 mA. The diffraction intensity was used in the range of 10 to 30° (2θ-angle range). The crystallinity indices and crystal widths of cellulose I in HBH and TO-HBHs were calculated from the X-ray diffraction patterns according to reported methods [38,39]. Optical microphotographs of HBH and TO-HBHs dispersed in water were obtained after placing the sample between two glass plates by using an Olympus BX50 (Japan). Scanning electron microscopy (SEM) images of the HB and HBH samples were captured using a Hitachi SEM S 4000 (Japan), after being coated with platinum by a sputtering method.

The TO-cellulose nanofibril/water dispersions prepared from TO-HBHs were put into poly(methyl methacrylate) disposable cuvettes, and their light transmittances were measured from 300 to 900 nm using a Shimadzu UV-Vis spectrometer (UV-1700). The spectrum of a cuvette filled with distilled water was used as a reference and to correct the transmittance of the dispersion sample. A diluted 0.001% solid content of TO-cellulose nanofibril/water dispersion was put on a mica plate and dried at room temperature. Lengths and widths of the nanofibrils were measured from AFM height images obtained in tapping mode using a Nanoscope III Multimode (Digital Instruments, USA). Approximately 150 nanofibrils were used in the measurements for each sample by using ImageJ free program. The preparation and isolation of TEMPO-oxidized cellulose nanofibrils are shown in Figure 5.

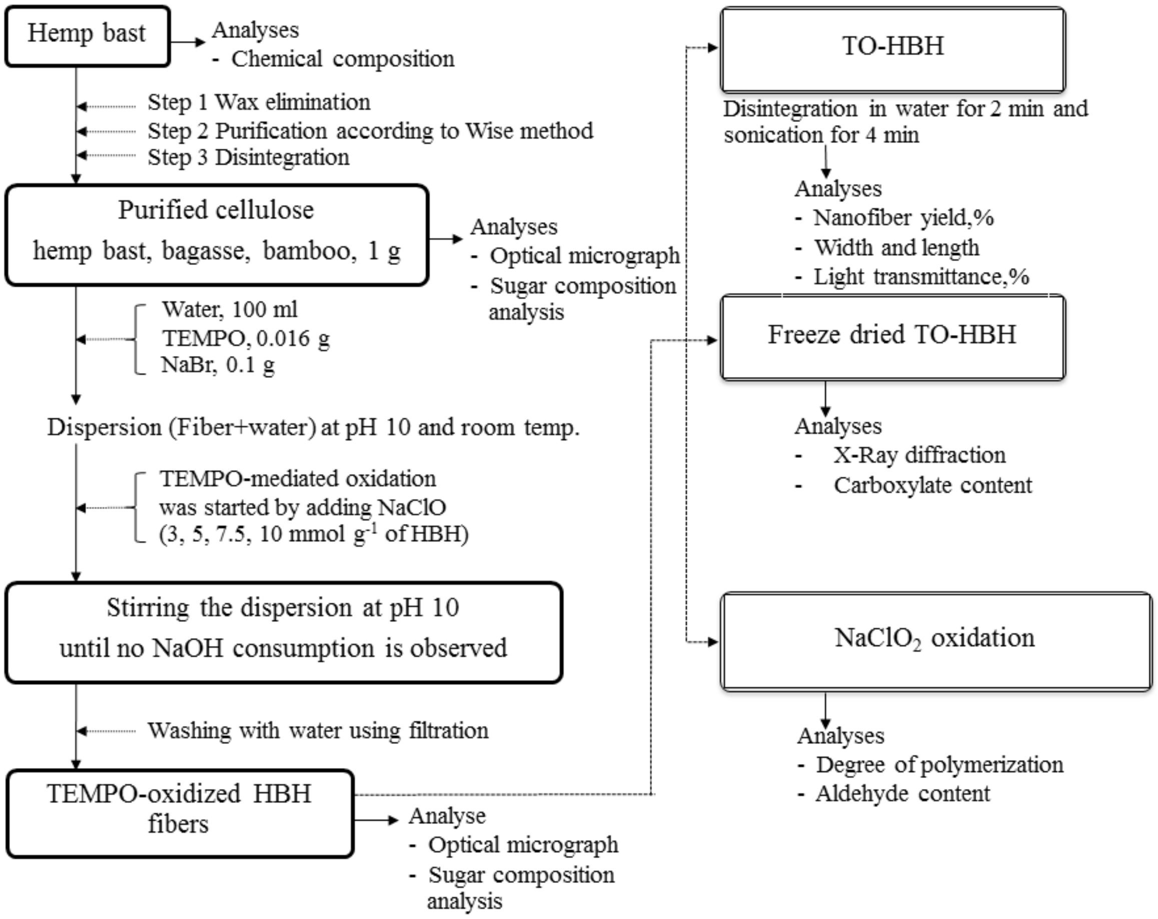

Figure 5. Scheme of preparation and characterization of TEMPO-oxidized cellulose nanofibrils

4.3 RESULTS AND DISCUSSION

4.3.1 Characterization of hemp bast holocellulose

Figure 6A shows a schematic illustration of hemp stem when demonstrated in cross section from the outer to the center, the hemp stem consists of a bast, core, pith and hollow, respectively. The bast layer is the thinnest compared with the core and pith layers. The SEM image shows that the hemp bast (HB) sample had a dense structure consisting of the HB fiber and matrix component probably originating from lignin and others substances (Figure 6B).

As to the individualization of fiber from plants cell wall, there are various methods. In this work, fibers form HB was isolated using Wise method [31] to remove lignin component which binds the fibers together in the plant cell walls.

Figure 6C shows scanning electron micrographs (SEM) of the hemp bast holocellulose (HBH) after the delignification treatment. HBH was obtained in the yield of 85.7±0.3%. The HBH had α-cellulose and lignin contents of 79.4±4.2 and 0.2±0.0%, respectively. It was shown that the holocellulose treatment removed almost all of the lignin from HB (Table 2). Individualization of the HBH fiber was achieved by mechanical disintegration in water. The diameter of single HBH fibers has been reported to be about 20-30 µm.

Table 2. Chemical compositions of hemp bast holocellulose.

		Chemical components, %		
Sample	Yield	α-cellulose	Hemicellulose	Insoluble lignin
Hemp bast holocellulose	85.7±0.3	79.6±4.2	20.2±4.2	0.2±0.0

The errors are expressed as standard deviations

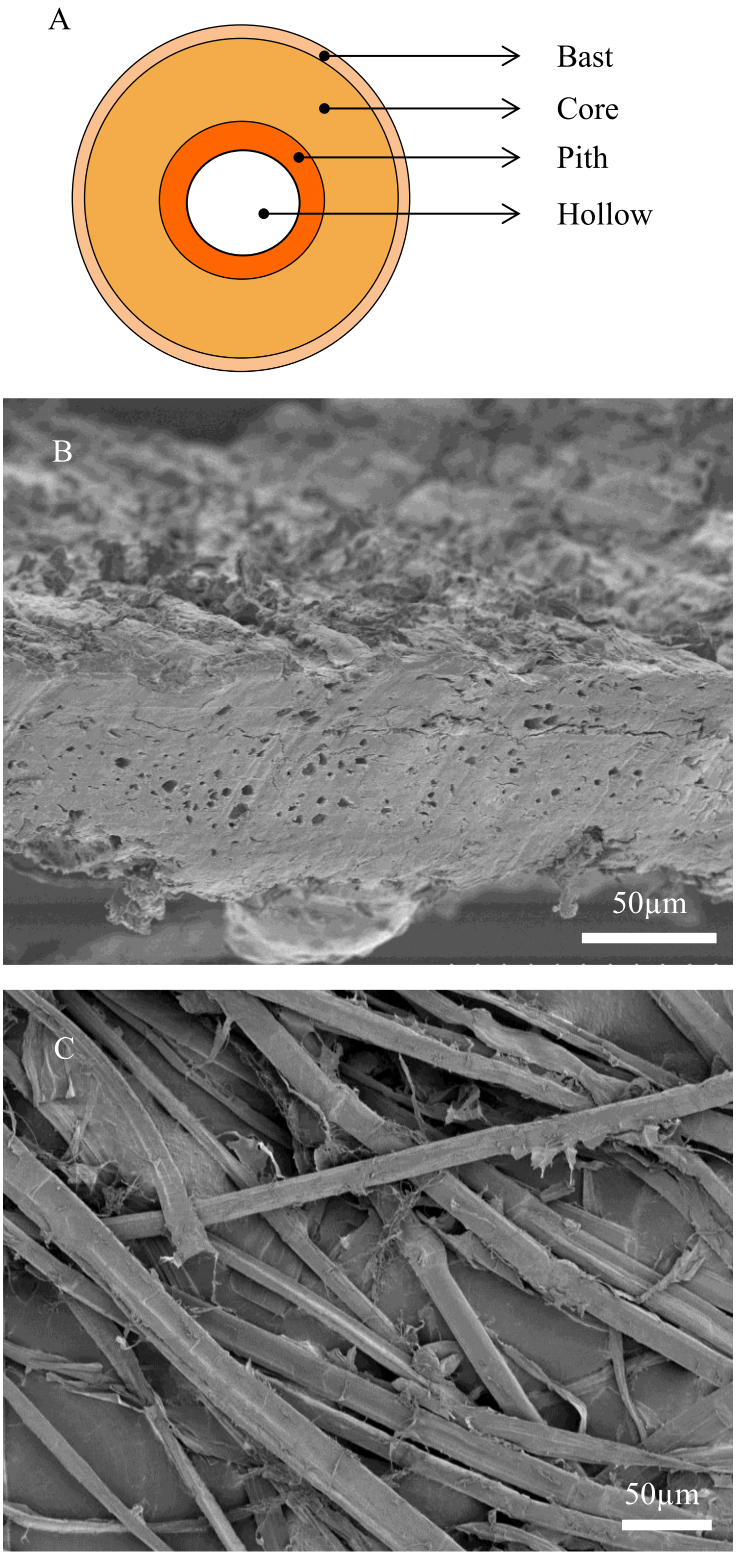

Figure 6. (A) Schematic illustration of cross section of hemp stem and SEM images of (B) cross section of hemp bast and (C) hemp bast holocellulose fiber.

4.3.2 TEMPO-mediated oxidation of hemp bast holocellulose

The HBH fiber was oxidized by the TEMPO/NaBr/NaClO system in water at pH 10 and room temperature, in which the NaClO addition level varied from 3 to 12.5 mmol/g-HBH. Optical microphotographs of the original hemp holocellulose fibers and TO-HBHs prepared at different NaClO addition levels are also shown in Figure 7. The TO-HBH (c), TO-HBH (d) and TO-HBH (e) are strongly swollen in water, forming balloons, when the addition level of NaClO was increased up from 7.5 to 12.5 mmol/g-HBH. Figure 8 shows the relationship between the NaClO addition level in the oxidation and either weight recovery ratio of the TEMPO-oxidized HB holocelluloses (TO-HBHs) or the time required for oxidation.

