Prevention of Aggregation of Pectin-Containing Cellulose Nanofibers Prepared from Mandarin Peel

Shou Hiasa*1,*2, Akio Kumagai*2, Takashi Endo*2,x, and Yusuke Edashige*1

*1 The United Graduate School of Agricultural Sciences, Ehime University, 3-5-7 Tarumi Matsuyama, Ehime 790-8566, Japan
*2 Research Institute for Sustainable Chemistry Department of Materials and Chemistry, National Institute of Advanced Science and Technology, 3-11-32, Kagamiyama, Higashihiroshima, Hiroshima 739-0046, Japan

Abstract: In this study, pectin-containing cellulose nanofibers (CNFs) were prepared from mandarin peel (MP), and the effect of pectin, which remained in purified cellulose after a purification treatment, on the aggregation of CNFs was investigated. Cellulose samples with different pectin concentrations were prepared by bleaching and a hydrothermal treatment with an acid solvent. Following the preparation of the different pectin-containing cellulose samples, each cellulose sample was fibrillated by a high-pressure homogenizer treatment. The morphological structures of CNF samples were observed using atomic force microscopy (AFM) and scanning electronic microscopy (SEM). The AFM images of the pectin-containing CNFs showed that the pectin covered the surfaces of the CNFs and that the CNFs obtained from MP were finer than those obtained from wood cellulose. Furthermore, the SEM images of the oven-dried samples showed that the pectin-containing CNFs were finer than the purified CNFs obtained from MP. Moreover, the oven-dried pectin-containing CNFs could be redispersed in water. This indicated that pectin has a potential to prevent the aggregation of CNFs. However, the addition of commercial pectin to a CNF suspension did not inhibit CNF aggregation. Although an interaction between the CNFs and pectin were confirmed in the case of the pectin-containing CNFs prepared from MP, the commercial pectin did not interact with the purified CNFs. This CNF-pectin interaction, which is based on their original structures, probably results in the pectin covering the surfaces of the CNFs. It is likely that this is the reason that the aggregation of the CNFs was inhibited.

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1. Introduction

There has been an increase in the demand to use more environmentally friendly biomass materials such as forest resources and agricultural by-products. The main component of these materials is cellulose, which is the most abundant organic material on earth. Cellulose is a homopolymer consisting of linear chains of repeated β-linked (1-4) glucose units. Cellulose forms hierarchical structures consisting of crystalline cellulose nanofibers (CNFs) [1]. CNFs are extremely fine and have widths of 3-5 nm [2]. Recently, CNFs have gained much attention owing to their numerous desirable properties. For instance, CNFs have a large surface area, low thermal expansivity, and high tensile strength [3,4]. These characteristics make CNFs a suitable material for use as plastic filler and functional films [5,6]. CNFs have previously been isolated from plants by mechanical and chemical treatments [3,7,8]. For example, CNFs were produced from wood after removing the other embedded substances such as hemicellulose and lignin. Subsequently, the purified wood cellulose, which was kept in water, was fibrillated by repeated grinding using a high-pressure homogenizer and an ultrasonic treatment [9,10]. After a series of such treatments, CNFs with a uniform width could be obtained. On the other hand, fruit tissue contains large amounts of cellulose and pectin, which is mainly composed of galacturonic acid [11]. Therefore, the isolation of CNFs from fruit requires the removal of the pectin [8,12]. Ifuku et al. [8] and Hiasa et al. [13] have reported CNFs with a width of 2-3 nm synthesized from fruits by disk milling and ultrasonic treatments after the

# corresponding author : ☎ 739-0046 広島県東広島市鏡山 3-11-32
repeated removal of pectin.

Preventing the aggregation of CNFs is a challenging problem in utilizing CNFs. Once CNFs have been dried, they aggregate through the formation of hydrogen bonds and lose their characteristic features, such as their large surface area and small width [14]. Many studies have been performed on ways to prevent the aggregation of CNFs and improve their dispersion properties. For example, the dispersibility of CNFs can be improved by chemical treatments and by adding a disperser. Saito et al. [2] prepared CNFs through TEMPO-mediated oxidation. The oxidized CNFs had high dispersibility owing to the electronic repulsion of the carboxy group. Lowys et al. [15] have reported that the aggregation properties of CNFs can be improved by adding a water-soluble polyelectrolyte to the CNF suspension. It is assumed that the aggregation of the CNFs, which is caused by hydrogen bond formation, was prevented by the formation of a water-soluble interfacial film around the CNFs while they were being dried. It has also been found that the dispersibility of CNFs is improved by the presence of noncellulosic materials in the cellulose after the purification treatment. Agoda-Tandjawa et al. [16] reported that the negative charge carried by the CNFs, attributable to the residual galacturonic acid, allows the fibers to electrostatically repulse each other. Therefore, pectin, which contains galacturonic acid, has the potential to improve the dispersibility of CNFs.

