

## Article

# Acetylation of Scaled-Down Chitin Nanofiber Films to Improve Mechanical Properties

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**Abstract:** A flexible chitin nanofiber (ChNF) film with a thin fiber morphology, named, scaled-down (SD)-ChNF film, was previously found to be formed via successive partial deacetylation of the parent self-assembled ChNFs, cationization/dispersion via electrostatic repulsion in aqueous acetic acid, and suction filtration/drying. In this study, acetylation of a SD-ChNF film using acetic anhydride in pyridine was carried out to improve the mechanical properties. The FT-IR spectra of the acetylated SD-ChNF films suggested that acetylation progressed from the surface to the interior of the films with the increasing amounts of pyridine and elevating temperatures. The degrees of acetylation (DA) strongly affected the chitin crystallinity and surface morphology of the acetylated SD-ChNF films. Tensile testing of the acetylated SD-ChNF films indicated that the mechanical properties were improved by adjusting the DA values of the films. For example, the acetylated SD-ChNF film with an 1.84 DA value on surface showed values of 44.1 MPa and 24.9% for tensile strength and elongation at break, respectively.

**Keywords:** acetylation; chitin nanofiber; mechanical properties; scaled down



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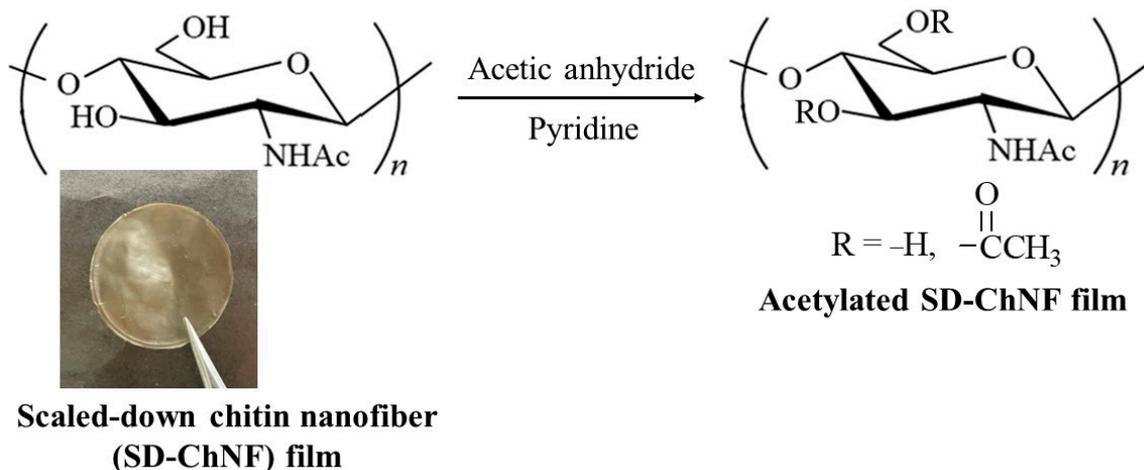
## 1. Introduction

Polysaccharides are widely present on the earth and act representatively as reservoirs of water/energy and structural materials [1–3]. Among such polysaccharides, chitin, which is composed of a  $\beta(1\rightarrow4)$ -linked N-acetyl-D-glucosamine chain, is a vital biomass resource because it is one of the most abundant polysaccharides, found mainly in the exoskeletons of crustaceans, shellfish, and insects [4–6]. However, chitin remains mostly unutilized because of its poor feasibility and processability owing to its robust fibrous chain packing, which is structured by numerous intra- and intermolecular hydrogen bonds. Recently, it has been identified that nanofibrillation, such as the fabrication of nanofibers and nanowhiskers, is one of the useful approaches to increase the functions of chitin [7–13], based on the remarkable properties of bio-based nanomaterials, such as biocompatibility, high tensile strength and low weight [14–20].

