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Preparation of high-strength transparent chitosan film reinforced with surface-deacetylated chitin nanofibers

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ABSTRACT

Surface-deacetylated chitin nanofiber reinforced chitosan films were prepared. The nano-composite films were highly transparent of approximately 84% at 600 nm due to the nanometer-sized fillers and chitosan matrix, which were embedded in the cavities and on the rough surface of the nanofiber networks. Due to the extended crystalline structure, the nanofibers worked effectively as reinforcement filler to improve the Young's modulus and the tensile strength of the chitosan film. After 10% blending of nanofiber, these properties were increased by 65% and 94%, respectively. Moreover, thermal expansion was also significantly decreased from 35.3 to 26.1 ppm K^{-1} after 10% addition of nanofibers. Surface-deacetylated chitin nanofiber and the nano-composite films showed antifungal activity against *A. alternata*.

1. Introduction

Although chitin, which is the main component of exoskeletons of crustaceans such as crab and shrimp, is the second most abundant biopolymer in nature, synthesized at a rate of 10^{10}–10^{11} tons per year (Muzzarelli, 2012), most chitin is thrown away as industrial waste. Chitosan is produced by deacetylation of chitin and has a cationic linear polysaccharide structure. Due to its biocompatible and biodegradable, non-toxic, anti-bacterial, and anti-fungal properties (Dutta, Tripathi, Mehrotra, & Dutta, 2009), chitosan has great potential for many uses, especially in food and agriculture applications (Ziani, Fernández-Pan, Royo, & Maté, 2009), since chitosan is allowed for food use in some countries, including Japan. Chitosan is soluble in acidic water and the viscous polymer solution can be used to make functional films. The chitosan-based films can potentially be used as a packaging material for the quality preservation of a variety of food and agricultural products such as fruits and vegetables. However, the uses of chitosan films are limited because of the low mechanical properties (Park, Marsh, & Rhim, 2002).

We have recently prepared chitin nanofibers (NF-chitin) from chitin extracted from crab shell (Ifuku et al., 2009; Ifuku and Saimoto, 2012). The NF-chitins have a highly uniform structure with approximately 10 nm thickness. The nanofibers have excellent mechanical properties due to their aniti-parallel extended crystalline structure. NF-chitins are therefore useful as reinforcement filler for creating high-performance composite materials. We have previously reported NF-chitin-reinforced plastics and organic-inorganic hybrid films (Ifuku, Morooka, Nakagaito, Morimoto, & Saimoto, 2011; Ifuku et al., 2012). More recently, surface-deacetylated chitin nanofibers have also been reported (Fan, Saito, & Isogai, 2010). Although the nanofiber surface in this study was transformed into chitosan by deacetylation, the core part was maintained as chitin crystal. Due to the cationic electrostatic repulsive forces, the surface-deacetylated NF-chitins were homogeneously dispersed in water. The high dispersion ability in water is useful for homogeneous blending with a water-soluble polymer to prepare a composite film through the casting process. Thus, the findings of these previous studies are applicable for using chitosan to create a high-performance bio-based film. In the present study, we prepared a chitosan film reinforced with surface-deacetylated NF-chitins to improve the mechanical properties, thus increasing the potential for using chitosan as an environmentally friendly multi-performance material.

2. Experimental

2.1. Materials

Chitin powder from crab shells was purchased from Nacalai Tesque, Inc. The degree of amino groups of the chitin was 3.9%.

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Chitosan and sodium hydroxide was purchased from Tokyo Chemical Industry Co., Ltd. (degree of deacetylation = 74%, M_W = 56,000) and used as received.

2.2. Preparation of surface-deacetylated NF-chitins

Surface-deacetylated chitin nanofibers were prepared as described previously and modified in NaOH concentration, reaction time, and temperature (Fan et al., 2010). Chitin powder (40 g) was stirred in 20% (w/w) NaOH (3.0 L) for 6 h at 160 °C under an argon atmosphere. After deacetylation, the supernatant was removed by decantation. The precipitate was thoroughly washed with distilled water by centrifuge to remove any water-soluble products. For nano-fibrillation, the deacetylated chitin was dispersed in 2.0 L of aqueous acetic acid (1.0% (w/w)). The sample was passed through a grinder (MKCA6–3; Masuko Sangyo Co., Ltd.) at 1500 rpm three times. Grinder treatment was performed with a clearance gauge of 1.5 (corresponding to a 0.15 mm shift) from the zero position. The position was determined as the point of slight contact between the two grinding stones. The concentration and degree of deacetylation of the obtained deacetylated chitin nanofibers were 0.62 wt% and 10%, respectively.