In this study, pectin-containing CNFs with different pectin concentrations were prepared from mandarin peel (MP) waste, and the effect of pectin on the aggregation of the CNFs was investigated by atomic force microscopy (AFM) and scanning electron microscopy (SEM). Further, the interaction between the CNFs and pectin was investigated through quartz crystal microbalance (QCM) monitoring. Since raw MP consists of 22.5% cellulose, 16.0% pectin, and 6.0% hemicellulose [11], MP was used to investigate the effect of pectin on CNF aggregation. Global industrial citrus peel wastes draw attention as a potential feedstock for bioethanol as their amount is estimated to be than 15×10^6 tons [17]. By elucidating the effect of pectin on the aggregation of the CNFs from MP, effective methods for utilizing MP wastes (other than as feedstock for bioethanol) may be discovered.

2. Materials and methods

2.1 Materials

The MP sample (peel of Citrus unshiu) used was the waste from citrus juice production and was provided by Hiroshima Cooperative Stock Company, Japan. The MP sample was stored in a freezer at -60°C. Before being used, the material was pulverized into particles using a cutter mill (3M7-40, Masuko Sangyou Co., Ltd., Japan). Powdered bleached wood pulp (W-400, Nippon Paper Chemicals Co., Ltd., Japan) and commercial pectin (pectin, from Citrus, Wako Pure Chemical Industries Co., Ltd., Japan) were used as reference materials.

2.2 Preparation of pectin-containing CNFs

Purified cellulose was prepared from the MP sample as per a previously reported process [18]. The treatments performed to prepare the pectin-containing cellulose are listed in Table 1. The lignin and coloring substances in the MP sample were bleached with 1.8 % (w/v) sodium chlorite and 0.4 % (w/v) acetic acid. The extraction process was performed at 80°C for 60 min. The solid concentration was 18 g/L. The bleached MP sample was collected by centrifugation (8000 rpm, 5 min, 25°C) and washed with distilled water five times.

After the extraction of the lignin and coloring substances, the pectin was removed through a hydrothermal treatment. During the hydrothermal treatment, the bleached MP sample was added to 0.05 M hydrochloric acid in a solid concentration of 10 g/L. The treatment was performed at 120°C for 10 or 120 min using an autoclave (MC-3032S, ALP Co., Ltd., Japan). After the treatment, the sample was washed with distilled water through centrifugation (8000 rpm, 5 min, 25°C) and dried.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Purification treatments used to prepare the pectin-containing samples.</th>
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<tbody>
<tr>
<td></td>
<td>Bleaching treatment</td>
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<tr>
<td>Pectin-MP-CNFs I</td>
<td>60 min</td>
</tr>
<tr>
<td>Pectin-MP-CNFs II</td>
<td>60 min</td>
</tr>
<tr>
<td>Pectin-MP-CNFs III</td>
<td>60 min</td>
</tr>
<tr>
<td>Purified-MP-CNFs</td>
<td>60 min</td>
</tr>
</tbody>
</table>

Table 1
5 min, 25°C) five times.

The MP sample hydrothermally treated for 120 min was treated with an alkaline solution (1.0 M potassium hydroxide) at a solid concentration of 10 g/L to obtain highly purified cellulose. The treatment was performed at 80°C for 120 min. After the treatment, the sample was washed with distilled water through centrifugation (8000 rpm, 5 min, 25°C) five times.

After the pectin-containing cellulose samples had been prepared, they were fibrillated using a high-pressure homogenizer (MMX-L200-D10, Masuko Sangyou Co., Ltd., Japan). The different fibrillated pectin-containing cellulose samples are labeled as Pectin-MP-CNFS I (bleached), Pectin-MP-CNFS II (bleached and hydrothermally treated for 10 min), Pectin-MP-CNFS III (bleached and hydrothermally treated for 120 min), and Purified-MP-CNFSs (bleached, hydrothermally treated, and then treated with an alkaline), as shown in Table 1.