Based on a bottom-up approach, we previously achieved a facile and efficient procedure to fabricate chitin nanofibers (ChNFs) with a length of several hundred nanometers and a width of approximately 20–60 nm. Regeneration through nanoscale self-assembling from a chitin ion gel with an ionic liquid, namely 1-allyl-3-methylimidazolium bromide (AMIMBr), was successful using methanol [7,10]. This was based on our previous findings that AMIMBr readily dissolves and swells chitin [21]. The resulting dispersion of ChNF in methanol was subjected to suction filtration to isolate ChNFs, which formed a ChNF film with a heavily entangled nanofiber morphology. Furthermore, self-assembled ChNFs were found to comprise a bundle assembly of thinner fibrils [22]. When treatment of ChNF films with an aqueous NaOH was carried out, amino groups were partially generated on the chitin chains (i.e., partially deacetylated chitin nanofibers (PDA-ChNFs)), leading to the successful disentanglement of the bundles through cationization and electrostatic

repulsion in an 1.0 mol/L aqueous acetic acid with ultrasonication, giving rise to individual thin fibril materials, particularly named “scaled-down ChNFs (SD-ChNFs)”. Furthermore, SD-ChNFs were isolated from the resulting dispersion via suction filtration to form a film with a heavily condensed nanofiber morphology, which showed quite a flexible property.

Previously, derivatization and modification on ChNFs have been investigated to improve their properties and introduce new functions [23–27]. For example, acetylation on ChNFs was carried out, which were manufactured through the disentanglement of native chitin microfibrils using a top-down approach [28]. The acetylated ChNFs were well composited with acrylic resin to obtain transparent nanocomposites. In this study, we demonstrated the acetylation of SD-ChNF films using acetic anhydride in pyridine under several conditions to improve the mechanical properties (Figure 1). A reaction using acetic anhydride in pyridine is one of the most well-known acetylation procedures for polysaccharides, such as a chitin powder [29]; pyridine acts as both base and solvent for efficient progress of the reaction. FT-IR analysis of the products indicated that acetylation gradually progressed from the surface to the interior of the films. Consequently, we found that, by adjusting the degrees of acetylation (DAs), the produced films exhibited superior mechanical properties to those of the parent SD-ChNF film. Because the SD-ChNF film, presented in the previous study, has been reported to show excellent flexibility, that was not previously reported for a nanochitin film or sheet [30], the present films will extend the application potentials for ChNF-based functional materials.



**Figure 1.** Acetylation of scaled-down chitin nanofiber (SD-ChNF) film using acetic anhydride in pyridine.

## 2. Materials and Methods

### 2.1. Materials

The chitin powder from the crab shells was purchased from FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan. An ionic liquid, AMIMBr, was prepared via the reaction of 1-methylimidazole (Sigma-Aldrich, Darmstadt, Germany, >99.0%) with 3-bromo-1-propene (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan, 97.0%) according to a method adapted from the literature [31]. Authentic chitin acetates with different DA values were prepared according to the method used for AMIMBr with different reaction times adapted from our procedure in the literature [32]. Other reagents and solvents were commercially available and used without further purification.

### 2.2. Preparation of a Film of Partially Deacetylated Chitin Nanofiber (PDA-ChNF)

A mixture of chitin (0.120 g, 0.59 mmol)/AMIMBr (1.00 g, 4.92 mmol) was allowed to stand for 24 h at room temperature and then heated with stirring for 24 h at 100 °C to obtain a chitin ion gel (10 wt%). This gel was then immersed in methanol (30 mL, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan, 99.8%) for 72 h at room temperature for

regeneration progress, followed by ultrasonication (Branson 1510 (42 kHz, 70 W, Emerson Japan, Ltd., Tokyo, Japan)) for 10 min to produce a self-assembled ChNF dispersion with methanol. The resulting dispersion was subjected to suction filtration to separate the ChNFs, which were washed with methanol and dried under reduced pressure to obtain a self-assembled ChNF film.

A mixture of the resulting film (0.120 g, 0.59 mmol)/aqueous NaOH (30 wt%, 20 mL, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan, 97%) was heated for 5 h at 80 °C. The film obtained was isolated using suction filtration, immersed in water (30 mL) for 10 min via ultrasonication (Branson 1510 (42 kHz, 70 W)), filtered, and washed with water. The resulting film was dried under reduced pressure to obtain a PDA-ChNF film. <sup>1</sup>H NMR (DCI/D<sub>2</sub>O) δ 2.1–2.3 (CH<sub>3</sub>), 3.0–4.0 (H2-6), 4.5–5.2 (H1). The degree of deacetylation (DDA) value of the product was 23.0% for the total repeating units (via <sup>1</sup>H NMR spectrum in DCI/D<sub>2</sub>O).