2.3. Preparation of surface-deacetylated NF-chitin/chitosan composite film

Chitosan was dissolved in 1.0% (v/v) aqueous acetic acid with a 1 wt% concentration. The chitosan solution was blended with the surface-deacetylated NF-chitin suspension (0.62 wt%) and treated with a super stirrer (Awatori-rentaro, ARE-301, THINKY Ltd.) to obtain a homogeneous mixture and to remove air bubbles. The mixtures were prepared with a dry weight ratio of surface-deacetylated NF-chitin/chitosan = 10/0, 9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8, 1/9, and 0/10 with a total dry weight of 20 mg. The mixtures were poured into glass Petri dishes coated with release agent and dried under ambient conditions (40 °C, 3 days). Thus, eleven different types of composite films were prepared with a thickness of 20 μm and a weight of 20 mg.

2.4. Measurements

The regular light transmittances of surface-deacetylated NF-chitin/chitosan composite films were measured by a UV–vis spectrophotometer (JASCO-V550) in the wavelength range of 400–800 nm. Tensile strengths and Young's moduli were measured by a universal testing instrument (AG-X, Shimadzu) for samples 50 mm long and 10 mm wide at a cross head speed of 1 mm min^{-1} with a gage length of 30 mm. For data accuracy, at least three specimens were used for testing. The coefficients of thermal expansion (CTE) were evaluated with a thermomechanical analyzer (Q400, TA instruments). Specimens for CTE measurement were 30 mm long and 3 mm wide with a 20 mm span. The measurements were carried out from 30 to 165 °C by raising the temperature at a rate of 5 ∘C min^{-1} in a N_2 atmosphere in tensile mode under a load of 0.05 N. The CTE values were determined in the second run. At least three specimens were used for testing. The degree of deacetylation was calculated from the C and N contents in the elemental analysis data by using an elemental analyzer (Elementar Vario EL III, Elementar). For field emission scanning electron microscopic (FE-SEM) observation, the sample was coated with an approximately 2 nm layer of Pt by an ion sputter coater and observed by FE-SEM (JSM-6700F; JEOL, Ltd.) operating at 2.0 kV.

2.5. Antifungal activity test

The strain O-276 of *Alternaria alternata*, which causes black spot disease on susceptible Japanese pear cultivars, was maintained on potato dextrose agar (Difco, Detroit, MI, USA) slopes or as 15% glycerol stocks at 80 °C. For antifungal activity tests, spores of the fungus were prepared as previously described (Johnson, Johnson, Itoh, Kodama, Otani, & Kohmoto, 2000). A spore suspension (10^5 spores/ml) was dropped on surface-deacetylated NF-chitin/chitosan composite films in the proportions 10/0, 8/2, 5/5, 2/8, and 0/10. A spore suspension was placed on a cellulose membrane (Seamless cellulose tubing, Wako Pure Chemicals) as a control. After incubation for 24 h, spore germination was observed under a light microscope.

3. Results and discussion

3.1. Transparency of surface-deacetylated NF-chitin/chitosan composite films

Partial deacetylation of chitin was successful by aqueous NaOH treatment with the degree of deacetylation of 10%. The partially deacetylated chitin was treated by using a grinder instrument under acidic conditions (Fan et al., 2010). By assistance of electrostatic repulsion caused by cationization of the amino group at the C2 position, the deacetylated chitin was thoroughly disintegrated into nanofibers and homogeneously dispersed in water after the grinder treatment. The sample was highly viscous and had a fine nanofiber structure with a width of approximately 10 nm. From the X-ray diffraction patterns, it can be determined that deacetylation mostly took place on the surfaces of the chitin nanofibers.

Nano-composite films were prepared from the aqueous mixture of surface-deacetylated NF-chitins and chitosan in proportions from 10/0 to 0/10 by casting and drying under ambient temperature. Fig. 1 shows the regular light transmittance spectra of the nanocomposite films. The transmittance of neat surface-deacetylated NF-chitin film was highly transparent (79.8%) at 600 nm (Fan, Fukuzumi, Saito, & Isogai, 2012). The mechanism causing the high levels of transparency is as follows. Since the highly dispersed surface-deacetylated NF-chitin was allowed to