As the NaClO addition level was increased from 3 to 12.5 mmol/g-HBH, the time required for oxidation increased up to 6 h, and the weight recovery ratio decreased from 98 to 59%. It has been reported that most of hemicelluloses in bleached kraft pulps are degraded during the oxidation and removed as water-soluble fractions during washing process of the TEMPO-oxidized pulps by filtration [7]. Because the α-cellulose content of the HBH was 79%, not only hemicelluloses but also a part cellulose in HBH was removed by the oxidation at the NaClO addition level of >7.5 mmol/g-HBH and the successive washing process. As the NaClO addition level was increased, not only hemicelluloses but also some oxidized cellulose molecules are partly depolymerized [32] and become water-soluble, resulting in the decreases in the weight recovery ratio.

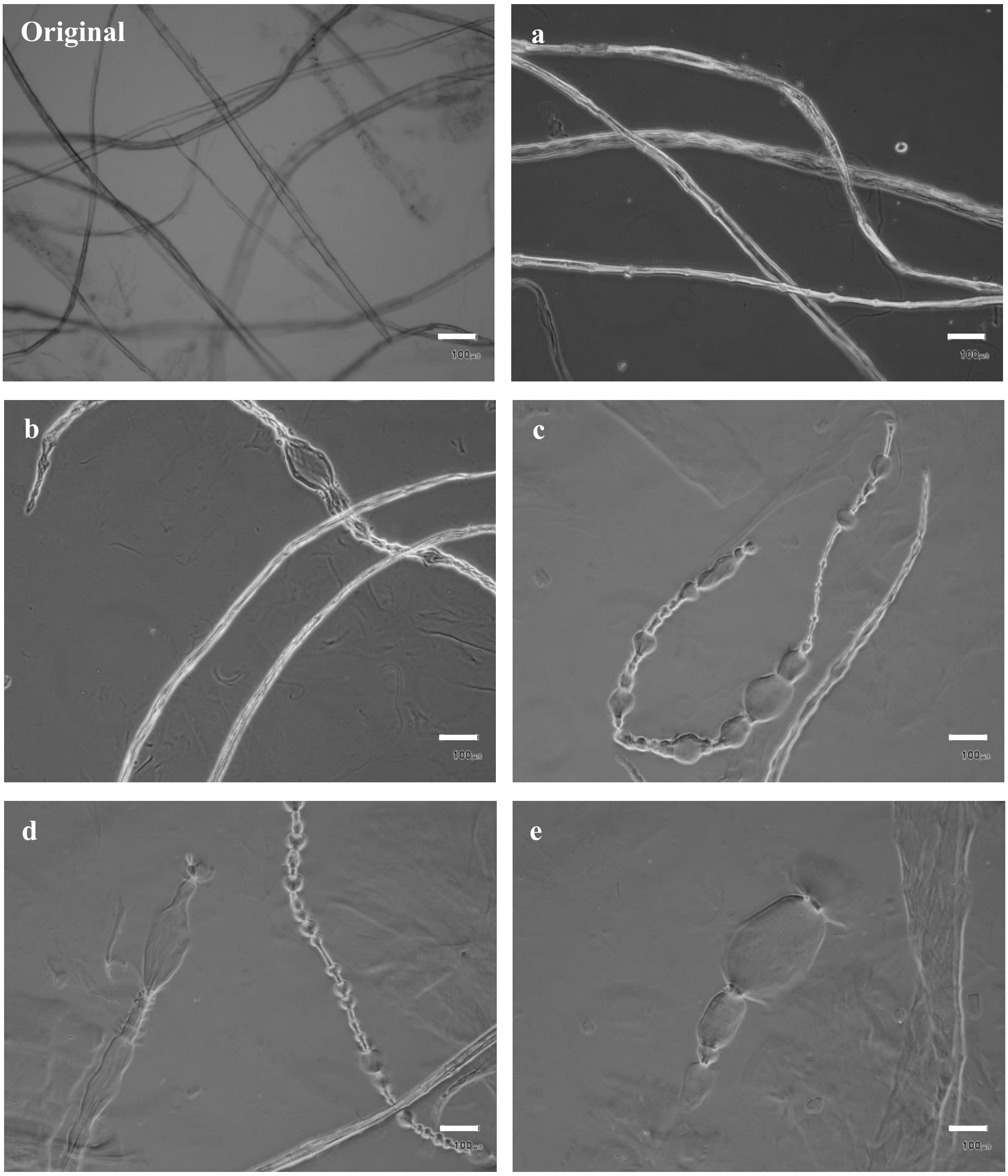

Figure 7. Optical microphotographs of original hemp bast holocellulose and the oxidized products. a) TO-HBHs prepared with NaClO 3 mmol g^{-1} b) 5 mmol g^{-1} c) 7.5 mmol g^{-1} d) 10 mmol g^{-1} and e) 12.5 mmol g^{-1}. The scale bar corresponds to 100 μm.

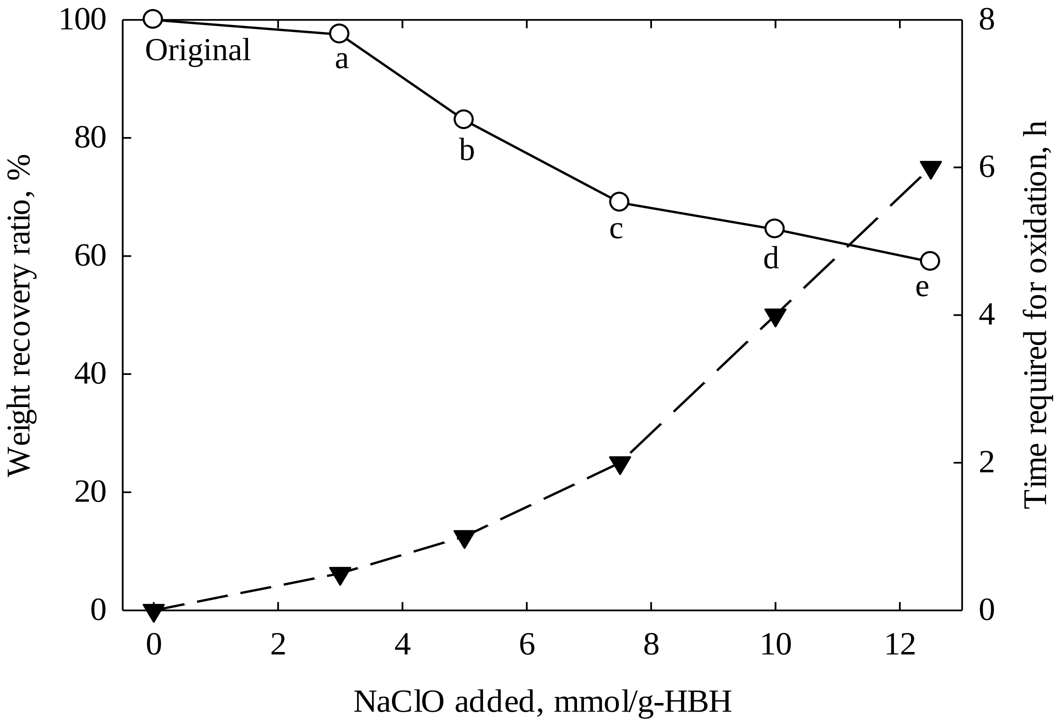

Figure 8. Relationship between the NaClO addition level in TEMPO-mediated oxidation of hemp bast holocellulose and either weight recovery ratio of the oxidized products or the time required for oxidation. The samples a-e corresponds to those in Figure 7.

Figure 9 shows carboxylate and aldehyde contents and DP_v value of the TO-HBHs prepared at various NaClO addition levels in the TEMPO-mediated oxidation. Carboxylate content of the TO-HBHs increased from 0.22 to 1.73 mmol/g with increasing the NaClO addition level from 0 to 12.5 mmol/g-HBH. Small amounts of C6-aldehydes, which can be converted to C6-carboxylates by the post-$NaClO_2$ oxidation, were present in the TO-HBHs. The C6-aldehyde content had the maximum value of 0.27 mmol/g at the NaClO addition level of 5 mmol/g-HBH and gradually decreased with increasing the NaClO addition level. The C6-aldehydes formed as intermediates can be thus oxidized to C6-carboxylate groups, when the NaClO addition level is more than 7.5 mmol/g-HBH. Not only the TEMPO/NaBr/NaClO oxidation but also NaClO itself can oxidize the C6-aldehydes to C6-carboxylates [39]. The relationship between the NaClO addition level in the TEMPO-mediated oxidation and either carboxylate or aldehyde content of the TO-HBHs in Figure 9 is similar to that for the TEMPO-oxidized softwood bleached kraft pulp [7]; the HBH behave like the kraft pulp in the TEMPO-mediated oxidation. Because almost no lignin was present in the HBH in this study, oxidation efficiency to form carboxylate and aldehyde groups in the TEMPO-oxidized products was much higher than that for TEMPO-mediated oxidation of lignin-containing hemp bast fibers at the same NaClO addition levels [30].

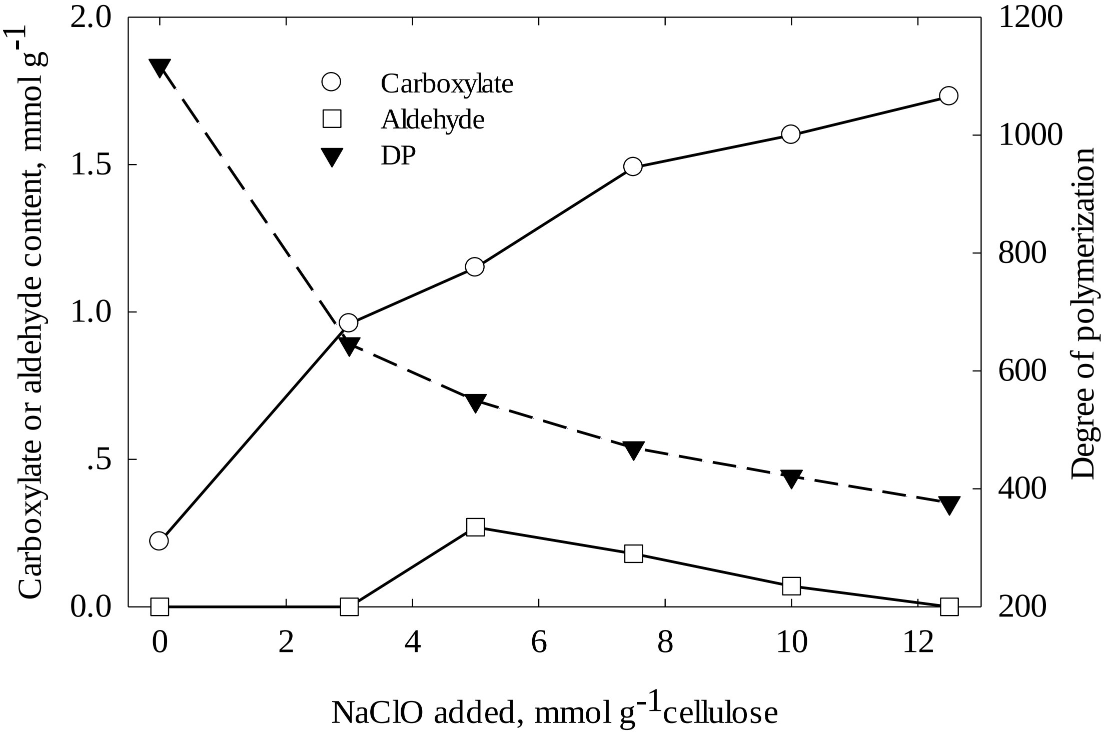

Figure 9. Carboxylate and aldehyde contents and viscosity-average degree of polymerization (DP_v) of TEMPO-oxidized hemp bast holocelluloses.