Water slurries (0.2% (w/v)) of the MP samples and wood pulp were passed once and ten times through the high-pressure homogenizer at 180 MPa, respectively. Since the high-pressure homogenizer treatment usually has to be repeated to obtain uniform and fine nanofibrils from wood cellulose [19,20], the fibrillation treatment was performed ten times.

2.3 Concentrations of neutral sugars and galacturonic acid

The contents of the neutral sugars in the samples were analyzed after the samples had been subjected to acid hydrolysis [21]. A certain amount of the dried sample in question (50 mg) was hydrolyzed with 600 L sulfuric acid (26.2 mol/L) at 50°C for 90 min. After being diluted with 16.8 mL distilled water, the sample solution was hydrothermally treated at 120°C for 60 min using an autoclave. After the acid hydrolysis process, the sample was neutralized with calcium carbonate. The contents of the neutral sugars in the hydrolyzed samples were measured using high-performance liquid chromatography (HPLC) with a refractive index (RI) detection system (RI-2031 plus, JASCO Co. Ltd., Japan). The measurements were performed at 80°C using an HPLC column (HPX-87P, Bio-Rad Laboratories Inc., USA). Ultrapure water was used as the eluent. The flow rate was 0.6 mL/min.

The galacturonic acid contents of the samples were determined after subjecting the samples to an enzymatic treatment [22]. The polygalacturonic acid of the pectin in the samples was degraded to galacturonic acid using pectinase (from Aspergillus niger, Sigma-Aldrich Co. LLC, USA). The enzymatic reaction mixture, which contained 20 mg of the solid sample, 10 mg of pectinase, and 25 mL sodium acetate buffer (pH 4.0), was incubated at 50°C for 2 days. After the enzymatic treatment, the galacturonic acid content was measured using the HPLC instrument with the RI detection system (RI-8020, TOSOH Co., Ltd., Japan). The measurements were performed at 60°C using an HPLC column (HPX-87H, Bio-Rad Laboratories Inc., USA). Sulphide acid (0.05 N) was used as the eluent. The flow rate was 0.6 mL/min.

2.4 Morphological structure

2.4.1 Atomic force microscopy (AFM)

AFM was used to observe the structures of the CNF samples. First, 0.01 wt% water suspensions of the fibrillated samples were spin-coated onto Au wafers precoated with polyethyleneimine (PEI) using an Opticoat spin-coater (MS-A 100, MIKASA Co., Ltd., Japan). The PEI acted as an absorbent on the Au wafers. After the water had been allowed to evaporate at room temperature, the samples were observed using AFM (JSPM-5200, JEOL Co., Ltd., Japan). The scan area was 3.50 μm × 3.50 μm, and the drive frequency was 298–311 kHz. A silicon cantilever (Type: PPP-NCHR, NANOWORLD Co., Ltd., Switzerland) with a spring constant of 42 N/m was used for the purpose. The observations were performed in the tapping mode at 25°C. The relative humidity was kept at approximately 30%.

2.4.2 Scanning electron microscopy (SEM)

SEM (S-4800, Hitachi High-Technologies Co., Ltd., Japan) was used to observe the morphological structures of the CNFs, which were freeze dried and oven dried for the purpose. The freeze-dried CNFs were prepared by freeze dehydration, after the water in the CNF suspensions had been replaced with tert-butyl alcohol. On the other hand, CNF films were used to prepare the oven-dried CNF samples. After the completion of the high-pressure homogenizer treatment, the CNF suspensions (0.2% (w/v)) were poured into petri dishes (20 mL) and dried in a ventilated oven without a forced air flow at 40°C for 3 days. This yielded films of the CNFs. These freeze-dried and oven-dried samples were coated with osmium using an osmium coater (NEOC-AN, Meiwafosis Co., Ltd., Japan). Finally, the osmium-coated samples were observed using SEM.
2.5 Redispersibility of CNF samples
2.5.1 Observation of redispersed CNFs

The redispersibility of the oven-dried CNFs was evaluated through visual and SEM observations. Each oven-dried CNF sample was immersed in water, and 0.2 wt% suspensions of the redispersed CNFs were prepared. The redispersed CNF samples were freeze dried, once the water in the CNF suspensions had been replaced with tert-butyl alcohol. They were then observed using SEM.