### 2.3. Preparation of Scaled-Down Chitin Nanofiber (SD-ChNF) Dispersion and Film

A mixture of the PDA-ChNF film (0.120 g)/aqueous acetic acid (1.0 mol/L, 20 mL, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan, 99.7%) was ultrasonicated using a homogenizer (Branson Advanced-Digital Sonifier 450 (20 kHz, 400 W)) for 10 min at room temperature to produce a SD-ChNF dispersion. After the resulting dispersion was subjected to suction filtration, the residue was washed with water and dried under reduced pressure to obtain a SD-ChNF film.

### 2.4. Acetylation of SD-ChNF Film

A typical experimental procedure was followed (entry 4, Table 1). A mixture of the SD-ChNF film (0.050 g, 0.26 mmol)/acetic anhydride (1.47 mL, 15.6 mmol, 60 equiv. with a repeating unit, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan, 97.0%)/pyridine (2.00 mL, 26.0 mmol, 100 equiv. with a repeating unit, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan, 99.5%) was heated for 1 h at 80 °C and then cooled for 30 min at room temperature. The obtained film was washed with water and dried under reduced pressure to produce an acetylated SD-ChNF (Ac-SD-ChNF) film.

**Table 1.** Conditions in the acetylation of the SD-ChNF film and properties of the acetylated SD-ChNF films <sup>(a)</sup>.

Entry	Equiv. of Pyridine <sup>(b)</sup>	Temp. (°C)	DA <sup>(c)</sup> (KBr Pellet Method)	DA <sup>(c)</sup> (ATR Method)	CI <sup>(d)</sup>	Tensile Strength (MPa)	Elongation at Break (%)
SD-ChNF film	-	-	-	-	95.7	38.6	17.2
1	0.6	50	0.81	1.82	94.7	28.5	21.4
2	10	50	0.96	1.83	93.8	45.0	21.8
3	10	80	1.18	1.84	93.6	44.1	24.9
4	100	80	1.22	1.86	92.7	14.0	15.4

<sup>(a)</sup> Reaction was carried out using 60 equiv. of acetic anhydride with a repeating unit for 1 h at desired a temperature followed by cooling for 30 min at room temperature. <sup>(b)</sup> With a repeating unit. <sup>(c)</sup> Degree of acetylation, determined via FT-IR analysis. <sup>(d)</sup> Crystalline index of the chitin crystal in the 110 plane, calculated from the XRD result according to the method mentioned in the literature [33].

### 2.5. Measurements

FT-IR spectra were recorded on a PerkinElmer Spectrum Two spectrometer (PerkinElmer Japan Co., Ltd., Yokohama, Japan). The KBr pellet and attenuated total reflection (ATR) methods were used for the evaluation of DAs of the entire material and film surfaces, respectively. The <sup>1</sup>H NMR spectra were recorded using an ECX400 instrument (JEOL, Akishima, Tokyo, Japan). Powder X-ray diffraction (XRD) measurements were performed using a PANalytical X'Pert Pro MPD instrument (PANalytical B.V., Almelo, The Netherlands) with Ni-filtered Cu-

K $\alpha$  radiation ( $\lambda = 0.15418$  nm). The crystalline index (CI) of the chitin crystal was calculated according to a method mentioned in the literature using the following equation [33].

$$\text{CI (\%)} = [(I_{110} - I_{\text{am}})/I_{110}] \times 100;$$

where  $I_{110}$  is the maximum intensity (arbitrary units) of the diffraction (110) at  $2\theta = 19.3^\circ$  and  $I_{\text{am}}$  is the intensity of the amorphous diffraction in the same unit at  $2\theta = 12.6^\circ$ .

SEM images were obtained using a Hitachi S-4100H electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan). The stress–strain curves were measured using a tensile tester (Little Senstar LSC-1/30, Tokyo Testing Machine, Tokyo, Japan).