The DPv values clearly decreased from 1120 to 380 as the NaClO addition level in the TEMPO-mediated oxidation was increased from 0 to 12.5 mmol/g-HBH; remarkable depolymerization of cellulose chains in the TO-HBHs is inevitable during the TEMPO/NaBr/NaClO oxidation in water at pH 10. The β-elimination at the C6-aldehyde groups formed as intermediates under alkaline conditions and/or oxidative depolymerization by NaClO are likely to cause the depolymerization [32].

Figure 10 shows X-ray diffraction patterns and crystallinity indices/crystal widths of cellulose I calculated from the X-ray diffraction patterns of the TO-HBHs. All the TO-HBHs had the same cellulose I crystal structure as that of the original HBH. The crystallinity index (C.I.) slightly decreased from 0.45 to 0.41-0.43 by the TEMPO-mediated oxidation. However, all the TO-HBHs had similar C.I. values and almost the same crystal widths of 3.7-3.9 nm, although the oxidized products prepared at the different NaClO addition levels had different weight recovery ratios (Figure 8), carboxyl contents (Figure 9) or DPv values (Figure 9). These results indicate that the formation of C6-carboxylate groups by the TEMPO-mediated oxidation takes place selectively at the C6-OH groups exposed on the cellulose microfibril surfaces in HBH in a similar manner to those of other native celluloses [40].

4.3.3 Sugar composition analysis of TEMPO-oxidized hemp bast holocelluloses

Sugar compositions of the HBH and TO-HBHs are listed in Table 3. The HBH had a glucose content of 88.7%, which was much greater than its α-cellulose content, 79.4%. Thus, the HBH may have some low-molecular-weight celluloses, which are soluble in 17.5% NaOH during the α-cellulose content measurement. Even though the HBH had small amounts of neutral sugars originating from hemicelluloses (xylose, mannose and rhamnose), none of these sugars was detected in the TO-HBHs prepared at the NaClO addition levels of 7.7-12.5 mmol/g-HBH, indicating that these hemicelluloses were degraded to water-soluble fractions during the TEMPO-mediated oxidation in water at pH 10 and removed by the washing process. Thus, almost pure TEMPO-oxidized celluloses can be prepared from the HBH by the TEMPO-mediated oxidation.

As the NaClO addition level was increased from 3.0 to 12.5 mmol/g-HBH, the glucose content decreased from 68 to 52% and vice versa the others fraction content increased from 30 to 49%. Assuming that one cellulose microfibril of HBH consists of 6×6 cellulose chains in the cross section according to the typical higher plant cellulose microfibril model and the TEMPO-mediated oxidation selectively takes place at the C6-OH groups exposed on the crystalline microfibril surface [7,40], the glucose and glucuronic acid contents are calculated to be 68 and 32%, respectively. Because the others fraction contents for the TO-HBH(c), TO-HBH(d) and TO-HBH(e) were 47-49%, which are higher than 32%, the others fraction probably consisted of glucuronic acids formed from cellulose by the TEMPO-mediated oxidation of HBH and unhydrolyzed dimers-oligomers having both glucose and glucuronic acid units. It has been reported that glycoside bonds of poly-(1→4)-β-glucuronic acids (cellouronic acids) have higher resistance to acid hydrolysis [41] than cellulose so that complete hydrolysis of glucuronate-containing molecules in TO-HBHs to monomers may have been difficult under the acid hydrolysis conditions for neutral sugar composition analysis.

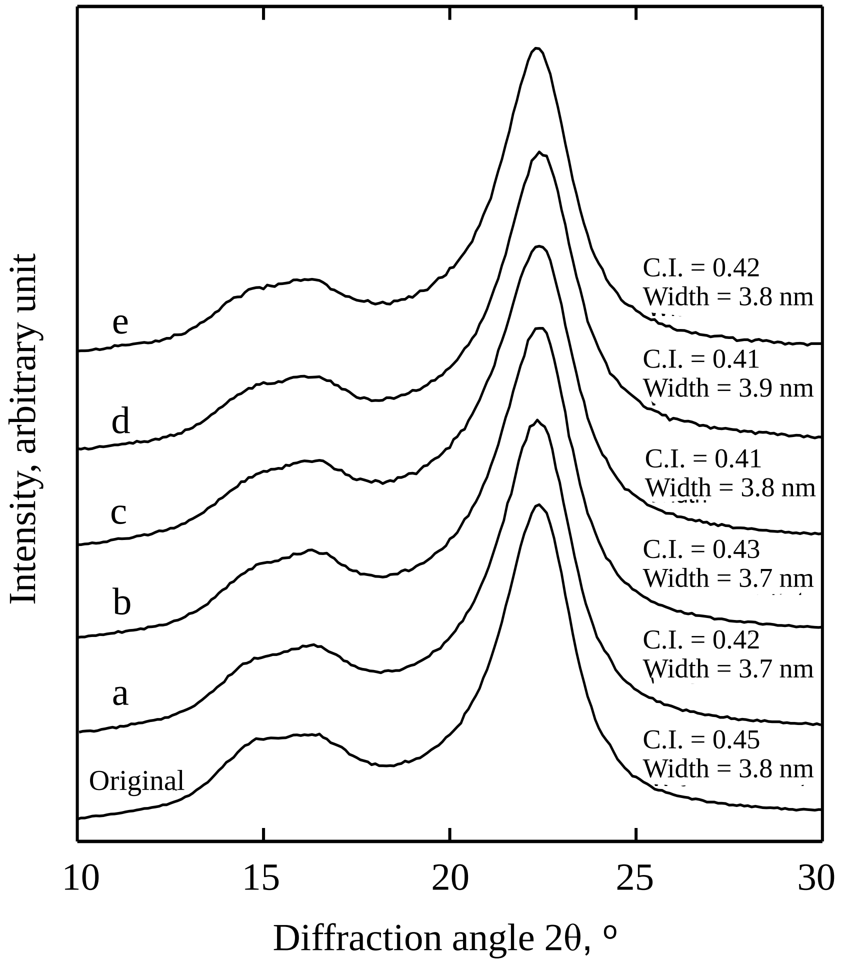

Figure 10. X-ray diffraction pattern, crystallinity index and (2 0 0) crystal width of TEMPO-oxidized hemp bast holocelluloses.

Table 3. Sugar compositions (%) of hemp bast holocellulose and TEMPO-oxidized hemp bast holocelluloses. The samples a-e corresponds to those in Figure 8.

	NaClO added (mmol/g)	Glc	Xyl	Man	Rha	Ara	Gal	Others[a]
Hemp bast holocellulose (HBH)								
	–	88.7±0.2	1.8±0.2	2.7±0.2	0.4±0.1	0.0	0.0	6.4
TEMPO-oxidized hemp bast holocellulose (TO-HBH)								
TO-HBH(a)	3.0	67.9±2.1	1.0±0.2	0.7±0.2	0.2±0.2	0.0	0.0	30.2
TO-HBH(b)	5.0	60.3±0.2	0.5±0.2	0.1±0.2	0.0	0.0	0.0	39.1
TO-HBH(c)	7.5	53.5±0.2	0.0	0.0	0.0	0.0	0.0	46.5
TO-HBH(d)	10.0	51.6±0.2	0.0	0.0	0.0	0.0	0.0	48.4
TO-HBH(e)	12.5	51.5±0.2	0.0	0.0	0.0	0.0	0.0	48.5

[a] The “Others” fraction was calculated by the following equation: Others (%) = 100 − (Glc) − (Xyl) − (Man) − (Rha) − (Ara) − (Gal)

4.3.4 Characterization of TEMPO-oxidized hemp bast holocellulose nanofibrils

When the TO-HBHs were mechanically disintegrated in water, highly viscous gels were obtained by conversion of the TO-HBH fibers to the individual TEMPO-oxidized cellulose nanofibrils [7,22-25]. Figures 11A and 11B show UV-Vis transmittance spectra and photographs, respectively, of the 0.1% TO-HBH nanofibril/water dispersions prepared by mechanical disintegration under the same conditions. Figure 11C depicts the corresponding nanofibrillation yields. When the C6-carboxylate contents of the TO-HBHs were more than approximately 1.5 mmol/g, the corresponding dispersions had light-transmittances at 600 nm and nanofibrillation yields of >73% and >96%, respectively (Figures 11A and 11C). Thus, the carboxylate content of the TO-HBHs, which is controllable by the NaClO addition level in the TEMPO-mediated oxidation process, is the primary factor influencing the nanofibrillation behavior of TO-HBHs. When a sufficient amount of C6-carboxylate groups is formed on crystalline cellulose microfibril surfaces in the HBH by the TEMPO-mediated oxidation, electrostatic repulsion effectively works between anionically-charged TEMPO-oxidized cellulose nanofibrils in water, achieving the almost complete individualization of cellulose nanofibrils in the TO-HBHs.

Figure 11D shows AFM images of TO-cellulose nanofibrils prepared from the TO-HBHs by mechanical disintegration under the same conditions. When the TO-HBHs were prepared with NaClO of <7.5 mmol/g-HBH, some aggregates of cellulose nanofibrils were clearly present. Average nanofibril widths were obtained from the AFM height images for all TO-HBH nanofibril samples. Average nanofibril lengths were also obtained from the AFM images but only for the TO-HBHs (c, d and e); it was difficult to estimate the accurate nanofibril lengths for the TO-HBHs (a and b) due to the presence of fibril aggregates. The results are listed in Table 4. All the samples had similar average nanofibril widths of 2.7-2.9 nm, while average nanofibril lengths decreased with increasing the NaClO addition level in the TEMPO-mediated oxidation. There were some discrepancies between the cellulose I crystallite widths determined by X-ray diffraction method (3.7-3.9 nm, Figure 10) and nanofibril widths measured by the AFM height method (2.7-2.9 nm, Table 4).