The redispersibility of a mixture of the CNFs and pectin was also observed for reference. The commercial pectin was dissolved at 80°C in water, and a 0.2 wt% pectin solution was prepared. This pectin solution was mixed with a suspension of the CNFs obtained from wood pulp, and the mixture was dried in a ventilated oven without a forced air flow at 40°C for 3 days.

2.5.2 Tensile testing

Tensile tests were performed on the CNF films, in order to evaluate the degree of hydrogen bond formation of the dried CNFs. The tensile strengths and Young’s modulus of the CNF films were measured using a tensile tester (AGS-5kNG, Shimadzu Co., Ltd., Japan). Specimens with a length of 20 mm and width of 5 mm were measured at a cross-head speed of 10 mm/min; the span length was 10 mm. The thickness of each film was measured using a micrometer (µ-mate, SONY Magnoscale Co., Ltd., Japan). All the films were kept at 25°C in a room with an RH of 20% for 7 days. Finally, each specimen was tested three times.

2.6 Interaction between CNFs and pectin
2.6.1 Quartz crystal microbalance (QCM) measurements

The QCM measurements were performed to elucidate the interaction between the CNFs and pectin; this was done by monitoring the amount of pectin adsorbed on a CNF-coated sensor. The CNF-coated sensor was prepared using a previously reported method [23]. PEI was used to improve attachment of the CNF samples to the gold sensor (QSX 301, Biolin Scientific AB, Göteborg, Sweden). The sensor was immersed in a 1 wt% PEI/Milli-Q water solution for 15 min, rinsed with Milli-Q water, and then dried with nitrogen gas. The suspension of CNFs obtained from wood pulp was diluted with Milli-Q water to a concentration of 0.2 wt% and agitated with an ultrasonic homogenizer for 15 s, in order to improve the dispersibility of the CNFs. This sample was then centrifuged for 30 min at 10000 rpm to remove the fibril aggregates. The centrifuged supernatant was used for coating the sensor. The sensor was coated using a spin-coater (Opticoat MS-A 100, Mikasa Co., Ltd., Tokyo, Japan). Finally, the CNF-coated sensor was heat treated in an oven at 80°C for 10 min.

The amount of pectin adsorbed onto the CNF-coated sensor was measured using a QCM instrument (Q-Sense E1, Biolin Scientific AB, Göteborg, Sweden). The sample solution of commercial pectin and the CNFs obtained from wood pulp was produced as follows. The CNF suspension was diluted with Milli-Q water to a concentration of 0.2 wt% and agitated with an ultrasonic homogenizer for 15 s, in order to improve the dispersibility of the CNFs. The suspension was then centrifuged for 30 min at 10000 rpm to remove the fibril aggregates. The centrifuged supernatant (5.0 × 10⁻³ wt%) was used as the sample solution. The pectin solution was prepared by melting commercial pectin in Milli-Q water at 80°C and diluting it to the same concentration as that of the CNF supernatant (5.0 × 10⁻¹ wt%). The sample solution was injected continuously into the flow cell at a flow rate of 50 μL/min at 25°C for 40 min. Thereafter, the flow of the sample solution was stopped, and Milli-Q water was introduced at a flow rate of 50 μL/min, in order to rinse the system. The frequency and dissipation changes were monitored at the fundamental resonance frequency (5 MHz). An overtone of 15 MHz was used for data evaluation. The experiments were repeated a number of times, and the variation across all the experiments was found to be negligible. The change in the frequency (Δf, Hz) was calculated in terms of the change in the adsorbed mass per unit surface (Δm, µg/cm²); the Q-tools software (Biolin Scientific AB, Göteborg, Sweden) was employed for the purpose.