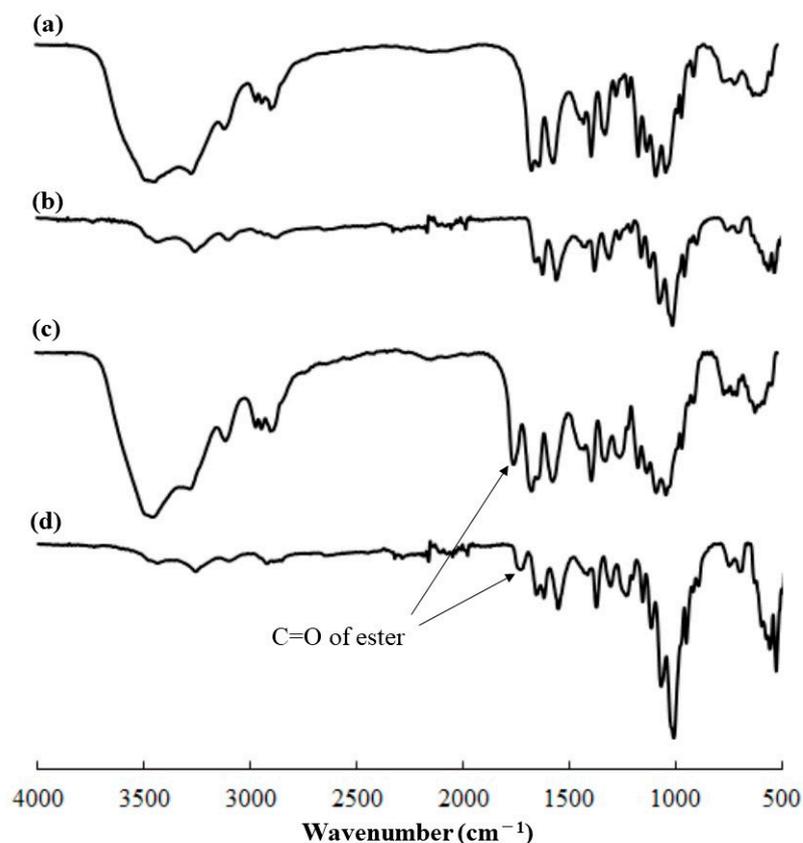
### 3. Results and Discussion

The PDA-ChNF film was first prepared through successive partial deacetylation by treating the self-assembled ChNF film with aqueous NaOH (30 wt%) for 5 h at  $80^\circ\text{C}$ , suction filtration, and drying (DDA value = 23.0%, determined via  $^1\text{H}$  NMR spectrum in  $\text{DCl}/\text{D}_2\text{O}$ ). The resulting film was then treated with aqueous acetic acid (1.0 mol/L) for 10 min at room temperature using ultrasonication (20 kHz, 400 W) to produce the SD-ChNF/aqueous acetic acid dispersion. The dispersion was then subjected to suction filtration, and the residue was dried under reduced pressure to obtain the SD-ChNF film.