The reason for these discrepancies is unknown at present, but a cantilever pressure on the cellulose nanofibrils during the AFM measurement may have brought about such smaller nanofibril width values. Figure 12 depicts the relationship between the DP_v value of the TO-HBHs and either average length of TEMPO-oxidized cellulose nanofibrils or cellulose chain length calculated from the DP_v values, in which cellulose chains are assumed to be fully and straightly extended, keeping the monomer length of 0.518 nm [32]. Even though all average cellulose nanofibril lengths had quite large fluctuations, a roughly linear relationship was obtained between the DP_v of the TO-HBHs and the average cellulose nanofibril length. Because the average cellulose nanofibril length was always greater than the cellulose chain length at the corresponding DP_v value (Figure 12), each nanofibril in TO-HBHs consists of cellulose chains shorter than the nanofibril length.

Table 4. Number-average length and width of cellulose nanofibrils prepared from TEMPO-oxidized hemp bast holocelluloses (TO-HBHs).

Sample	NaClO added (mmol/g)	Number-average length (nm)	Number-average width (nm)
TO-HBH(a)	3.0	few microns	2.7±1.2
TO-HBH(b)	5.0	few microns	2.9±0.9
TO-HBH(c)	7.5	585±420	2.9±1.0
TO-HBH(d)	10.0	435±311	2.7±1.0
TO-HBH(e)	12.5	402±263	2.9±0.7

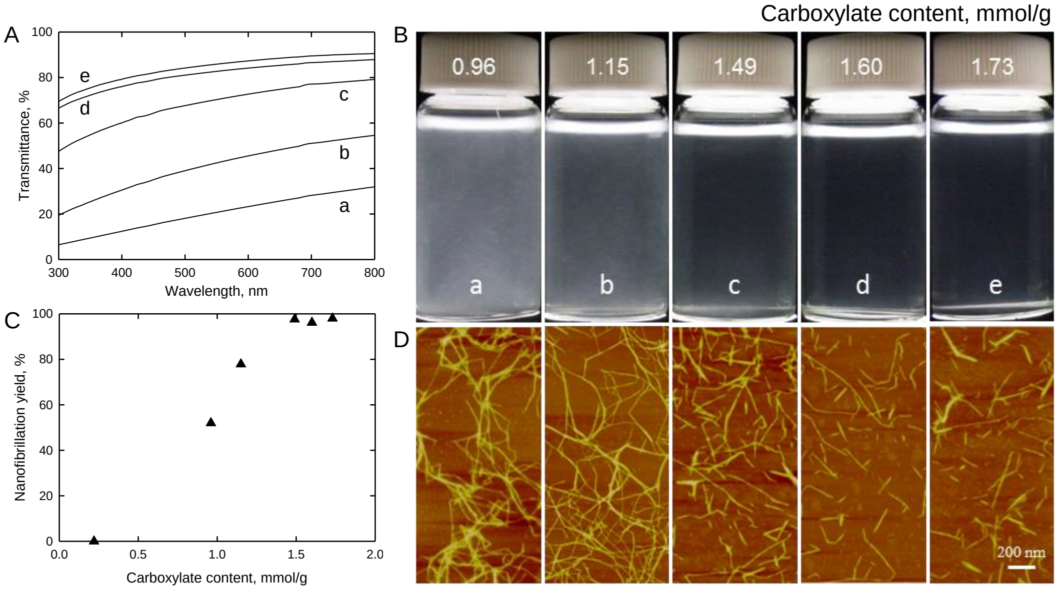

Figure 11. (A) Light transmittance spectra and (B) photographs of 0.1% cellulose nanofibril/water dispersions prepared from TEMPO-oxidized hemp holocelluloses. (C) Nanofibrillation yields and (D) AFM images of cellulose nanofibrils are also shown.

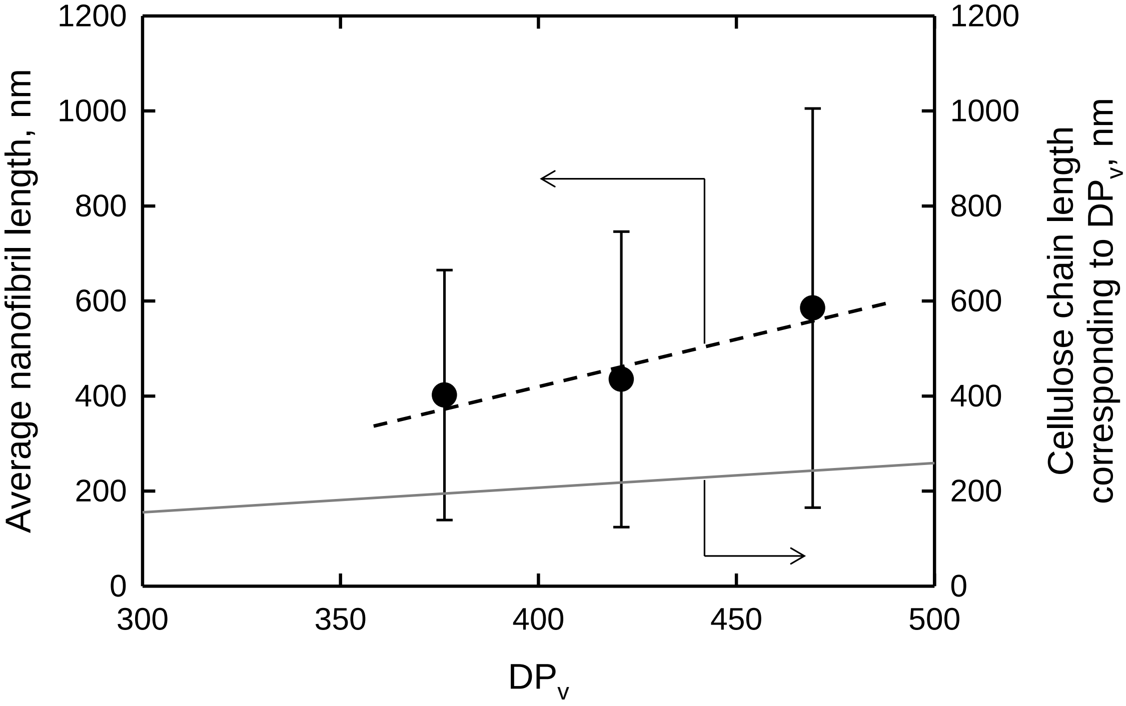

Figure 12. Relationship between DP$_v$ of TEMPO-oxidized hemp bast holocelluloses and either average cellulose nanofibril length measured from AFM height images or cellulose chain length corresponding to each DP$_v$ value.

4.4 CONCLUSION

The hemp bast holocellulose (HBH) fiber was oxidized by the TEMPO/NaBr/NaClO system in water at pH 10 to prepare individualized TEMPO-oxidized cellulose nanofibrils. When the NaClO addition levels were 7.5-12.5 mmol/g-HBH, the oxidized HBHs were converted to transparent dispersions by mechanical disintegration in water to form almost completely individualized TEMPO-oxidized cellulose nanofibrils in the nanofibrillation yields of 98-100%. However, the weight recovery ratios and DP_v values of the TEMPO-oxidized HBHs decreased to 69-59% and 470-380 from 1120, respectively, while their carboxylate contents increased to 1.5-1.7 mmol/g. All the TEMPO-oxidized (TO) HBHs had the same cellulose I crystal structure and similar cellulose I crystal widths and crystallinity indices of 3.7-3.9 nm and 0.41-0.43, respectively. Most of hemicelluloses originally present in the HBH were removed during the oxidation and washing processes, showing that almost pure TEMPO-oxidized celluloses were obtained from the HBH by the TEMPO-mediated oxidation under suitable conditions and the successive mechanical disintegration of the oxidized products in water. AMF height images revealed that the nanofibril widths were 2.7-2.9 nm, which were smaller than those (3.7-3.9 nm) obtained from X-ray diffraction patterns. The average nanofibril lengths decreased from 590 to 400 nm as the NaClO addition level was increased from 7.7 to 12.5 mmol/g-HBH in the TEMPO-mediated oxidation process.

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5. COMPARATIVE CHARACTERIZATION OF CELLULOSE NANOFIBRIL FILMS PREPARED FROM NON-WOOD CELLULOSES BY TEMPO-MEDIATED OXIDATION

ABSTRACT

Three non-wood celluloses, hemp bast holocellulose, and commercial bamboo and bagasse bleached kraft pulps, were oxidized by TEMPO-mediated oxidation in water at pH 10. The water-insoluble TEMPO-oxidized celluloses thus obtained were converted to aqueous dispersions of TEMPO-oxidized cellulose nanofibrils (TOCN) and then to self-standing TOCN films. Weight recovery ratios of the TEMPO-oxidized celluloses decreased to 70−80% and their carboxylate contents reached 1.5−1.7 mmol g^{-1} by the TEMPO-mediated oxidation. The viscosity-average degrees of polymerization remarkably decreased from 800−1100 to 200−480 by partial depolymerization occurring during the oxidation, depending on the non-wood celluloses used as the starting materials. The average lengths and widths of the TOCNs were estimated to be 500−650 nm and 2.4−2.9 nm, respectively, from their atomic force microscopy images. The self-standing TOCN films had high light-transparencies (>87% at 600 nm), high tensile strengths (140−230 MPa), high Young's moduli (7−11 MPa), low coefficients of thermal expansion (4−6 ppm K^{-1}) in spite of low densities of 1.4−1.7 g cm^{-3}. In particular, the TOCN films prepared from the hemp bast holocellulose had clearly high works of fracture (~30 MJ m^{-3}), whereas those prepared from other two non-wood celluloses had 2−8 MJ m^{-3}.

5.1 INTRODUCTION

The production of nanomaterials from renewable cellulose, which are the most abundantly present biopolymer on earth, has gained an increasing attention in recent years. Plant cellulose fibers consist of highly crystalline microfibrils 3−4 nm in width, each of which constitutes 30−40 cellulose chains fully extended to the longitudinal direction of microfibril [1−4]. One of the most promising, efficient and energy-saving pretreatments to convert plant cellulose fibers to nanocelluloses is position-selective surface oxidation of C6-primary hydroxyls exposed on crystalline cellulose microfibril surfaces to C6-carboxylate groups by catalytic oxidation with TEMPO in water [2]. When wood cellulose fibers or bleached wood kraft pulps for papermaking are oxidized using a TEMPO/NaBr/NaClO system in water at room temperature and pH 10 for 2−4 h, C6-carboxyl groups are selectively formed on the crystalline cellulose microfibrils and carboxyl contents reach up to 1.8 mmol g^{-1}, depending on the oxidation conditions [5−7]. The oxidized wood celluloses, even though they have high amounts of hydrophilic carboxyl groups, maintain their original fibrous morphologies, and thus can be purified and obtained as wet fibers by repeated washing and filtration with water [2].