2.6.2 Analysis of constituent glucose before and after centrifuge treatment

The pectin-containing CNFs obtained from MP and the mixture of CNFs obtained from wood pulp and the commercial pectin were centrifuged for 30 min at 15000 rpm and 25°C to prepare precipitates of the CNF samples. The glucose contents of the precipitates were analyzed by the HPLC method in the manner described above.
Table 2  Concentrations of neutral sugars and galacturonic acid in the pectin-containing samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Arabinose</th>
<th>Galacturonic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pectin-MP-CNFs I</td>
<td>38.6±0.3</td>
<td>1.6±0.1</td>
<td>0.5±0.2</td>
<td>12.1±0.4</td>
<td>30.5±1.5</td>
</tr>
<tr>
<td>Pectin-MP-CNF II</td>
<td>77.1±2.1</td>
<td>8.4±0.3</td>
<td>6.0±0.2</td>
<td>-</td>
<td>4.9±0.3</td>
</tr>
<tr>
<td>Pectin-MP-CNFs III</td>
<td>88.3±2.3</td>
<td>5.0±0.3</td>
<td>3.2±0.1</td>
<td>-</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Purified-MP-CNFs</td>
<td>92.9±0.2</td>
<td>1.5±0.1</td>
<td>4.5±0.5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are mean and SD (n=3)

3. Results and discussion

3.1 Preparation of pectin-containing CNFs from MP

Pectin-containing cellulose was prepared from MP by controlling the treatment conditions. The bleaching treatment was performed to remove the coloring materials and lignin from the MP sample. After the bleaching treatment had been performed, the pectin content was adjusted by hydrothermal treatment using an acid solvent. Since the industrial process for extracting pectin employs an acid and involves a hydrothermal treatment [24], the acid treatment is considered a practical method for solubilizing pectin. It is for this reason that the acid treatment was employed. In addition, cellulose-containing pectin has been prepared from MP by a hydrothermal treatment using an acid solvent previously too [13]. Table 1 shows the treatment conditions used for preparing the pectin-containing cellulose samples. The pectin content was controlled by varying the hydrothermal treatment time. Further, we attempted to remove the pectin and hemicellulose remaining through an alkaline treatment after the hydrothermal treatment. Table 2 shows the concentrations of the neutral sugars and galacturonic acid in the prepared pectin-containing samples. As cellulose is a polysaccharide consisting entirely of glucose, the glucose content can serve as an index of cellulose purity. Further, the main components of hemicellulose and pectin are other sugars and galacturonic acid, respectively. After the hydrothermal treatment, the glucose content increased, while the contents of the other sugars and galacturonic acid decreased. This result confirmed that cellulose samples with different pectin contents could be prepared successfully from MP by controlling the duration of the hydrothermal treatment. Further, highly purified cellulose could be obtained by performing an alkaline treatment after a 120-min hydrothermal treatment. Figure 1 shows AFM height images of the CNF samples and pectin. Figures 1a and b show the CNFs prepared from wood pulp (Purified-WP-CNFs) and commercial pectin, respectively. The Purified-WP-CNFs had a fibrous structure, while the commercial pectin had a nonfibrous structure. Figures 1c and d show images of the Pectin-MP-CNFs I and Purified-MP-CNFs, respectively. The AFM image of Pectin-MP-CNFs I (Fig. 1c) showed that a nonfibrous substance covered the surfaces of the CNFs. On the other hand, the Purified-MP-CNFs, which did not contain pectin (see Fig. 1d), had a fibrous structure similar to that of Purified-WP-CNFs (Fig. 1a). These results indicated that the nonfibrous substance observed on the surfaces of the fibers of Pectin-MP-CNFs I was pectin. Thus, pectin covered the surfaces of the CNFs prepared from MP.

The widths of the CNFs were determined from the AFM height images (Figs. 1a and d). The widths of the CNFs in the Purified-WP-CNFs and Purified-MP-CNFs were 3–8 nm and 2–3 nm, respectively.
Although continuous fibrillation was required to prepare fine CNFs from wood pulp, finer CNFs could be obtained from MP by simply performing fibrillation once, and the yield of CNFs on the basis of dried-MP was 15.8%. These results indicated that fine CNFs can be obtained more readily from MP than from wood pulp. Wanga et al. [25] reported that it is easier to isolate CNFs from parenchymal cells of bamboo than from bamboo fibers. According to Wanga and coworkers, the loose and porous structure of parenchymal cells greatly facilitates the isolation of CNFs. Since MP was also parenchymal cells, the fibrillation of MP could be achieved easily, and finer fibers were obtained. In addition, the original width of the fibers of fruit cellulose (1–2 nm) is smaller than that of wood cellulose (3–5 nm) [226].