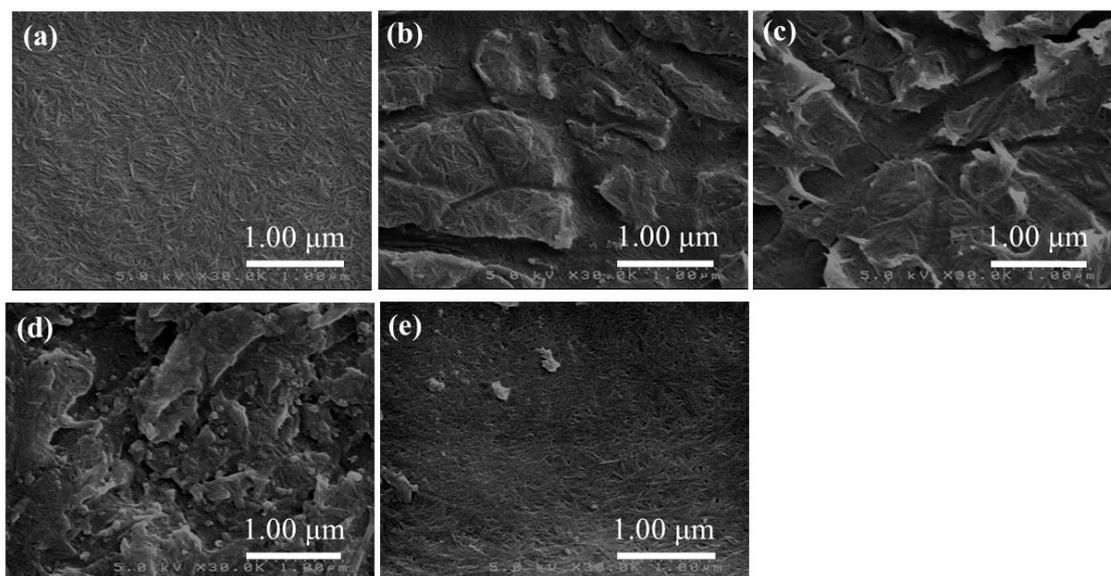
Acetylation of the resulting SD-ChNF film was carried out using acetic anhydride (60 equiv. with a repeating unit) in different amounts of pyridine for 1 h at  $50\text{--}80^\circ\text{C}$ , followed by cooling for 30 min at room temperature (Figure 1, entries 1–4, Table 1). The FT-IR spectra of all the newly acetylated products, measured using the KBr pellet method (Figure 2c, entry 4), exhibited the C = O absorptions derived from the ester groups around  $1740\text{ cm}^{-1}$  compared to those of the chitin power (Figure 2a), suggesting progress of acetylation; a similar spectroscopic pattern was also observed in the FT-IR spectrum of chitin powder, measured using the ATR method (Figure 2b) [30] (Found in the Supplementary Materials of this publication). DA values were calculated from the FT-IR spectra using the following equation:  $R = 0.975 - 0.003\text{ DA}$ , as previously reported in the literature [34], where  $R$  is the absorbance ratio ( $D_{1740}/D_{1658}$ ), which increased with increasing amounts of pyridine and increasing temperatures for acetylation. The DA values of the film surfaces were also evaluated using FT-IR spectra, measured via the ATR method. To create a calibration curve for evaluation, we prepared authentic chitin acetates with different DA values via the acetylation method using acetic anhydride in AMIMBr with different reaction times, adapted from our procedure in the literature [32]. The exact DA values were then calculated via  $^1\text{H}$  NMR spectra after acidic hydrolysis and dissolution of the chitin acetates in  $\text{DCl}/\text{D}_2\text{O}$  (Supplementary Figure S1). Based on the resulting DA values and FT-IR spectra of the chitin acetates, measured using the ATR method, the following calibration curve was obtained:  $R = 5.5369 - 9.8114\text{ DA}$ . Based on this equation, the DA values of the film surfaces were estimated from the FT-IR spectra of the acetylated SD-ChNF films measured using the ATR method (Figure 2d, entry 4), which were not mostly affected by the reaction conditions and were close to the quantitative DA value of chitin (~2) as shown in Table 1. These FT-IR results supported the fact that the acetylation reactions occurred almost quantitatively on the surface and progressed from the surface to the interior of the films according to increasing amounts of pyridine and elevating temperatures (from entry 1 to entry 4).

The XRD measurement of the acetylated SD-ChNF films was then performed to evaluate the chitin crystallinity. The CI values in the 110 planes of the chitin crystals ( $19^\circ$ ), which were calculated based on the XRD results with typical diffraction patterns assigned to the intrinsic crystalline structure of the chitin (Supplementary Figure S2), decreased with increasing DA values of the entire product (Table 1, from FT-IR spectra, measured using the KBr pellet method). These results indicated that the progress of acetylation inside the film gradually disrupted the crystalline structures of the chitin chains. The surface morphologies of the acetylated SD-ChNF films were then evaluated using SEM measurement. The SEM image of the parent SD-ChNF film showed a heavily condensed

nanofiber morphology (Figure 3a), as previously reported in the literature [30]. On the other hand, all the SEM images of the acetylated SD-ChNF films did not exhibit clear nanofiber morphologies (Figure 3b–e), owing to the almost quantitative acetylation on the surfaces of the films, regardless of the reaction conditions.