The TEMPO-oxidized wood celluloses having carboxylate contents of more than ~1 mmol g^{-1} are convertible to highly viscous and transparent gels consisting of mostly individual TEMPO-oxidized cellulose nanofibrils (TOCNs) with uniform 3−4 nm widths and lengths > 500 nm by mild mechanical disintegration in water [2,5−7]. Moreover, self-standing TOCN films prepared from the aqueous TOCN dispersions by casting and drying have high mechanical strengths and high oxygen barrier properties under dry conditions [8,9]. Thus, wood TOCN/water dispersions and wood TOCN films have wide potential applications as new bio-based nanomaterials in the fields of packaging, light-weight and strong composites, electric devices, flat display panels and others [10−12].

In Thailand, more effective utilization of non-wood celluloses originating from, for instance, hemp, bamboo and bagasse to commodity goods and high-tech materials have become significant for further improvement of both agriculture and industrials [13−17].

TEMPO-mediated oxidation to prepare TOCN/water dispersions and TOCN films has been studied using wood celluloses, cotton linters and bacterial celluloses to accumulate fundamental and scientific information. Milanovic et al. [18] studied TEMPO-mediated oxidation of lignin-containing hemp bast fibers to add new functionalities to hemp fibers for textile use.

In a previous section, a holocellulose was prepared from hemp bast (*Cannabis sativa L.* Subsp. Sativa), and oxidized using the TEMPO/NaBr/NaClO system in water at pH 10. Fundamental information concerning the effects of various oxidation conditions on characteristics of TEMPO-oxidized hemp bast holocelluloses and TOCNs obtained by mechanical disintegration of the oxidized hemp bast holocelluloses in water. When carboxylate contents of the TEMPO-oxidized hemp bast holocelluloses were 1.5–1.7 mmol g^{-1}, TEMPO-oxidized hemp bast cellulose nanofibrils almost completely dispersed at the individual nanofibril were obtained by mechanical disintegration in water. Atomic force microscopy height images showed that the nanofibril widths were 2.7–2.9 nm, and the average nanofibril lengths decreased from 590 to 400 nm as the NaClO addition level was increased from 7.5 to 12.5 mmol g^{-1} of dry weight of the hemp bast holocellulose in the TEMPO-mediated oxidation.

In this section, comparative characterizations of TEMPO-oxidized cellulose nanofibril films prepared from hemp bast holocellulose, and commercial bamboo and bagasse bleached kraft pulps are carried out. Hemp, bamboo and bagasse have been become significant cellulose resources alternative to wood pulps particularly in developing countries, and development of advanced utilization of these non-wood and fast-growing celluloses in terms of both qualities and quantities has been of importance [13].

5.2 EXPERIMENTAL

5.2.1 Materials

The hemp bast holocellulose, which was prepared according to a previously described method, and commercial bamboo and bagasse bleached kraft pulps were used as the non-wood cellulose sources in TEMPO-mediated oxidation. The bamboo and bagasse pulps were provided by Industrial Krungthai Co. Ltd., Pathum Thani, Thailand, and Environment Pulp and Paper Co. Ltd, Nakornsawan, Thailand, respectively. TEMPO, sodium bromide, sodium chlorite, a 13% sodium hypochlorite solution, sodium hydroxide and other chemicals and solvents were of laboratory grade (Wako Pure Chemicals, Co. Ltd, Japan) and used without further purification.

5.2.2 TEMPO-mediated oxidation of cellulose

Cellulose sample (1 g) was suspended in water (100 mL), containing TEMPO (0.016 g) and sodium bromide (0.1g). TEMPO-mediated oxidation was started by adding NaClO solution containing 7.5 mmol g^{-1} of cellulose to the cellulose suspension at room temperature. The mixture was stirred at pH 10 by continuous addition of 0.5 M NaOH using a pH stat until no NaOH consumption was observed. The TEMPO-oxidized cellulose thus obtained was washed thoroughly with water by filtration and stored at 4°C before further treatment or analysis. The weight recovery ratios of the water-insoluble TEMPO-oxidized celluloses were calculated from dry weights before and after the TEMPO-mediated oxidation. Carboxylate contents of the TEMPO-oxidized celluloses were determined using electric conductivity titration [19].

5.2.3 Preparation of TEMPO-oxidized cellulose nanofibril Films

The never-dried and fibrous TEMPO-oxidized cellulose corresponding to 25 mg dry weight was suspended in water (25 mL) at a 0.1% (w/v) solid content, and the suspension was disintegrated at 7,500 rpm for 2 min at room temperature using a double-cylinder-type homogenizer (Physcotron, Microtec Nition Co. Ltd., Japan). The gel thus obtained was then sonicated for 4 min using an ultrasonic homogenizer with a 7-mm-diameter probe tip at 19.5 kHz and 300 W output power (US-300T, Nissei, Japan) to prepare an aqueous dispersion of TEMPO-oxidized cellulose nanofibrils (TOCN). Unfibrillated or partly fibrillated fraction was removed from the dispersion by centrifugation at 12,000*g* for 20 min, and the nanofibrillation yield was measured from dry weight of the centrifugally removed fraction. The TOCN/water dispersion thus obtained was cast on a poly(styrene) petri dish and dried in an oven at 45°C without air-flow for 3−7 days until the film was dried and naturally detached from the petri dish to obtain a self-standing TOCN film. All films were preserved at temperature 23±1°C and relative humidity 50±2% before testing.

5.2.4 Analyses

The neutral sugar composition analysis was carried out using a high-performance liquid chromatograph (HPLC) according to the previously described method. The original and TEMPO-oxidized celluloses after post $NaClO_2$ treatment to oxidize residual C6-aldehyde groups present in the TEMPO-oxidized celluloses to C6-carbxyls (0.04 g) were dissolved in 0.5 M copper ethylenediamine (cuen, 10 mL) and distilled water (10 mL) by continuous stirring for 30 min. Viscosity numbers (η_{sp}/c) of the solutions were obtained using a Cannon-Fenske-type capillary viscometer at 20°C, and these values were converted to viscosity-average degrees of polymerization (DP_v) according to the reported method [7]. The original and TEMPO-oxidized celluloses obtained by freeze-drying (0.1 g each) was pressed into tablets using a hydraulic press at 600 MPa for 1 min. X-ray diffraction patterns were collected for the tablets using a Rigaku RINT 2000 X-ray diffractometer with monochromator-treated CuKα radiation (λ 0.15418 nm) at 40 kV and 40 mA by the reflection mode. The crystallinity indices (C.I.) and crystal widths of (2 0 0) planes of cellulose I were determined for the samples from the X-ray diffraction patterns according to the previously described method.

Scanning electron microscopy (SEM) images of the original cellulose samples were captured using a Hitachi SEM S 4000 (Japan) after being coated with platinum using sputtering technique. Atomic force microscopy (AFM) images of TOCNs and surfaces of self-standing TOCN films were observed using a NanoScope III Multimode (Digital Instruments, USA). Lengths and heights of approximately 150 nanofibrils were measured by using ImageJ free program from the AFM height images for each sample.

Thermal expansion behavior of TOCN films pre-heated at 120°C for 50 min were analyzed using a Shimadzu TMA-60 instrument with a 0.05 N load cell in a nitrogen atmosphere from 25 to 120°C at 10°C min^{-1}. The light transmittances of self-standing TOCN films were measured from 200 to 900 nm using a Shimadzu UC-1700 UV-vis spectrometer. Moisture contents of the films conditioned at 23°C and 50% relative humidity were calculated from their weights before and after heating at 105°C for 3 h. Tensile strength, elongation at break, Young’s modulus and works of fracture of the films 2 mm wide and at least 20 mm long were measured at a speed of 1.0 mm min^{-1} and a span length of 10 mm using a Shimadzu EZ-TEST instrument equipped with a 500 N load cell. The preparation of non-wood TEMPO-oxidized cellulose nanofibrils and their films is shown in Figure 13.

5.3 RESULTS AND DISCUSSION

5.3.1 TEMPO-mediated oxidation of non-wood celluloses

The hemp bast holocellulose and two commercial non-wood bleached kraft pulps produced from bamboo and bagasse were used as the starting samples in this section. SEM images of the three non-wood celluloses are shown in Figure 13. Although small amounts of fines/particle fractions and film-like fragments probably originating from parenchyma cells [20] were present in the SEM images, the major components were long fibers separated from each other. Fiber diameters of the hemp bast holocellulose and bagasse pulp were 20−30μm, and those of the bamboo pulp were 10−20 μm.

The weight recovery ratios and carboxylate contents of TEMPO-oxidized celluloses obtained as water-insoluble fractions are depicted in Figure 14. When the TEMPO-mediated oxidation with the same amount of NaClO (7.5 mmol g^{-1} per gram of each sample) was applied to the non-wood celluloses, the carboxylate contents of the oxidized celluloses ranged from 1.5 to 1.7 mmol g^{-1}, which are due to glucuronosyl units formed from glucosyl units of cellulose by the TEMPO-mediated oxidation. Weight recovery ratios decreased to 70−80% by the TEMPO-mediated oxidation, probably because a part of hemicelluloses and low-molecular-weight celluloses degraded to water-soluble fractions during the oxidation.