3.2 Effect of pectin on aggregation of CNFs

Figure 2 shows the SEM images of the purified CNF samples prepared from wood pulp (Figs. 2a and b) and MP (Figs. 2c and d). It has been reported that CNFs aggregate after being dried in an oven [14]. Therefore, the CNF samples were freeze dried after the water in the CNF suspensions had been replaced with tert-butyl alcohol, in order to prevent their aggregation. The freeze-dried CNF samples obtained from wood pulp (Fig. 2a) and MP (Fig. 2c) consisted of a fibrous network. Further, even though the oven-dried CNF samples obtained from wood pulp (Fig. 2b) and MP (Fig. 2d) also formed a fibrous network, these fibers were thicker than the freeze-dried CNF samples (Figs. 2a and c). This result indicated that if CNFs obtained from MP are purified, they will aggregate like the CNFs obtained from wood pulp and purified by a pulping process.

Fig. 2 SEM images of the purified CNF samples prepared from (a, b) wood pulp and (c, d) MP. Drying method : (a, c) freeze drying and (b, d) oven drying.

3.3 Redispersibility of oven-dried CNF samples

The redispersibility of the pectin-containing CNFs in water was evaluated using the oven-dried pectin-containing CNF samples. Figure 4 shows the CNF suspensions formed after the immersion of the oven-dried CNF samples in water. In the case of

Fig. 3 SEM images of the oven-dried pectin-containing CNF samples obtained from MP. The pectin contents are (a) 30.5% (Pectin-MP-CNPs I), (b) 4.9% (Pectin-MP-CNPs II), and (c) 0.0% (Pectin-MP-CNPs III).

Figure 3 shows SEM images of the oven-dried pectin-containing CNF samples with different pectin contents. It was confirmed that these pectin-containing CNF samples also formed a fibrous network; in contrast, a nonfibrous substance was observed in the case of Pectin-MP-CNPs I (Fig. 3a). Although the fibers of Pectin-MP-CNPs III (Fig. 3c) were thick like those of the oven-dried purified CNF samples (Figs. 2b and d), the fibrous networks observed in Pectin-MP-CNPs I (Fig. 3a) and Pectin-MP-CNPs II (Fig. 3b) were relatively finer, unlike those of the oven-dried CNF samples. It can be inferred from this observation that pectin prevented the aggregation of CNFs during the drying process. Thimm et al. [27] reported that the pectin in the parenchymal cells of celery affects the aggregation of cellulose fibrils. According to Thimm and coworkers, removing the pectin allows the cellulose microfibrils to move closer, probably resulting in their aggregation.

Fig. 4 CNF samples redispersed in water : (a) Pectin-MP-CNPs I, (b) Pectin-MP-CNPs II, and (c) Purified-MP-CNPs.
Purified-MP-CNFS, CNF aggregation was observed. However, the CNF samples could be redispersed uniformly as the pectin content was increased. For instance, the oven-dried Pectin-MP-CNFS I could be dispersed uniformly in water without exhibiting precipitation. In addition, the redispersibility of Pectin-MP-CNFS I was confirmed using polarization microscopy (data not shown). These results indicated that CNFs containing adequate amounts of pectin can be redispersed in water. Figure 5 shows SEM images of the redispersed CNF samples (a) Pectin-MP-CNFS I and (b) Purified-MP-CNFSs. Each SEM sample was freeze-dried after exchanging the water in CNF suspension with tert-butyl alcohol, in order to ensure that their original morphology was maintained. A web-like structure was observed in the case of the redispersed Pectin-MP-CNFS I (Fig. 5a). On the other hand, aggregated fibers were observed in the case of the redispersed Purified-MP-CNFS (Fig. 5b). It has been reported that the aggregation of cellulose nanocrystals can be prevented by adding a disperser such as polyethylene glycol [28]. According to this study, the disperser covers the surfaces of the cellulose nanocrystals, thus preventing the formation of hydrogen bonds between the nanocrystals. As a result, the cellulose nanocrystals could be redispersed. Both CNFs and cellulose nanocrystals are cellulose nanostructures. Thus, in the case of the pectin containing CNFs, the pectin also functions like a disperser and covers the surfaces of the CNFs.