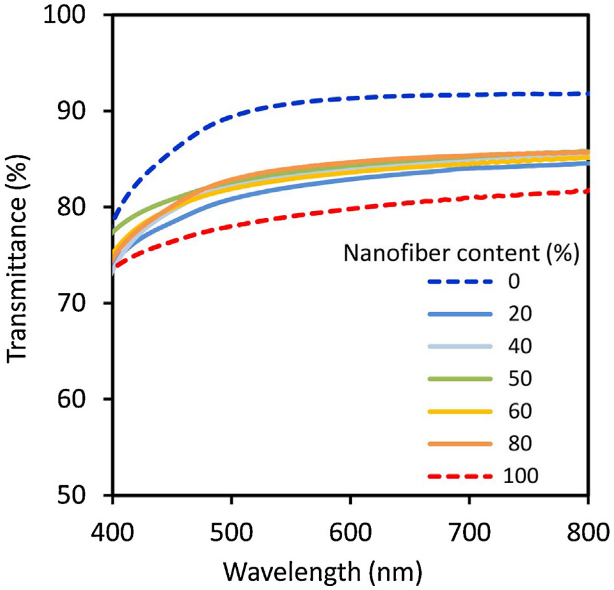

Fig. 1. Regular light transmittance spectra of nano-composite films with a different nanofiber content.

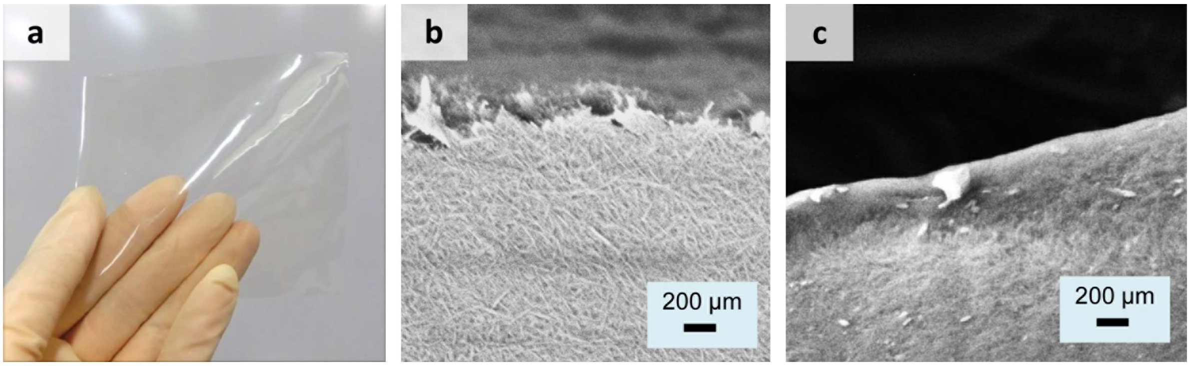

Fig. 2. (a) The appearance of nanocomposite film. (b and c) Surface images transparent film with nanofiber content of (b) 100% and (c) 50%.

dry slowly, the nanofibers were densely packed, and the cavities in the cast film were almost entirely removed. Since cavities cause light scattering at the nanofiber interfaces, densely packed nanofiber films will have less light scattering, resulting in a high level of transparency (Nogi, Iwamoto, Nakagaito, & Yano, 2009). Surface-deacetylated NF-chitin/chitosan composite had a higher transparency of approximately 84% at 600 nm than neat surface-deacetylated NF-chitin film regardless of nanofiber content (Fig. 2a). The improvement in transmittance is due to the chitosan molecules playing the role of a matrix to reduce light scattering. That is, chitosan was embedded in air cavities of the nanofiber network, which reduced light scattering in the film. Moreover, the chitosan matrix made the film surface smooth. In Fig. 2b, nanofiber elements can be observed on the neat NF-chitin film surface. On the other hand, due to the chitosan coating, the nanocomposite film had a flatter and smoother surface, resulting in higher transparency (Fig. 2c)