The sugar compositions of the three non-wood celluloses before and after TEMPO-mediated oxidation are listed in Table 5. The original hemp bast holocellulose had the highest glucose content (88.7%) followed by the bamboo pulp (87.2%) and bagasse pulp (77.8%). This order probably corresponds to that of their cellulose contents. Based on the high xylose contents of bamboo and bagasse pulps, xylan is the predominant hemicelluloses in these pulps, which is in agreement with literature [21,22]. After TEMPO-mediated oxidation, a part of glucosyl units present on cellulose microfibril surfaces are converted to glucuronosyl units,

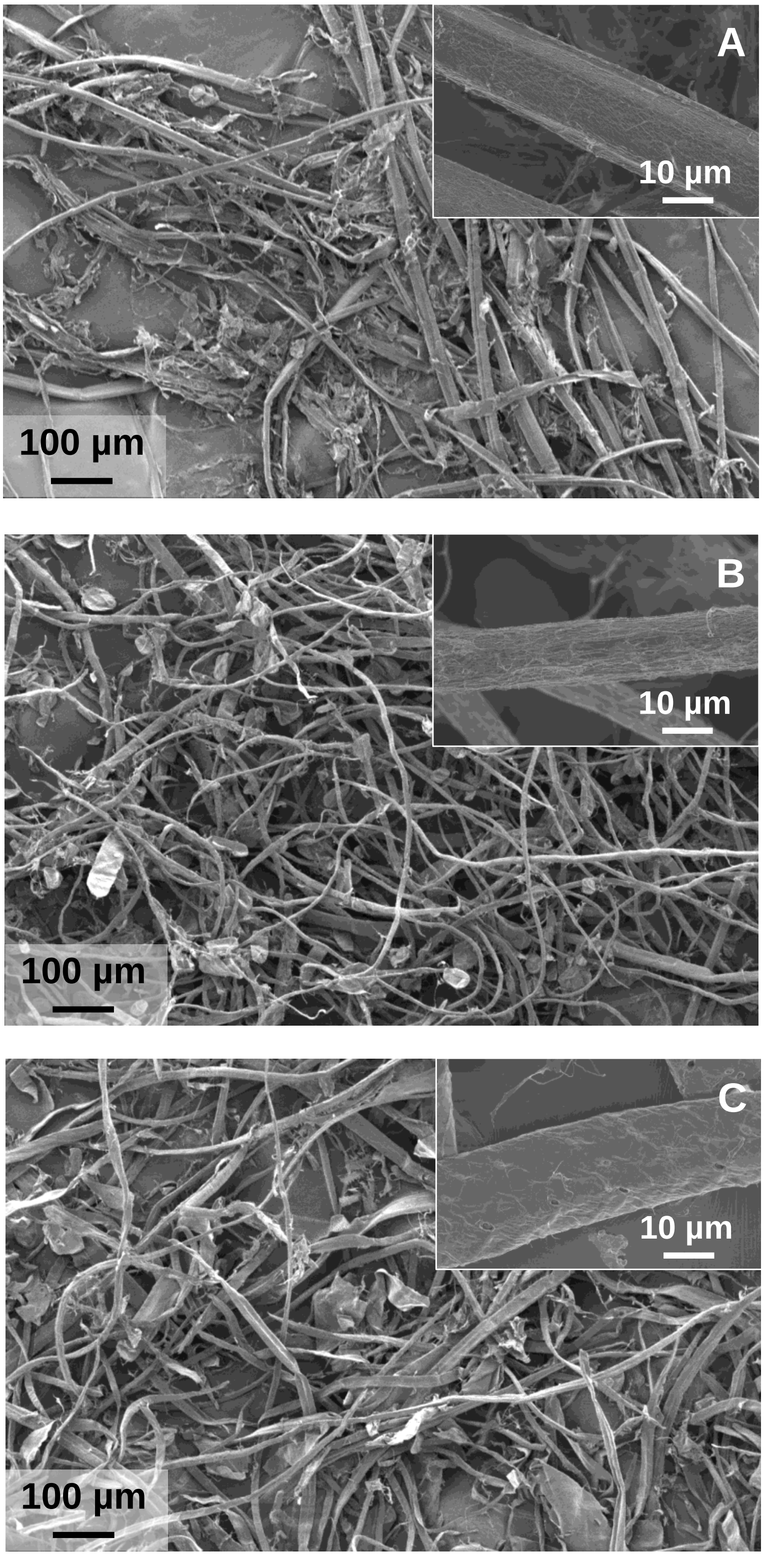

Figure 13. SEM images of (A) hemp bast holocellulose, and (B) bamboo and (C) bagasse pulps

which cannot be detected as neutral sugars by the HPLC system used in this study. Thus, the glucuronosyl units and some glucose units having resistance to acid hydrolysis in the neutral sugar analysis (due to the presence of glucuronosyl units linked to the glucosyl units) are included in the category of “Others” in Table 5. The amounts of glucose and “Others” were similar in each TEMPO-oxidized cellulose; approximately a half of glucosyl units in the original non-wood cellulose turned to the “others” fraction by the TEMPO-mediated oxidation, which is roughly consistent with the TEMPO-oxidized cellulose nanofibril model proposed for higher plant celluloses [2,23]. More than half amounts of xylose units present in the original non-wood celluloses were degraded and removed as water-soluble fractions by the TEMPO-mediated oxidation.

The viscosity-average degrees of polymerization (DP_v) of the three original non-wood celluloses and the corresponding TEMPO-oxidized celluloses are shown in Figure 15. Although the original non-wood celluloses had DP_v values of 800−1100, the corresponding TEMPO-oxidized celluloses remarkably decreased to 200−480. The mechanism for decreasing DP_v of TEMPO-oxidized celluloses is hypothesized in terms of the following two factors; 1) the presence of somewhat disordered regions periodically along longitudinal direction of the crystalline cellulose microfibrils in higher plants, and 2) oxidative depolymerization of the disordered regions takes place to some extent during TEMPO-mediated oxidation [7].

X-ray diffraction patterns of the non-wood celluloses before and after the TEMPO-mediated oxidation are shown in Figure 16 together with their crystallinity indices and crystal widths of (2 0 0) diffraction plane of cellulose I. In all cases, the original cellulose I crystal structure was maintained in the oxidized celluloses. The crystallinity indices of the original samples slightly decreased from 0.42−0.46 to 0.36−0.42 by the TEMPO-mediated oxidation, indicating that some of the crystalline cellulose molecules in the original non-wood celluloses turn to disordered structures having sodium glucuronosyl units by the oxidation. The slight decreases in the (2 0 0) crystal width from 3.7−3.9 nm to 3.5−3.6 nm also support the above hypothesis. Nevertheless, because the levels of decreasing crystallinity index or crystal width by the TEMPO-mediated oxidation are sufficiently small, it can be concluded that the oxidation of glucosyl units to glucuronosyl units or C6-primary hydroxyls to C6-carboxylates mostly takes place on crystalline microfibril surfaces of these non-wood celluloses by the TEMPO-mediated oxidation.

Table 5 Sugar compositions of hemp bast holocellulose, and bamboo and bagasse pulps before and after TEMPO-mediated oxidation

	Hemp bast holocellulose		Bamboo pulp		Bagasse pulp	
	before ox.	after ox.	before ox.	after ox.	before ox.	After ox.
Glucose	88.7	53.5	87.2	44.9	77.8	49.3
Xylose	1.8	0.0	12.8	5.3	21.7	9.2
Mannose	2.7	0.0	0.0	0.0	0.0	0.0
Rhamnose	0.4	0.0	0.0	0.0	0.0	0.0
Arabinose	0.0	0.0	0.0	0.0	0.0	0.0
Galactose	0.0	0.0	0.0	0.0	0.0	0.0
Others[a]	6.4	46.5	0.0	49.8	0.5	41.5

[a] The “Others” fraction was calculated by the following equation: Others (%) = 100 − (Glc) − (Xyl) − (Man) − (Rha) − (Ara) − (Gal)

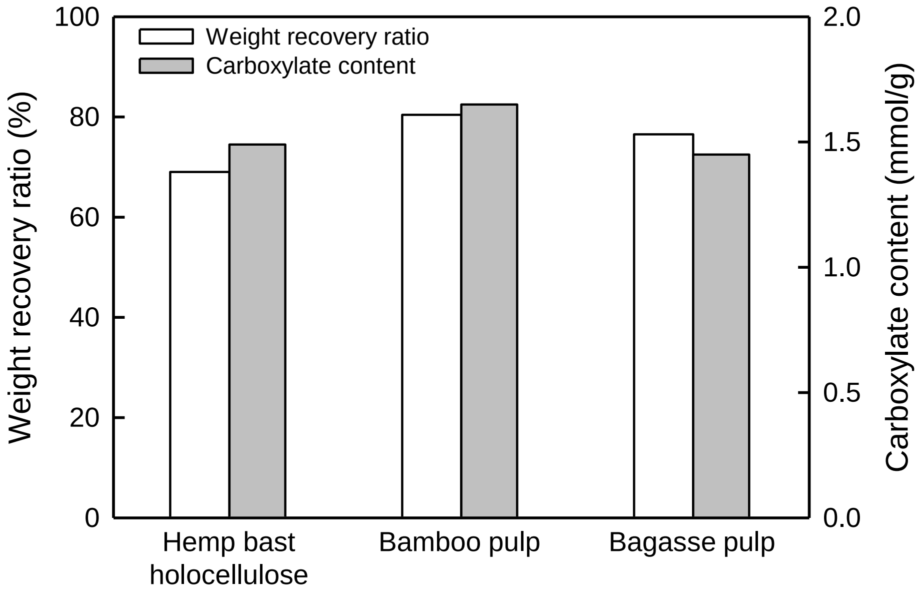

Figure 14. Weight recovery ratio and carboxylate content of TEMPO-oxidized celluloses

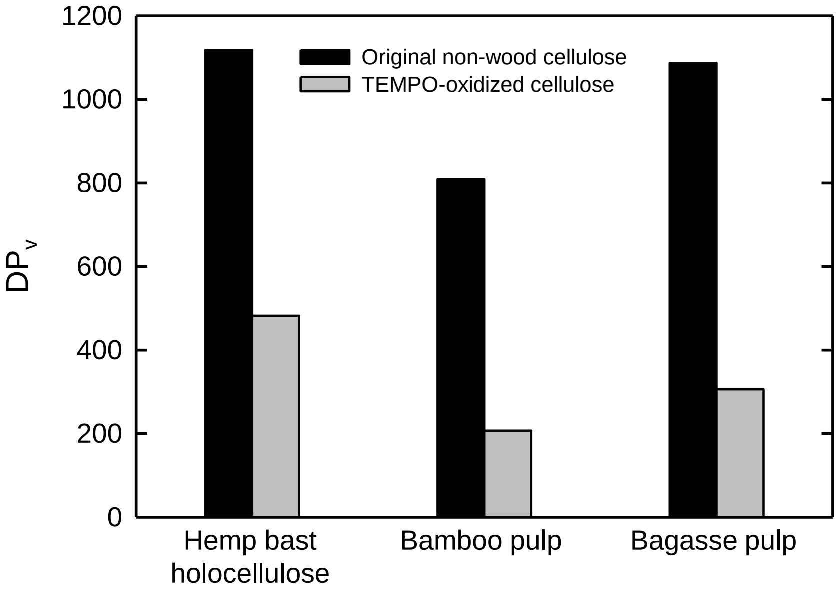

Figure 15. Viscosity-average degrees of polymerization (DP$_v$) of hemp bast holocellulose, and bamboo and bagasse pulps before and after TEMPO-mediated oxidation