The aggregation of CNFs depends on the formation of hydrogen bonds between them. Further, once dried, CNFs are difficult to disperse in water again [14]. Therefore, in order to be able to fabricate redispensible CNFs, it is necessary to prevent the formation of hydrogen bonds between the CNFs during the drying process. Sehaqui et al. [29] reported that the mechanical properties of cellulose films are affected by the hydrogen bonds formed between the CNFs and that an increase in the number of fiber-fiber bonding sites resulted in the high mechanical properties of the films. For this reason, the extent of hydrogen bonding between the CNFs was evaluated on the basis of the mechanical properties of films of the produced CNFs.

CNF films with different pectin contents were prepared, and tensile tests were performed to determine their mechanical properties. Table 3 shows the results of the tensile tests performed on the CNF films prepared from MP and wood pulp. The tensile strength and Young’s modulus of the CNF films increased with an increase in the purity of the cellulose, and the mechanical properties of the Purified-MP-CNFS film were comparable to that of the Purified-WP-CNFS film prepared from wood pulp. This indicated that the mechanical properties of the films increased with a decrease in the pectin content. Therefore, the removal of pectin increases the number of hydrogen bonds between the cellulose fibers. In the case of wood pulp paper, it was found that the strength is increased by hemicellulose [30]. The presence of the hydroxyl group in the hemicellulose molecules, which caused the formation of a greater number of hydrogen bonds between cellulose and hemicelulloses, increased the degree of bonding between the fibers. However, the pectin present in MP is composed of galacturonic acid, which causes electrostatic repulsion, which is attributable to the acid groups of hemicellulose such

<table>
<thead>
<tr>
<th>Table 3 Mechanical properties of films of CNF samples.</th>
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<tr>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Pectin-MP-CNFS I</td>
</tr>
<tr>
<td>Pectin-MP-CNFS II</td>
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<tr>
<td>Pectin-MP-CNFS III</td>
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<tr>
<td>Purified-MP-CNFS</td>
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<td>Purified-WP-CNFS</td>
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Values are mean and SD (n=3)

![Fig. 5 SEM images of the redispersed CNF samples: (a) Pectin-MP-CNFS I and (b) Purified-MP-CNFSs.](image-url)
as the 4-O-methyl glucuronic acid substituent. Therefore, the results suggested that the galacturonic acid of pectin also decreased the number of hydrogen bonds formed between pectin-containing CNFs.

The prevention of hydrogen bond formation between CNFs is crucial for preparing fine CNFs. In case of the pectin-containing CNFs prepared from MP, it was assumed that the pectin physically coats the CNFs and weakens the hydrogen bonds formed between the CNFs through electrostatic repulsion. It is for this reason that the pectin-containing CNFs could be redispersed in water after being dried in an oven.

3.4 Effect of adding commercial pectin on aggregation of CNFs

As mentioned above, it was found that pectin has a potential to prevent the aggregation of CNFs. Thus, we attempted to use commercial pectin as an inhibitor of CNF aggregation. Since the glucose/galacturonic acid ratio (w/w) in Pectin-MP-CNPs I was nearly 4/3, a Purified-WP-CNPs/commercial pectin mixture (w/w = 4/3) was prepared. After the preparation of this CNF/pectin mixture, its suspension was oven dried and redispersed in water. Figure 6 shows the redispersed suspensions of (a) oven-dried Purified-WP-CNPs and (b) the Purified-WP-CNPs/commercial pectin mixture. CNF aggregation was observed in the cases of the Purified-WP-CNPs/commercial pectin mixture as well as in the case of Purified-WP-CNPs, even though commercial pectin was added as an inhibitor to prevent the CNFs from aggregating. This indicated that just mixing commercial pectin does not inhibit CNF aggregation.

According to Cheng [28], the redispersibility of cellulose nanocrystals can be improved by adding a disperser. Cheng reported that the disperser is adsorbed on the surfaces of the cellulose nanocrystals, thus effectively inhibiting the aggregation of the nanocrystals. For this reason, the interaction between the cellulose nanocrystals and the disperser is critical for preventing the aggregation of the cellulose nanocrystals. Thus, the interaction between the CNFs and pectin was investigated, in order to evaluate the effect of pectin on the prevention of CNF aggregation.