3.2. Mechanical properties of surface-deacetylated NF-chitin/chitosan composite films

Figs. 3 and 4 show the tensile Young's moduli and fracture strengths, respectively, of the surface-deacetylated NF-chitin/chitosan composite films in the proportions from 10/0 to 0/10. Since chitin nanofiber has an extended crystalline structure, surface-deacetylated NF-chitin also has efficient mechanical properties due to the core part of the chitin crystal. Therefore, Young's modulus and the tensile strength of neat surface-deacetylated NF-chitin film were very high, 8832 MPa and 156.5 MPa, respectively. These values came close to those of cellulose nanofiber films (Fukuzumi, Saito, Iwata, Kumamoto, & Isogai, 2009; Abe & Yano, 2009), and were higher than previously reported for surface-deacetylated NF-chitin film (Fan et al., 2012). The high mechanical properties of the film were due to the hydrogen bonding between strong NF-chitin caused by the ambient drying process. In addition, the improvement of mechanical properties would be caused by modification of the deacetylation process. On the other hand, Young's modulus and the tensile strength of neat chitosan film were quite low, 2640 MPa and 40.0 MPa, respectively, since the crystallinity of chitosan is low. However, these properties significantly increased to 4406 MPa and 77.6 MPa, respectively, after only 10% blending of surface-deacetylated NF-chitin. These values corresponded to a 65% and 94% increase. These significant improvements indicate that surface-deacetylated NF-chitins were dispersed homogeneously and worked effectively as reinforcement fillers in the chitosan film. These properties proportionally increased with increasing nanofiber content in the films. Thus, the mechanical properties of chitosan films can easily be controlled by changing the content of NF-chitin according to the application of the chitosan film.

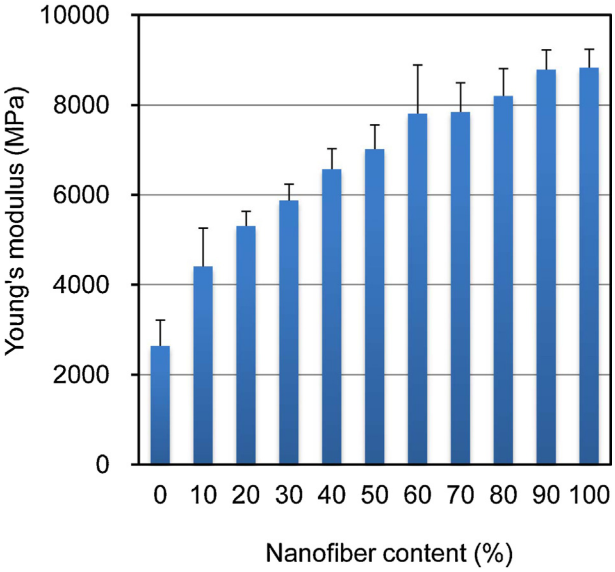

Fig. 3. Young's modulus of nano-composite films with a different nanofiber content.

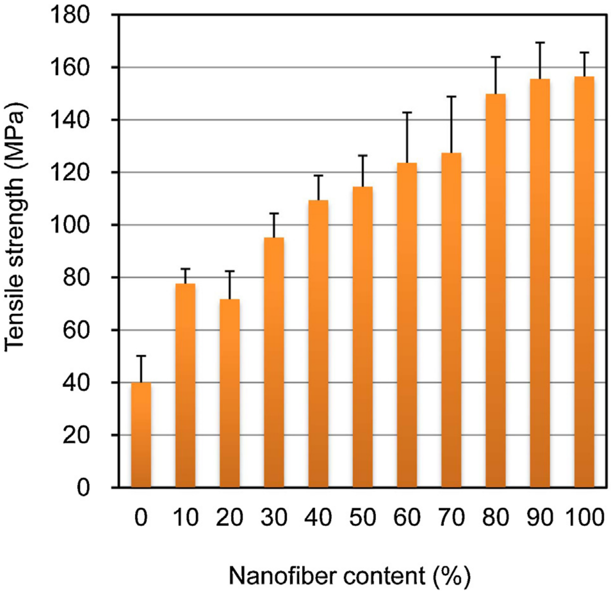

Fig. 4. Tensile strength of nano-composite films with a different nanofiber content.

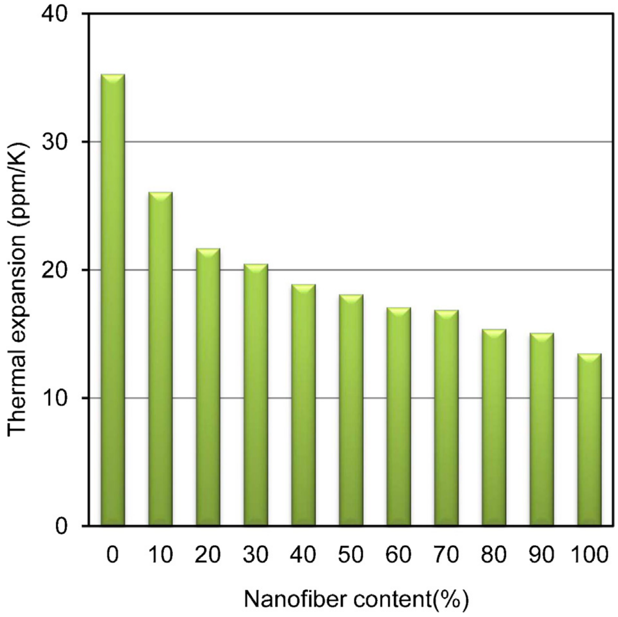

Fig. 5. Coefficient of thermal expansion of nano-composite films with a different nanofiber content.