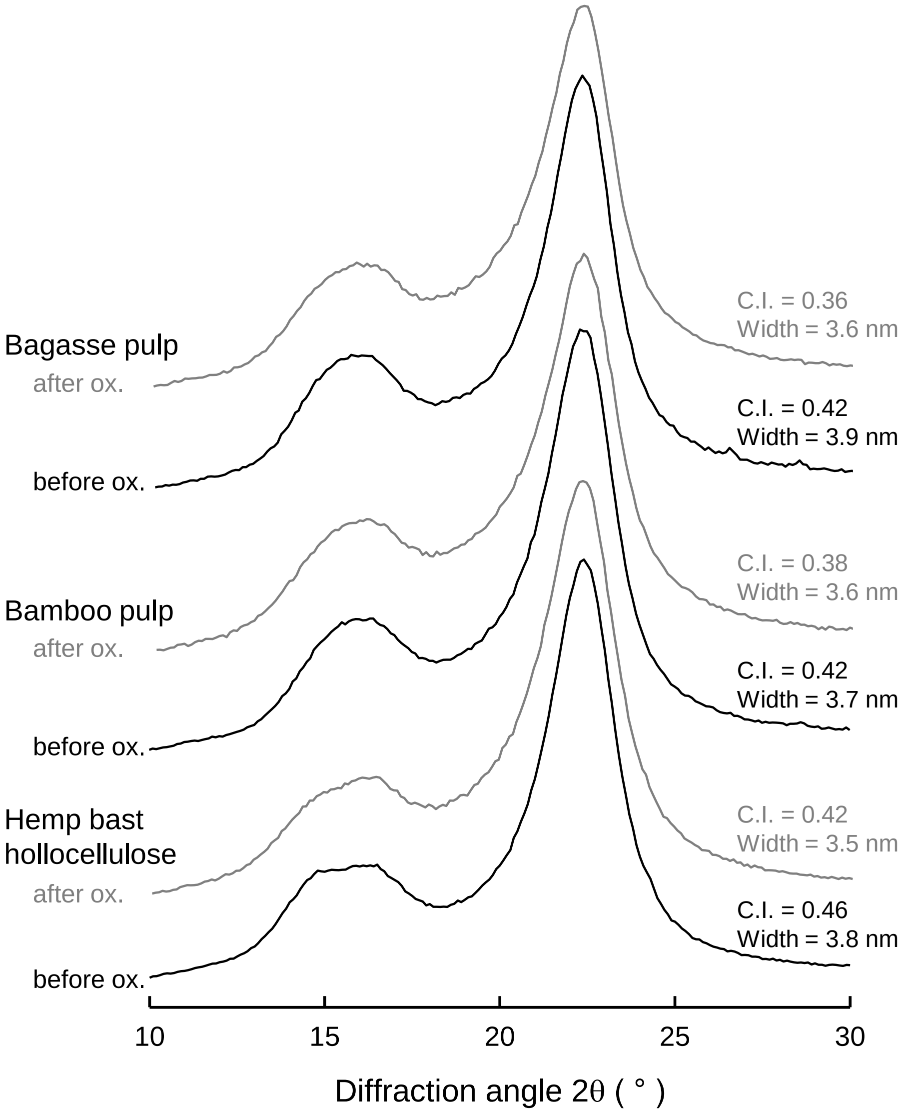

Figure 16. X-ray diffraction patterns of hemp bast holocellulose, and bamboo and bagasse pulps before and after TEMPO-mediated oxidation. Crystallinity index (C.I.) and (2 0 0) crystal width of cellulose I of the samples are depicted

5.3.2 TEMPO-oxidized cellulose nanofibrils prepared from non-wood celluloses

The 0.1% (w/v) TEMPO-oxidized cellulose/water slurries were mechanically disintegrated under the same conditions. The partly fibrillated or unfibrillated fraction slightly present in the obtained gel-like dispersion was removed by centrifugation. The nanofibrillation yields were 98, 98 and 82% for TEMPO-oxidized celluloses prepared from the hemp bast holocellulose, and bamboo and bagasse pulps, respectively. The relatively high xylose content in the TEMPO-oxidized cellulose prepared from bagasse pulp (Table 5) resulted in the low nanofibrillation yield, because xylan have somewhat hydrophobic and no C6-primary hydroxyl groups to be oxidized to hydrophilic carboxylate groups.

Figure 17 shows AFM images of TEMPO-oxidized cellulose nanofibrils (TOCNs) prepared from three non-wood celluloses. Length and width distributions of TOCNs determined from AFM height images are also attached to Figure 17. The TOCNs in each sample were mostly present as individual nanofibers. Some of them had kinks, although it is unknown whether these kinks originally existed in some TOCNs in the dispersion states or formed during drying process of the TOCN dispersions in AFM sample preparation. These kinks were ignored in measuring the TOCN lengths in this study; each nanofiber even having some kinks was regarded as one single TOCN in the length measurement.

In all samples, the TOCN lengths had wide distributions up to more than 1.6 μm, although the highest frequency existed in the same length range of 400−600 nm. The length-weighted average lengths were 654, 498 and 654 nm for TOCNs prepared from the hemp bast holocellulose, and the bamboo and bagasse pulps, respectively; the DP_v values of TEMPO-oxidized celluloses (Figure 15) were not necessarily correlate with the average lengths of the corresponding TOCNs. In contrast, TOCN widths of the three samples were similar to each

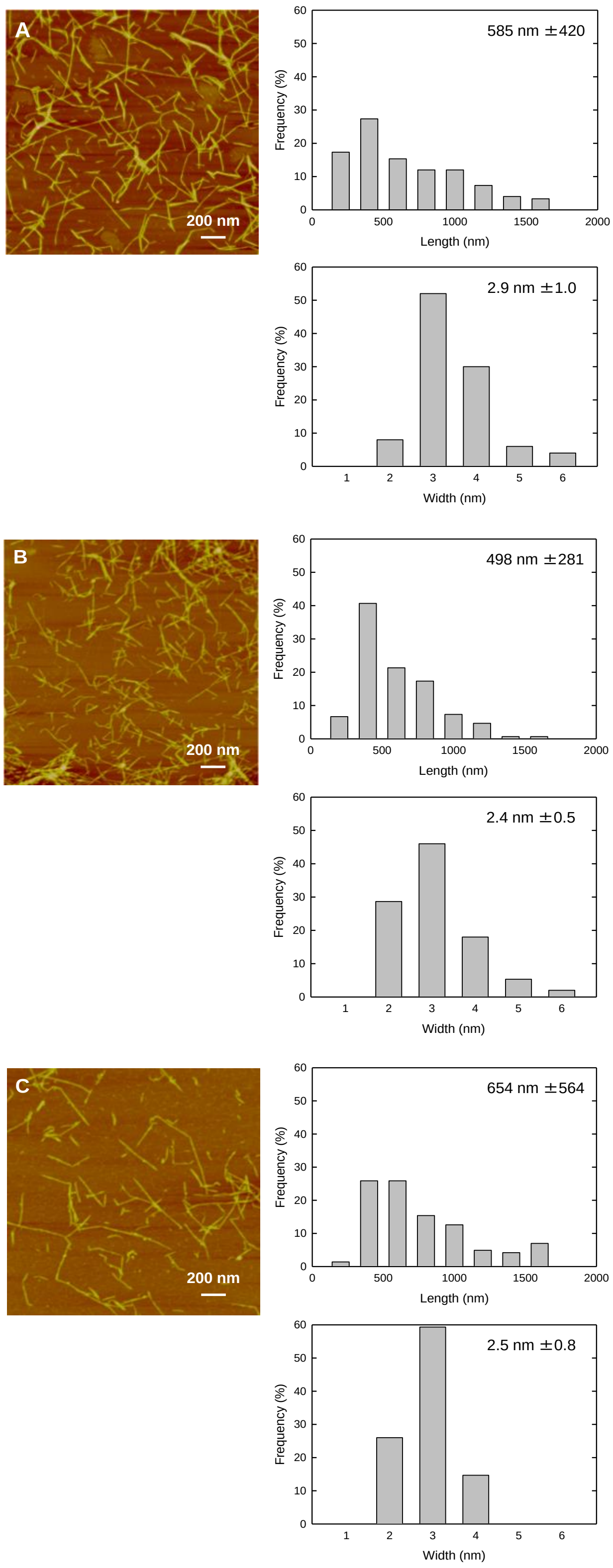

Figure 17. AFM height images, and length and width distributions of TOCNs prepared from their 0.1% aqueous dispersions. The original non-wood celluloses are (A) hemp bast holocellulose, and (B) bamboo and (C) bagasse pulp

other and ranged from 2.4 to 2.9 nm. The highest frequency of the TOCN widths was ~3 nm for all samples (Figure 17). It is generally observed that TOCN widths measured from AFM height images are somewhat smaller than (2 0 0) crystal widths determined from X-ray diffraction patterns [24].

5.3.3 Properties of TEMPO-oxidized non-wood cellulose nanofibril Films

The self-standing TOCN films were prepared by casting and drying of the TOCN/water dispersions on petri dishes. The UV-vis transmittance spectra of the TOCN films ~10 μm in thickness are shown in Figure 18. All the TOCN films had high light transmittances >87% at 600 nm, which are similar to those prepared from softwood and hardwood TOCN films [8]. Thus, almost complete nanofibrillation of non-wood celluloses can be achieved by the TEMPO-mediated oxidation and the successive mechanical disintegration of the oxidized celluloses in water under the conditions used in this study. When some unfibrillated or partly fibrillated bundles are present in the films, the sizes of which are close to or larger than wavelengths of irradiation light, the light transmittances remarkably decrease by scattering [25]. The AFM height images of the TOCN film surfaces (2 × 2 μm) are depicted in Figure 19. All TOCN film surfaces had network structures consisting of randomly oriented TOCN elements. Because the TOCNs prepared from the bamboo pulp had the shortest average length, the TOCN film had the smoothest surface.