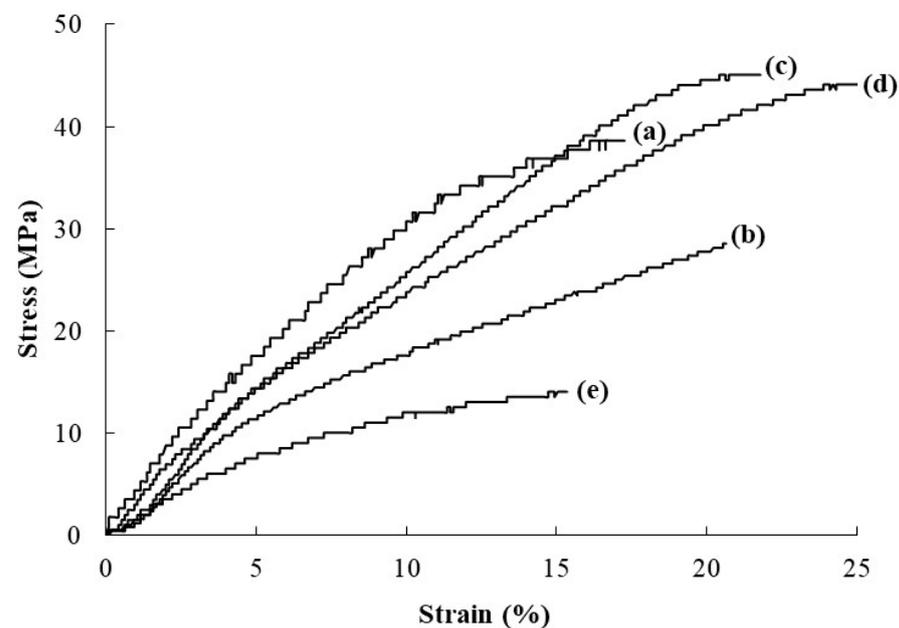


**Figure 2.** FT-IR spectra of (a,b) chitin using KBr pellet and ATR methods, respectively, and (c,d) acetylated SD-ChNF film of entry 4 using KBr pellet and ATR methods, respectively.



**Figure 3.** SEM images of the (a) SD-ChNF film and (b–e) acetylated SD-ChNF films of entries 1–4.

The mechanical properties of the acetylated SD-ChNF films were evaluated through tensile testing. The stress–strain curves of the acetylated SD-ChNF film of entry 1 showed a lower tensile strength than that of the parent SD-ChNF film (Figure 4a,b). With increasing DA values of the entire acetylated SD-ChNF films of entries 2 and 3, both tensile strength and elongation at break increased beyond those of the SD-ChNF film (Figure 4c,d). However, a further increase in the DA value drastically weakened the mechanical properties, as seen in the stress–strain curve of the acetylated SD-ChNF film of entry 4 (Figure 4e). These results strongly suggested that the optimized acetylation of chitin inside the films contributed to the enhancement in the mechanical properties of the SD-ChNF film. Acetylation on the surface probably caused the nanofibers at interfacial area to merge, resulting in a smooth surface of the film (Figure 3) due to an improvement in the mechanical properties. However, further progress of acetylation inside the film reduced the chitin crystallinity, which weakened the mechanical properties.



**Figure 4.** Stress–strain curves of the (a) SD-ChNF film and (b–e) acetylated SD-ChNF films of entries 1–4 under tensile mode.

#### 4. Conclusions

In this study, an improvement in the mechanical properties of the SD-ChNF films was obtained via acetylation along with adjusting the DA values. The acetylation of the SD-ChNF films was carried out using acetic anhydride in pyridine for 1 h at desired temperatures followed by cooling for 30 min at room temperature. The FT-IR spectra of the acetylated SD-ChNF films suggested the progress of acetylation from the surface to the interior of the films in response to increasing amounts of pyridine and elevating temperatures, which caused a disruption of the chitin crystals. The heavily condensed ChNF morphology on the surface of the SD-ChNF film became unclear after acetylation regardless of the DA values, because the surface was almost quantitatively acetylated in all cases. Tensile testing of the SD-ChNF films indicated that their mechanical properties were improved via acetylation with the optimized DA values. The presented ChNF materials with excellent mechanical properties have potentials for practical applications as new bio-based films in green and sustainable fields because of their eco-friendly and non-toxic properties. Furthermore, the present approach will be extended to use other acylation reagents, such as fatty acyl chlorides, to obtain new SD-ChNF materials with additional functions in the future.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/surfaces6030017/s1>, Figure S1: <sup>1</sup>H NMR spectrum of sample via acidic hydrolysis and dissolution of chitin acetate, prepared using acetic anhydride in 1-allyl-3-methylimidazolium bromide (AMIMBr), in DCl/D<sub>2</sub>O, Figure S2: XRD profiles of (a–d) acetylated SD-ChNF films of entries 1–4 and (e) SD-ChNF film.

**Author Contributions:** J.-i.K. conceived of the project, designed the experiments, directed the research, and wrote the manuscript. C.I. and A.N. performed the experiments. All authors have read and agreed to the published version of the manuscript.

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