3.3. Thermal expansion of surface-deacetylated NF-chitin/chitosan composite films

Thermal expansion is related to the depth of the atomic bond energy function and has an inverse relationship with Young's modulus. Since NF-chitins show a high Young's modulus, surface-deacetylated NF-chitins should also have a high Young's modulus and low thermal expansion. Thus, the coefficient of thermal expansion (CTE) of neat surface-deacetylated NF-chitin is only 13.5 ppm K^{-1} (Fig. 5). In contrast, the neat CTE of chitosan film was found to be considerably higher, 35.3 ppm K^{-1}. After a 10% addition of nanofibers, the CTE of the chitosan film decreased by 26% to 26.1 ppm K^{-1}. By increasing the nanofiber content, the CTE of chitosan film was proportionally decreased. Thus, NF-chitin effectively decreased the thermal expansion of chitosan film.

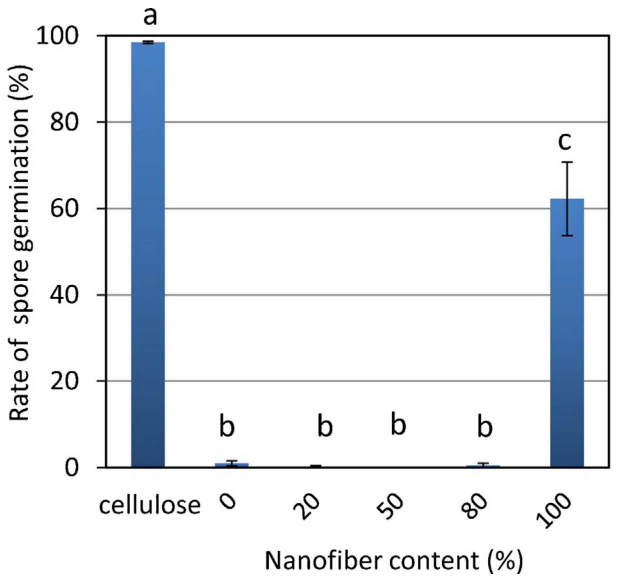

Fig. 6. Antifungal activity of nano-composite films with a different nanofiber content. Spore suspension (105 spores/ml) was dropped on surface deacetylated NF-chitin/chitosan composite films with the percentage of nanofiber content 0, 20, 50, 80 and 100%. Spore suspension was placed on a cellulose membrane as a control. After incubation for 24 h, spore germination was observed. Data represent the mean of four independent experiments. Means with the same letter are not significantly different according to Tukey's test ($P < 0.05$).

3.4. Antifungal activity of surface-deacetylated NF-chitin/chitosan composite films

Alternaria alternata is known as a plant pathogenic fungi that affects a broad range of host species at any stage of growth, including in harvested products. To assess the antifungal activity of the surface-deacetylated NF-chitin/chitosan composite films, the rate of spore germination of one strain of *A. alternata* was estimated. The rate of spore germination on the surface-deacetylated NF-chitin/chitosan composite film at a 100% nanofiber content decreased from 99% to 63% compared to that on cellulose membrane (Fig. 6). Antifungal activity was more effective on chitosan film. There were no differences in the inhibition rate (0 ~ 1%) among the levels of nanofiber content from 0 to 80%. These results indicate that NF-chitins contribute to an improvement in the physical properties of chitosan film without any impairment of antifungal activity.

4. Conclusion

Surface-deacetylated NF-chitin/chitosan composite films were prepared. When the cavities of the nanofiber networks and their surface were embedded with a chitosan matrix, the transparency of the nanofiber sheet increased. According to the nanofiber content, Young's modulus and tensile strength of the composite increased compared to neat chitosan film due to a reinforcement effect of strong nanofibers. The thermal expansion of composites decreased significantly due to the low thermal expansion of nanofiber fillers. The nano-composite films inhibited the spore germination of *A. Alternata*, known as plant pathogenic fungi. Thus, surface-deacetylated NF-chitins were found to improve the mechanical properties and thermal expansion of chitosan without reducing its transparency, flexibility and anti-fungal activity. This result of the present study should allow for increased use of chitosan film in food and agricultural packaging applications.

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