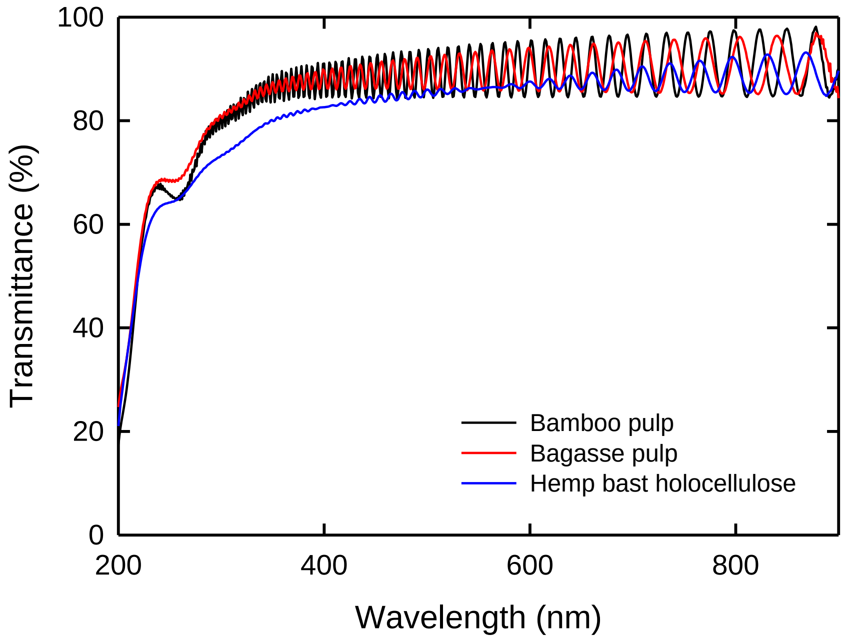

Figure 18. UV-vis transmittance spectra of TOCN films (~10 µm thickness) prepared from (A) hemp bast holocellulose, and (B) bamboo and (C) bagasse pulps

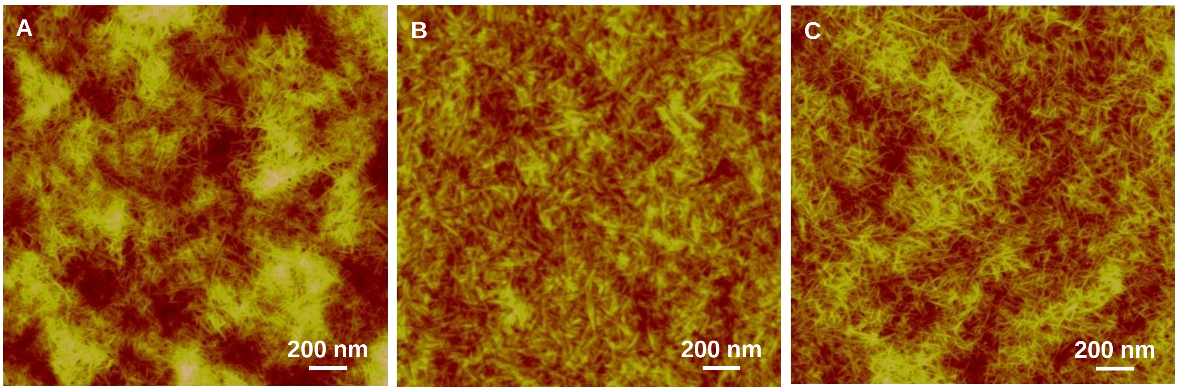

Figure 19. AFM height images of TOCN films prepared from (A) hemp bast holocellulose, and (B) bamboo and (C) bagasse pulps

Fundamental and mechanical properties of self-standing TOCN films are presented in Table 6. The bagasse TOCN films had the lowest density of 1.41 g cm^{-3} probably because of the longest average TOCN length (Figure 17). The hemp bast holocellulose TOCN films had the highest tensile strength (232 MPa) and the largest elongation at break (11%), which may be due to its high DP_v and high crystallinity index of cellulose I. Accordingly, the hemp bast holocellulose TOCN films had clearly high works of fracture of ~30 MJ m^{-3} compared with those of the bamboo and bagasse TOCN films (2−8 MJ m^{-3}). The mechanical properties of the hemp bast holocellulose TOCN films are similar to those prepared from wood TOCNs [8]. The relatively low Young's modulus of the hemp bast holocellulose TOCN films may be due to its high moisture content under the conditions at 23°C and 50% relative humidity. In contrast, the low tensile strengths and low elongations at break of the bamboo and bagasse TOCN films are likely to be due to their low DP_v values and low crystallinity indices. The high Young's moduli of these films are probably caused by their low moisture contents, which make the films more brittle. Coefficients of thermal expansion of the TOCN films were similar and ranged of 4−6 ppm K^{-1}, which are clearly low compared with other petroleum-based polymer films. The highly crystalline TOCN elements of the films lead to these results [2,8,26].

Table 6. Fundamental and mechanical properties of TEMPO-oxidized cellulose nanofibril films prepared from hemp bast holocellulose, and bamboo and bagasse pulps

	Hemp bast holocellulose	Bamboo pulp	Bagasse pulp
Density (g cm^{-3})	1.65	1.61	1.41
Moisture content at 23°C and 50% R.H. (%)	11.2	9.6	8.1
Tensile strength (MPa)	232 ± 7	170 ± 9	137 ± 14
Elongation at break (%)	11.2 ± 0.9	3.6 ± 0.6	1.4 ± 0.3
Young's modulus (GPa)	6.8 ± 1.5	9.5 ± 1.8	10.9 ± 0.5
Work of fracture (MJ m^{-3})	29.6 ± 1.5	7.8 ± 1.8	1.5 ± 0.3
Coefficient of thermal expansion (ppm K^{-1})	5.7	5.2	4.1

5.4 CONCLUSIONS

When the three non-wood celluloses were oxidized by the TEMPO/NaBr/NaClO system with 7.5 mmol g^{-1} of cellulose in water at pH 10, weight recovery ratios of the water-insoluble TEMPO-oxidized celluloses thus obtained decreased to 70−80% and their carboxylate contents reached 1.5−1.7 mmol g^{-1}. The DP_v values remarkably decreased from 800−1100 to 200−480 by partial depolymerization occurring during the oxidation. The TEMPO-oxidized cellulose maintained the original cellulose I crystal structure, while crystallinity index and crystal width were slightly decreased by the oxidation. TOCNs prepared by mechanical disintegration of the TEMPO-oxidized celluloses in water consisted of mostly individual TOCN elements and their nanofibrillation yields were 82−98%. The average lengths and widths of the TOCNs were 500−650 nm and 2.4−2.9 nm, respectively. The self-standing TOCN films prepared by casting and drying of the TOCN/water dispersions had high light-transparencies (>87% at 600 nm), high tensile strengths (140−230 MPa), high Young’s moduli (7−11 MPa), low coefficients of thermal expansion (4−6 ppm K^{-1}) in spite of low densities of 1.4−1.7 g cm^{-3}. In particular, the hemp bast holocellulose TOCN films had clearly high works of fracture (~30 MJ^{-1}) compared with those of the bamboo and bagasse TOCN films (2−8 MJ^{-1}). Thus, these non-wood celluloses can be used in place of wood cellulose pulps for preparation of TOCN/water dispersions and self-standing TOCN films.

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6. SUMMARY

The following conclusions concerning TEMPO-mediated oxidation of non-wood celluloses can be drawn from the results obtained in this study.

1. Because the non-wood agricultural products have potential application as cellulose sources, it would be beneficial especially for developing countries to use these agricultural products as fibers, chemical, biofuel, etc. In this context, fundamental properties of non-wood plant fibers must be understood from various aspects to use them as resources of nanocelluloses. In this work, therfore, I focused on the preparation and characterization of TEMPO-oxidized cellulose nanofibrils and their cast films from three non-wood plant resource, hemp bast, bagasse, and bamboo.

2. The HBHs is easily converted to individual fibers through mechanical disintegration treatment in water because most of lignin present in the original HB is removed by the Wise method. The HBH has widths of 20-30 micrometers and lengths of thousand micrometers. After delignification, α-cellulose and lignin content of the HBH is 79.4% and 0.2%, respectively. This cellulose content of HBH is similar to or slightly lower than that of bleached kraft pulps for papermaking.

3. Carboxylate contents in TEMPO-oxidized HBHs (TO-HBHs) are controllable by controlling TEMPO-mediated oxidation conditions. Because the TO-HBHs are strongly swollen in water, partly forming balloon-like structures, degree of oxidation of TO-HBHs should be adequately controlled to improve filtration efficiency and other handlings. The weight recovery ratio of the TO-HBHs decrease as the degree of oxidation is increased,

probably because hemicelluloses are degraded during the oxidation and removed as water-soluble compounds during washing. When harsh oxidation conditions are applied to HBH, not only hemicelluloses but also a part of cellulose fraction becomes water-soluble, resulting in lower weight recovery ratios.

4. The C6-primary hydroxyl groups exposed on crystalline microfibril surfaces of HBHs are selectively converted to C6-carboxylates groups through TEMPO-mediated oxidation, which is proved through X-ray diffraction analyses of TO-HBHs. Compared with TO-wood celluloses, the amounts of C6-aldehydes formed as intermediates are lower, indicating that C6-aldehydes are easily converted to C6-carboxylates during the TEMPO-mediated oxidation. This is a characteristic and beneficial point of TEMPO-mediated oxidation of HBH to prepare TO-HBHs.

5. Remarkable depolymerization of cellulose chains is inevitable during the TEMPO-mediated oxidation of HBH in water at pH 10. Thus, controlling DPv of TO-HBHs is one of the most challenging subjects for practical applications of TO-HBHs in future.

6. Neutral sugar composition analysis of TO-HBHs show the most of hemicelluloses are removed as water-soluble products from the water-insoluble TO-HBHs. Thus, almost pure TO-celluloses without containing any hemicelluloses can be obtained from HBH by TEMPO-mediated oxidation. Probably, non-crystalline hemicelluloses having lower degrees of polymerization are more suscepotible to TEMPO-mediated oxidation to form water-soluble oxidized products having lower degrees of polymerization.

7. The TO-HBHs are converted to transparent and highly viscous dispersions in water by mechanical disintegration treatment, consisting of mostly individualized TO-cellulose nanofibrils (TOCNs). The nanofibrillation yields can increase up to 98-100%, depending on carboxylate contents of TO-HBHs. Thus, not only wood cellulose but also HBS is plausible to be used as a resource for preparation of TOCNs. The TOCNs prepared from HBHs, bagasse, and bamboo pulp with sodium carboxylate groups of 1.5−1.7 mmol g^{-1} have almost similar nanofibrils widths of 2.4-2.9 nm and lengths of 500-650 nm, when observed with atomic force microscopy; these non-wood celluloses have crystalline microfibrils having the same widths. Because the DPv values of TO-non-wood celluloses remarkably decrease from 800−1100 to 200−480 through partial depolymerization occurring during the oxidation, controlling DPv values as high as possible is one of the key issure to be overcome in practical applications.

8. Because the TOCN films prepared from three non-wood celluloses show high light-transmittances, they have potential application as transparent and flexible films. As to mechanica properties of the TOCN films, the DPv values of TO-non-wood celluloses primarily govern the resultant properties of the films. Thus, again controlling DPv values are the key point to prepare TOCN films having sufficiently high mechanical properties. All TOCN films show lower coefficients of thermal expansion than those of other petroleum-based polymer films, irrespective of the non-wood resources used, showing potential applications as nanofiller components in electric devices.

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RESEARCH ACHIEVEMENT

LIST OF PUBLICATIONS

1. **Buapan Puangsin**, Shuji Fujisawa, Ryota Kuramae, Tsuguyuki Saito and Akira Isogai. 2012. TEMPO-mediated oxidation of hemp bast holocellulose to prepare cellulose nanofibrils dispersed in water. Journal of Polymers and the Environment. 21:555-563

2. **Buapan Puangsin**, Quanling Yang, Tsuguyuki Saito and Akira Isogai. 2013. Comparative Characterization of Cellulose Nanofibril Films Prepared from Mon-Wood Celluloses by TEMPO-Mediated Oxidation. International Journal of Biological Macromolecules. 59:208-213

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