The interaction between the CNFs and the commercial pectin was investigated using the QCM instrument. QCM is a tool for performing measurements during in-situ adsorption processes in aqueous solutions and for determining the biocompatibility of materials [32]. Eronen et al. [33] measured the amount of hemicellulose adsorbed onto cellulose by QCM using a CNF-coated sensor and discussed the interaction between the CNFs and hemicellulose. In this work, the amount of pectin adsorbed onto the CNFs was also measured by QCM using a CNF-coated sensor. Further, the interaction between the CNFs and pectin was elucidated. Figure 7 shows the QCM profiles corresponding to the adsorption of the CNFs and pectin onto the CNF-coated sensor. The mass increase of CNFs indicates that CNFs adsorbed on CNF coated-sensor well. Since cellulose fibers have a large surface area and contain the hydroxyl group [4], it is likely that the CNFs readily formed hydrogen bonds with the CNF-coated sensor. In contrast, an increase in mass was not observed in the case of the adsorption of pectin onto the CNF-coated sensor. This indicated that the affinity between the CNFs and pectin was low. In case of the adsorption of hemicellulose onto cellulose, the carboxyl groups in hemicellulose decrease the degree of adsorption onto cellulose, owing to electrostatic

![Fig. 6 Redispersed CNF samples: (a) purified-WP-CNPs and (b) purified-WP-CNPs/commercial pectin mixture.](image)

![Fig. 7 Amount of (a) commercial pectin and (b) purified-WP-CNPs adsorbed onto the CNF coated-sensor.](image)
repulsion [31]. Since pectin is composed of galacturonic acid, which also causes electrostatic repulsion [16], pectin was not absorbed onto the CNF-coated sensor.

Subsequently, the interaction between the CNFs and the pectin in the pectin-containing CNFs from MP was evaluated by investigating the amount of pectin retained on the CNFs after centrifugation; the above-mentioned Purified-WP-CNFS/commercial pectin mixture was used for the purpose. Table 4 shows the glucose contents in the sample before and after centrifugation. The glucose content in the precipitate of the Purified-WP-CNFS/commercial pectin mixture increased from 69.3% to 90.9%. This increase in the glucose content indicated that pectin was easily dissociated from the CNFs by centrifugation. Further, this result was in keeping with those of the QCM analyses. On the other hand, the glucose content in the precipitate of Pectin-MP-CNFS I remained almost unchanged after centrifugation. This result indicated that the CNFs and the pectin in MP were associated with each other through an interaction that was strong enough to endure the centrifugation process. Lin et al. [34] have reported that binding of pectin with high content of neutral sugar side chains to cellulose was greater than that of pectin with less neutral sugar side chains during cellulose biosynthesis. Since these side chains were removed during the pectin extraction process, the commercial pectin was not absorbed onto the CNFs. Thus, it can be assumed that the original structures of cellulose and pectin are responsible for the CNF-pectin interaction seen in the case of Pectin-MP-CNFS I.

### 4. Conclusion

Pectin-containing cellulose was obtained from MP by bleaching and a subsequent hydrothermal treatment, and fine CNFs were obtained from the resulting pectin-containing cellulose through a single fibrillation treatment. The width of the oven-dried pectin-containing CNFs was smaller than that of the oven-dried purified CNFs. Moreover, the pectin-containing CNFs could be redispersed in water. It was found that the pectin prevented the aggregation of the CNFs. However, the addition of pectin to a CNF suspension did not prevent CNF aggregation. An interaction between the CNFs and pectin was confirmed in the case of the pectin-containing CNFs prepared from MP; however, this was not the case when the purified CNFs and commercial pectin were mixed. The pectin in the pectin-containing CNFs prepared from MP could cover the surfaces of the CNFs owing to this CNF-pectin interaction, which is probably related to their original structures and results in the aggregation of the CNFs being inhibited.

### References

14. Y., Peng, D. J. Gardner, and Y. Han, Cellulose, 19.

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**Table 4** Glucose contents of the CNF samples before and after centrifugation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucose content (wt%)</th>
<th>Before centrifugation</th>
<th>After centrifugation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pectin-MP-CNFS I</td>
<td>38.6 ± 0.3</td>
<td>35.6 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Purified-WP-CNFS + commercial pectin</td>
<td>69.3 ± 0.4</td>
<td>90.9 ± 3.1</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean and SD (n=3)


